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High-precision sampling of trace gas-borne volatiles by the dynamic solvent effect with a comparative review of alternative techniques

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SUMMARY

The precise determination of traces of organic volatiles is particularly challenging in semiochemistry and clinical chemistry, where specimen sizes are intrinsically limited and amounts of trace components correspondingly very small. This demands a sampling technique that is fully compatible with the ability of capillary columns and gas chromatographic detectors to separate and quantify nanogram and sub-nanogram amounts from complex mixtures.

The quantitative precision of dynamic solvent-effect sampling of low parts per billion (10^9) aqueous carbonyl compounds, high ppb and low parts per million aqueous phenols, low ppb and high parts per trillion (10^{12}) airborne hydrocarbons and the volatiles from wine, human urine and a slow-release pesticide was tested with specimen sizes that yielded amounts of volatiles down to the sub-nanogram level.

Provided that sources of variability, such as temperature changes, adsorption on containers, incomplete peak resolution and changes in the specimens themselves, were adequately controlled, dynamic solvent-effect sampling consistently provided coefficients of variation in peak areas, peak percentage areas and peak-area ratios of less than 10% at nanogram and sub-nanogram levels. The literature was surveyed for data on the performance of other sampling systems. None of them have been demonstrated to match the precision of the dynamic solvent effect with such small amounts from such a wide range of materials.

INTRODUCTION

The use of films of liquid to extract volatiles from gases for chromatographic analysis extends back to at least 1964 when Pavelka[1] used a film of solvent spread on glass beads to trap airborne volatiles. Grob[2] demonstrated focusing by the solvent effect, on a 2-μl film of hexane on a capillary column, of low-boiling volatiles from 1

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cm^3 of the headspace of a spice. Jennings[3] trapped food headspace volatiles on a refluxing film of Freon 12. Roerade and Blomberg[4] mentioned the possibility of using the solvent effect to focus gas-phase volatiles, but presented no experimental results. Pretorius and Bertsch[5] provided a theoretical treatment but their requirement that the carrier gas be saturated with solvent to prevent evaporation of the solvent film is impractical, and imcompatible with sampling from live animals and any specimen that cannot be enclosed and pressurized. Pretorius and Lawson[6] considered theoretically a more versatile method in which the solvent film is allowed to evaporate during sampling. Neither of these theoretical papers included any experimental findings.

The dynamic solvent effect accumulates gas-phase volatiles by trapping them on the evaporating edge of a film of pure solvent held in dynamic equilibrium between evaporation and capillary rise in an axially perforated, porous, packed bed[7]. The resulting sample consists of the trapped volatiles and approximately 20 μl of solvent; it can be transferred directly to a capillary column by carrying out static solvent-effect focusing with the bed in an inlet to which the column is connected[8,9].

The dynamic solvent effect was developed specifically to provide a sampling technique that allows full exploitation of the ability of capillary columns and gas chromatographic detectors to separate and quantify low- and sub-nanogram amounts of solutes in complex mixtures, a need which is keenly felt in work on semiochemicals[10] and clinical chemistry[11].

The quantitative precision of dynamic solvent-effect sampling of gas-borne volatiles from a range of specimens is reported here. The specimens were chosen to represent types of material commonly analysed by capillary gas chromatography and to provide a test of the performance of the dynamic solvent effect with small specimens and samples.

EXPERIMENTAL

Separations were carried out in a Varian 3700 gas chromatograph fitted with a dynamic solvent-effect inlet[8] and a 25 m × 0.3 mm I.D. capillary column coated with a 0.4-μm film of methylsilicone. The initial temperature of the inlet and column was 40°C, the inlet was heated ballistically to 220°C after a solvent evaporation time determined for each concentrator[8], and the column temperature was programmed at 10°C min^{-1} after 6 min for synthetic specimens, or at 5°C min^{-1} for natural specimens. The carrier gas was hydrogen with a linear velocity of 50 cm s^{-1}. A flame ionization detector was used at a sensitivity of 10^{-11} A mV^{-1} and chromatograms were recorded on a Varian 4270 integrator with a full-scale deflection of 2 or 4 mV. Eight dynamic solvent effect concentrators were used for the various specimens.

Attempts to make up standard specimens with low parts per billion (10^9) concentrations, which were accurate to within the 1–2% limits needed to test the accuracy of dynamic solvent-effect sampling, proved unproductive. Adsorption, the effects of temperature on density, limited volumetric accuracy and evaporation of volatile solvents all contribute to uncertainty in the concentration of standards[12–15]. Nor was it possible to measure the concentration of a standard independently because even the best of the alternative methods yield sampling variations as large as or larger than that provided by the dynamic solvent effect (see Table XIII and Discussion). Consequently, only the precision of dynamic solvent-effect sampling was investigated; the

concentrations of the specimens are presented only as guide to the levels at which the performance was obtained.

Similarly, the masses of each compound represented by a given peak are based on calibrations of detector response from split injections of relatively concentrated solutions. When the identity of component was unknown, as in the wine and urine samples, its quantification was based on the response of the detector to *n*-alkanes, so the amounts given are probably slightly higher than the true values. The figures for the mass of each component are offered only as a guide to the level of sensitivity at which the reported precision was obtained. As standard deviations were calculated from raw peak areas, these approximations do not affect the reported precision of the dynamic solvent effect.

The test specimens were prepared as follows.

Aqueous aldehydes and ketones

The standard solution contained (concentrations in ppb) 2-heptanone (40), heptanal (16), 2,6-dimethyl-4-heptanone (16), nonanal (48), decanal (16), undecanal (24) and dodecanal (8) in distilled water, which had been purged of organic volatiles by vigorous boiling and sparging with charcoal-filtered nitrogen.

Each specimen was a 5-cm^3 aliquot of the stock standard solution measured into a 10-cm^3 borosilicate glass bubbler (Fig. 1) using a borosilicate glass pipette. Both the pipette and bubbler were rinsed with 5 cm^3 of standard solution immediately before each specimen was measured. The test compounds were purged from the water with a 10 cm^3 min^{-1} flow of palladium-purified hydrogen for 10 min, and trapped by the dynamic solvent effect using *n*-hexane as solvent at 30-30.6°C. A series of five samples were run on each of three concentrators.

A further series of five samples, for which the pipette and bubbler were not rinsed, were run on one concentrator.

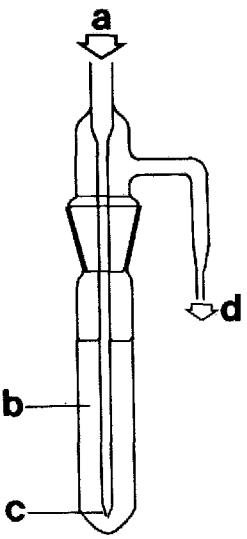

Fig. 1. Bubbler used to sparge volatiles from up to 10 cm^3 of liquid when sampling by the dynamic solvent effect. a = pure gas; b = liquid specimen; c = fine tip; d = to dynamic solvent effect concentrator.

Aqueous phenols

Precisely weighed portions (*ca.* 140–250 mg) of phenol, *p*-cresol and 2,4-dichlorophenol were each dissolved in 2 cm^3 of 5 *M* sodium hydroxide solution. Interfering impurities were extracted from the solutions with 5 × 100 μl of *n*-hexane. Sufficient of each solution was added to 1 l of water in a borosilicate glas flask to give concentrations of $1.67{:}10^6$ of phenol, $0.42{:}10^6$ of *p*-cresol and $0.13{:}10^6$ of 2,4-dichlorophenol. The water had been distilled three times from alkaline permanganate and percolated through activated charcoal.

Each sample was obtained by bubbling palladium-cell-purified hydrogen through the 1 l of solution at a flow-rate of 5 $cm^3\ min^{-1}$ for 10 min. The temperature was held at 29.6°C. Five samples were taken on each of three concentrators using *n*-hexane as solvent.

Airborne hydrocarbons

Low concentrations of a complex mixture of hydrocarbons in air were generated by passing a 10 $cm^3\ min^{-1}$ flow of charcoal-filtered air over the surface of *ca.* 10 cm^3 of white petroleum jelly in a 20-cm^3 tube (Fig. 2) at room temperature (26–27°C). The air flow was maintained for 1 week, with hydrocarbon concentrations monitored daily, to allow the system to stabilize before serial sampling was carried out. Sampling

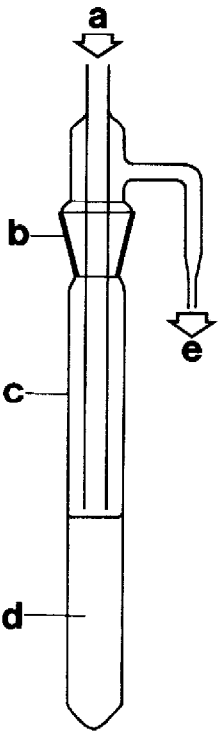

Fig. 2. Apparatus used to generate ppb concentrations of complex mixtures of airborne hydrocarbons. a = 10 $cm^3\ min^{-1}$ of charcoal-purified air; b = 14/23 joint; c = 20-cm^3 tube; d = 10 cm^3 white petroleum jelly; e = to dynamic solvent effect concentrator.

was carried out by passing the hydrocarbon-loaded air through a dynamic solvent-effect concentrator, with *n*-hexane as solvent, for 10 min. A series of five samples were taken on each of three concentrators.

Wine

Each specimen was 10 cm^3 of a red table wine (Tassenberg, Stellenbosch Farmers Winery, Oude Libertas, Stellenbosch, South Africa) dispensed directly from its commercial, laminated foil container into a graduated, borosilicate glass bubbler that had been rinsed with the same wine. Palladium-purified hydrogen was bubbled through the wine for 10 min at a flow-rate of 10 cm^3 min^{-1} at 29.6°C for each of a series of five samples on one concentrator.

Urine

Approximately 250 cm^3 of human urine were collected and kept at 0°C until analysis. Portions of 10 cm^3 were measured with a borosilicate glass pipette into a 50-cm^3 borosilicate pear-shaped flask. The urine specimen was allowed 10 min to warm to the sampling temperature of 29.6°C, then 10 cm^3 min^{-1} of palladium-purified hydrogen were bubbled through it for 10 min. Five samples were taken on one concentrator.

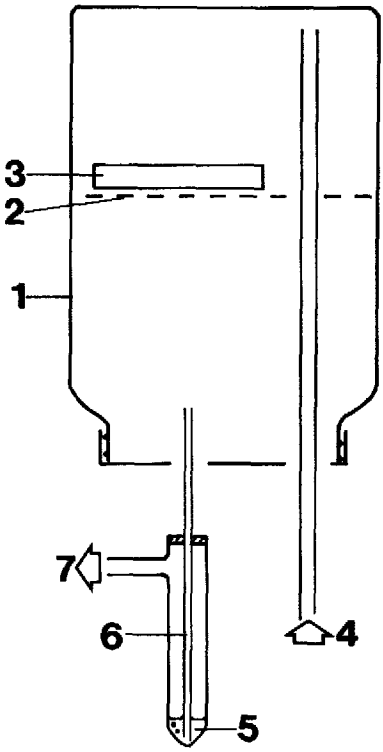

Fig. 3. Apparatus used for sampling volatiles from a slow-release pesticide strip by the dynamic solvent effect. 1 = 500-cm^3 jar; 2 = wire grid; 3 = pesticide strip; 4 = 1 l min^{-1} charcoal-filtered air; 5 = *n*-hexane; 6 = dynamic solvent-effect concentrator; 7 = 5 cm^3 min^{-1} gas flow to vacuum.

Dichlorvinphos

A commercial, slow-release pesticide strip, designed for small spaces and containing 19.5% of dichlorvinphos (Vapona Cupboard Exterminator, Shell Chemicals) was aged for 8 days in the open air and for a further 9 days in a glass container flushed with 1 l min^{-1} of charcoal-filtered air. On the 18th day eight samples were taken on one concentrator by sucking air from the container at 5 cm^3 min^{-1} for 5 min (Fig. 3). Over the sampling period the temperature varied between 23.6 and 24.1°C. The identity of the dichlorvinphos peak was confirmed by gas chromatography–mass spectrometry.

Chromatograms of the volatiles from each type of specimen are shown to illustrate the general quality of the separations achieved.

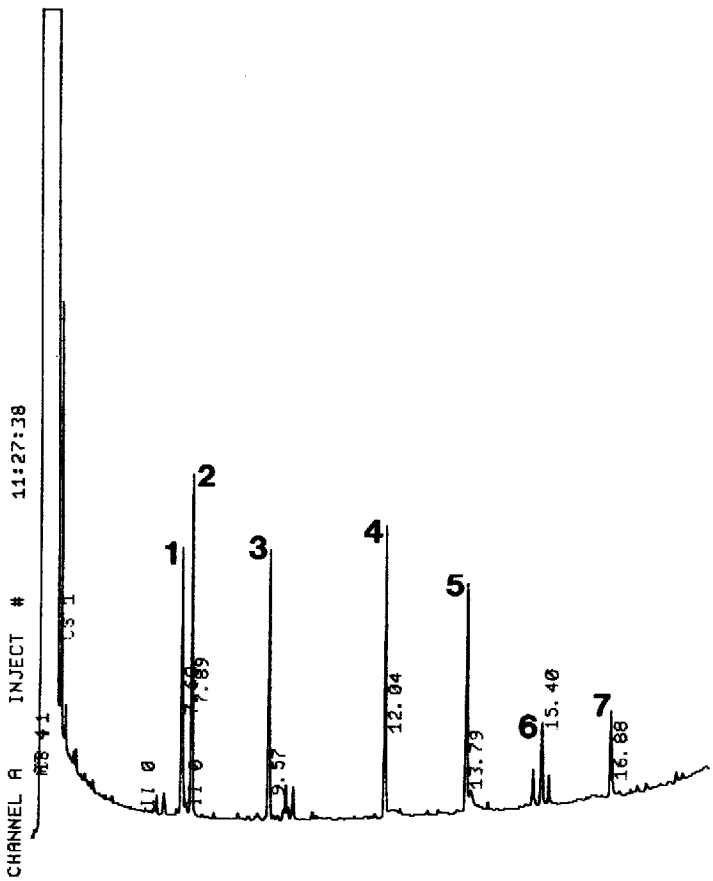

Fig. 4. Chromatogram of carbonyl compounds sampled from 5 cm^3 of aqueous solution by gas sparging at 10 cm^3 min^{-1} for 10 min at 30–30.6°C and trapping by the dynamic solvent effect using *n*-hexane as solvent. Peaks: 1 = *n*-heptan-2-one; 2 = *n*-heptanal; 3 = 2,6-dimethylheptan-4-one; 4 = *n*-nonanal; 5 = *n*-decanal; 6 = *n*-undecanal; 7 = *n*-dodecanal. Computer-printed values at peaks are retention times in min.

TABLE I

COEFFICIENTS OF VARIATION OF PEAK AREAS AND PERCENTAGE AREAS, OVER FIVE REPLICATES ON EACH OF THREE CONCENTRATORS, FOR AN AQUEOUS SOLUTION OF CARBONYL COMPOUNDS SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Glassware rinsed with specimen solution

Compound	*Approx. mass (ng)*	*Coefficient of variation (%)*					
		Concentrator Q2		*Concentrator Q3*		*Concentrator H7*	
		Area	*% Area*	*Area*	*% Area*	*Area*	*% Area*
n-Heptanone	2.5	3.87	4.02	3.94	2.79	6.25	3.20
n-Heptanal	3.5	3.22	2.83	2.43	1.79	7.63	7.17
Dimethylheptanone	3.1	4.30	5.66	3.88	2.83	6.14	2.96
n-Nonanal	3.0	3.92	1.27	5.29	2.31	8.84	3.02
n-Decanal	2.6	10.51	7.96	5.22	2.05	16.18	8.70
n-Undecanal	0.9	9.68	6.25	8.48	6.53	10.14	8.99
n-Dodecanal	1.5	10.88	8.06	9.61	7.58	12.94	11.44

Statistical analysis

Means, standard deviations and coefficients of variation [(S.D./mean) · 100] were calculated for peak areas, peak percentage areas and peak-area ratios from sets of five consecutive runs.

RESULTS

Aqueous aldehydes and ketones

For all the test components the peaks were sharp and symmetrical (Fig. 4). The coefficients of variation of the various statistics are given in Tables I–III.

Omitting the rinsing of the pipette and bubbler with specimen solution seriously degraded the precision of the peak areas (Table IV).

TABLE II

COEFFICIENTS OF VARIATION OF PEAK-AREA RATIOS, OVER FIVE REPLICATES ON EACH OF THREE CONCENTRATORS, FOR AQUEOUS CARBONYL COMPOUNDS SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Glassware rinsed with specimen solution.

Compound	*Coefficient of variation (%)*		
	Concentrator Q2	*Concentrator Q3*	*Concentrator H7*
Heptanone:heptanal	3.68	2.96	2.17
Heptanal:dimethylheptanone	5.83	2.92	2.34
Dimethylheptanone:nonanal	5.67	3.75	5.55
Nonanal:decanal	7.71	1.09	6.73
Decanal:undecanal	9.69	7.10	15.82
Undecanal:dodecanal	3.35	3.46	3.63

TABLE III

COEFFICIENTS OF VARIATION, POOLED FOR FIVE REPLICATES ON EACH OF THREE CONCENTRATORS, OF PEAK AREAS, PERCENTAGE AREAS AND PEAK-AREA RATIOS FOR DYNAMIC SOLVENT-EFFECT SAMPLING FROM AN AQUEOUS SOLUTION OF CARBONYL COMPOUNDS

Glassware rinsed with specimen solution.

Compound	*Coefficient of variation (%)*		
	Area	*% Area*	*Ratio*
n-Heptanone	8.09	17.04	
n-Heptanal	4.99	12.42	
Dimethylheptanone	12.06	4.18	
n-Nonanal	12.32	2.81	
n-Decanal	24.48	15.06	
n-Undecanal	14.83	7.33	
n-Dodecanal	38.11	31.93	
Heptanone:heptanal			5.64
Heptanal:dimethylheptanone			13.63
Dimethylheptanone:nonanal			4.70
Nonanal:decanal			14.69
Decanal:undecanal			15.45
Undecanal:dodecanal			34.50

Aqueous phenols

The phenols were eluted as sharp, symmetrical peaks (Fig. 5). All three concentrators yielded coefficients of variation of better than 10% for all three peak measurements (Tables V and VI). Analysis of the pooled data also produced coefficients of variation of less than 10% (Table VII).

Airborne hydrocarbons

Despite the complexity of the mixture of hydrocarbons (Fig. 6) and the small

TABLE IV

COEFFICIENTS OF VARIATION OF PEAK AREAS, OVER FIVE REPLICATES ON ONE CONCENTRATOR (Q2), FOR AN AQUEOUS SOLUTION OF CARBONYL COMPOUNDS SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Glassware not rinsed with specimen solution.

Compound	*Coefficient of variation (%)*
n-Heptanone	6.36
n-Heptanal	11.55
Dimethylheptanone	6.70
n-Nonanal	17.93
n-Decanal	28.65
n-Undecanal	27.02
n-Dodecanal	43.25

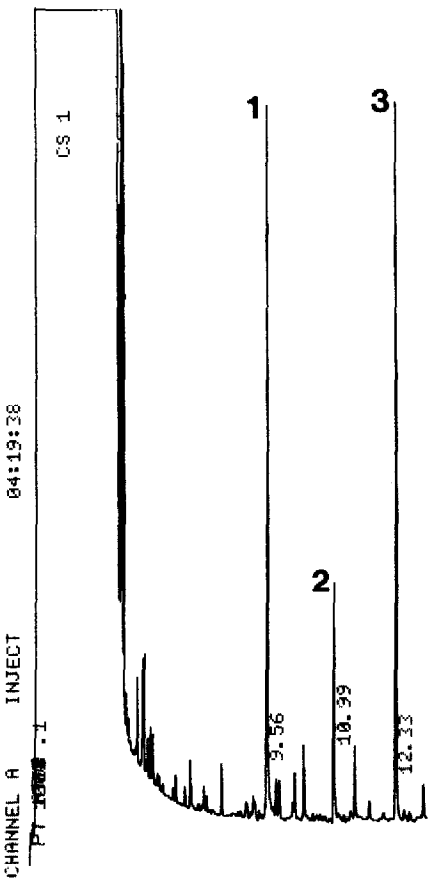

Fig. 5. Chromatogram of phenols sampled from an aqueous solution by gas sparging and the dynamic solvent effect with a flow-rate of 5 cm^3 min^{-1} for 10 min. Peaks: 1 = phenol (2.2 ng); 2 = *p*-cresol (0.8 ng); 3 = 2,4-dichlorophenol (2.5 ng).

amounts involved (0.6–2.0 ng), the coefficients of variation were below 10% in all instances (Tables VIII–X).

Wine

The wine samples yielded moderately complex chromatograms (Fig. 7). The coefficients of variation for the peaks which were above the integration threshold (0.5 ng) in all five runs are given in Table XI.

Urine

A chromatogram of the volatiles from the urine samples is shown in Fig. 8 and the coefficients of variation are given in Table XII.

Dichlorvinphos

In addition to its major pesticide component, the Vapona strip emitted a mix-

TABLE V

COEFFICIENTS OF VARIATION OF PEAK AREAS AND PERCENTAGE AREAS FOR FIVE REPLICATE SAMPLES, ON EACH OF THREE DYNAMIC SOLVENT-EFFECT CONCENTRATORS, FROM AN AQUEOUS STANDARD CONTAINING PHENOL AT $1.67{:}10^6$, *p*-CRESOL AT $0.42{:}10^6$ AND 2,4-DICHLOROPHENOL AT $0.13{:}10^6$

Compound	*Approx. mass (ng)*	*Coefficient of variation (%)*					
		Concentrator 4		*Concentrator 5*		*Concentrator 7*	
		Area	*% Area*	*Area*	*% Area*	*Area*	*% Area*
Phenol	2.2	3.5	2.3	1.3	1.3	4.3	4.6
p-Cresol	0.8	4.0	2.0	2.8	1.6	4.2	1.2
2,4-Dichlorophenol	2.5	2.8	2.2	1.7	0.9	7.6	4.7

TABLE VI

COEFFICIENTS OF VARIATION OF PEAK-AREA RATIOS FOR FIVE REPLICATE SAMPLES, ON EACH OF THREE DYNAMIC SOLVENT-EFFECT CONCENTRATORS, FROM AN AQUEOUS STANDARD CONTAINING PHENOL AT $1.67{:}10^6$, *p*-CRESOL AT $0.42{:}10^6$ AND 2,4-DICHLOROPHENOL AT $0.13{:}10^6$

Ratio	*Coefficient of variation (%)*		
	Concentrator 4	*Concentrator 5*	*Concentrator 7*
Phenol:*p*-cresol	3.0	4.8	2.6
Phenol:2,4-dichlorophenol	4.6	9.1	2.1
p-Cresol:2,4-dichlorophenol	3.6	4.8	2.1

TABLE VII

COEFFICIENTS OF VARIATION OF PEAK AREAS, PERCENTAGE AREAS AND PEAK-AREA RATIOS FROM AN AQUEOUS STANDARD CONTAINING PHENOL AT $1.67{:}10^6$, *p*-CRESOL AT $0.42{:}10^6$ AND 2,4-DICHLOROPHENOL AT $0.13{:}10^6$, FOR DATA POOLED FROM THREE SERIES OF FIVE REPLICATES ON THREE CONCENTRATORS

Compound	*Coefficient of variation (%)*		
	Area	*% Area*	*Ratio*
Phenol	7.94	4.62	
p-Cresol	7.97	2.00	
2,4-Dichlorophenol	7.50	4.32	
Phenol:*p*-cresol			5.08
Phenol:2,4-dichlorophenol			9.34
p-Cresol:2,4-dichlorophenol			5.16

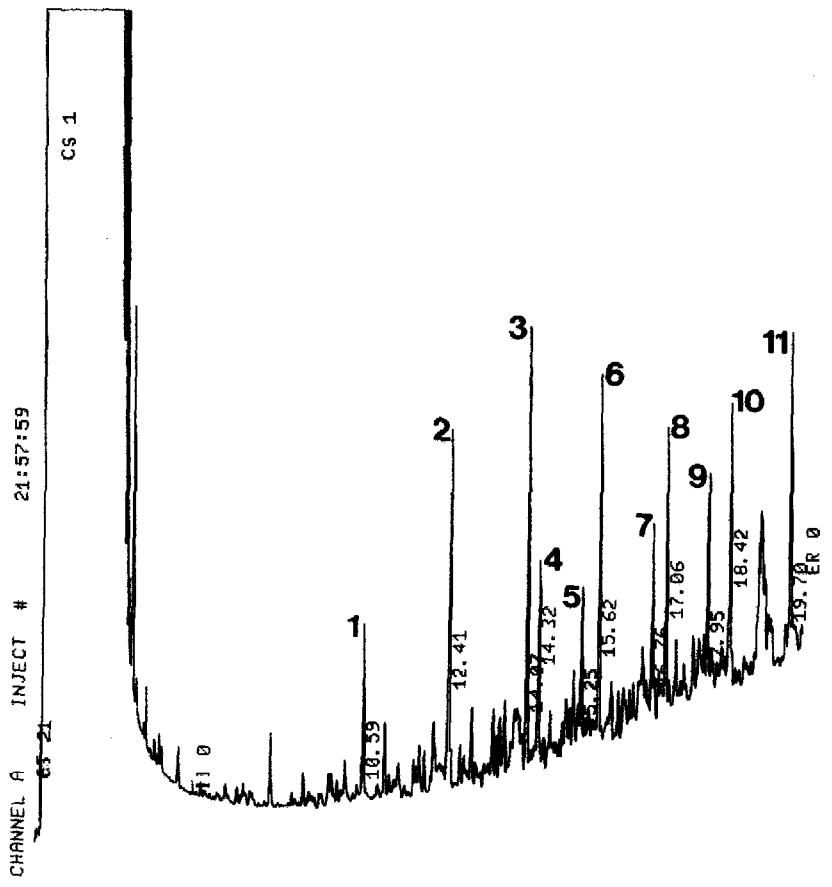

Fig. 6. Chromatogram of airborne hydrocarbons sampled by the dynamic solvent effect for 10 min with an air flow-rate of 10 cm^3 min^{-1}, using *n*-hexane as solvent at 26–27°C. Peaks: 1 = *n*-decane; 2 = *n*-undecane; 3 = *n*-dodecane; 6 = *n*-tridecane; 8 = *n*-tetradecane; 10 = *n*-pentadecane; 11 = *n*-hexadecane.

ture of hydrocarbons, presumably solvents (Fig. 9). Each sample contained *ca.* 90 ng of dichlorvinphos, giving a rate of emission from the strip of 60 ng s^{-1}. The coefficients of variation of the area and percentage area of the dichlorvinphos peak were both 1.61%. The ratio of the dichlorvinphos peak area to that of the largest hydrocarbon peak had a coefficient of variation of 2.46%.

DISCUSSION

The generation of accurately known concentrations of gas-phase volatiles in the ppb range presents considerable problems. Of the techniques available, the use of diffusion/permeation tubes appears to be the most accurate[12]. However, even at 50 ppm the calibration, by weight loss, of such a device is "tedious and time consuming"[16]. At ppb levels it would be almost impossible; an emission rate yielding 1 ng in a 100-cm^3 specimen sampled at 10 cm^3 min^{-1} would give a weight loss of 1 mg over a

TABLE VIII

COEFFICIENTS OF VARIATION OF PEAK AREAS AND PEAK PERCENTAGE AREAS OVER A SERIES OF FIVE REPLICATES ON EACH OF THREE CONCENTRATORS FOR A MIXTURE OF AIRBORNE HYDROCARBONS SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Peak[a]	*Concentration (ppb, v/v)*[b]	*Coefficient of variation (%)*					
		Concentrator Q2		*Concentrator H5*		*Concentrator H7*	
		Area	*% Area*	*Area*	*% Area*	*Area*	*% Area*
1	1.3	4.69	1.79	2.46	2.12	0.63	2.77
2	2.9	4.14	2.00	2.44	1.56	1.07	2.66
3	2.6	3.48	1.74	2.40	1.74	0.95	2.23
4	1.3	3.60	1.58	1.92	1.87	0.67	2.80
5	0.7	2.70	3.26	2.07	1.70	1.34	4.08
6	1.8	4.24	1.89	1.96	1.73	1.84	1.69
7	0.9	3.17	2.19	1.58	2.59	1.63	1.89
8	1.4	4.57	2.19	2.70	1.23	9.26	7.38
9	1.8	3.23	3.06	3.13	1.78	1.39	1.85
10	1.5	3.72	1.96	3.12	1.68	1.39	3.18
11	1.4	6.30	4.07	7.21	6.33	3.10	3.32

[a] Peak numbers correspond to Fig. 6.
[b] Approximate, based on assumption of no sampling losses.

period of 19 years! Serial dilution of a more concentrated vapour provides a solution to the weighing problem, but the accuracy with which the final concentration is known will be limited by the cumulative inaccuracies of gas flow measurement and regulation at each dilution step[17]. Crisp[18] suggested that diffusion standards be cali-

TABLE IX

COEFFICIENTS OF VARIATION OF PEAK-AREA RATIOS OVER FIVE REPLICATES ON EACH OF THREE CONCENTRATORS FOR DYNAMIC SOLVENT-EFFECT SAMPLING OF A MIXTURE OF AIRBORNE HYDROCARBONS

Peak[a]	*Coefficient of variation (%)*		
	Concentrator Q2	*Concentrator H5*	*Concentrator H7*
1:2	1.26	2.82	0.51
2:3	1.42	1.94	0.70
3:4	0.62	0.66	0.69
4:5	2.13	2.60	1.40
5:6	2.38	2.47	2.67
6:7	1.13	1.40	0.47
7:8	1.66	3.13	6.91
8:9	2.44	2.65	7.91
9:10	1.24	1.14	3.18
10:11	3.23	4.58	6.54

[a] Peak numbers correspond to Fig. 6.

TABLE X

COEFFICIENTS OF VARIATION, POOLED FOR FIVE REPLICATES ON EACH OF THREE CONCENTRATORS, OF PEAK AREAS, PERCENTAGE AREAS AND PEAK-AREA RATIOS FOR DYNAMIC SOLVENT-EFFECT SAMPLING OF AIRBORNE HYDROCARBONS

Peak[a]	*Coefficient of variation (%)*		
	Area	*% Area*	*Ratio*
1	2.85	2.62	
2	2.99	3.65	
3	2.30	2.10	
4	2.25	2.43	
5	3.25	3.74	
6	2.87	1.75	
7	2.77	2.08	
8	6.25	4.49	
9	3.14	2.18	
10	4.80	2.66	
11	7.30	5.48	
1:2			2.24
2:3			2.33
3:4			0.64
4:5			3.02
5:6			3.27
6:7			1.28
7:8			4.29
8:9			4.81
9:10			2.43
10:11			4.89

[a] Peak numbers correspond to Fig. 6.

brated analytically, a procedure which is neatly circular. Lieber and Berk[19] generated ppb "gas-phase" standards by injecting a calculated volume of liquid onto adsorbent traps, but still obtained accuracies of no better than 6% above the calculated levels.

Hence it appears that measurement of the accuracy of dynamic solvent-effect sampling of airborne volatiles will have to await the development of more accurate methods of generating such specimens.

The external standard method of quantitation involves dividing the area of an experimental peak by the area of a peak obtained from an independently estimated amount of the same compound[20]. Therefore, the coefficient of variation of the calculated mass will be an additive combination of that for the peak area (Tables I, III, V, VII, VIII, X, XI and XII) and for the standard amount. Internal standardization involves comparison of the areas of two peaks on the same chromatogram[21], in this instance the coefficients of variation of peak-area ratios (Tables II, III, VI, IX, X and XI) provide a direct measure of the highest available precision. The high precision of the dynamic solvent effect means that, in practice, the precision of both standardizations will probably be limited by errors in the estimates of the standard amounts rather than by variation in sampling.

Dynamic solvent-effect sampling from solvent specimens has been shown to be

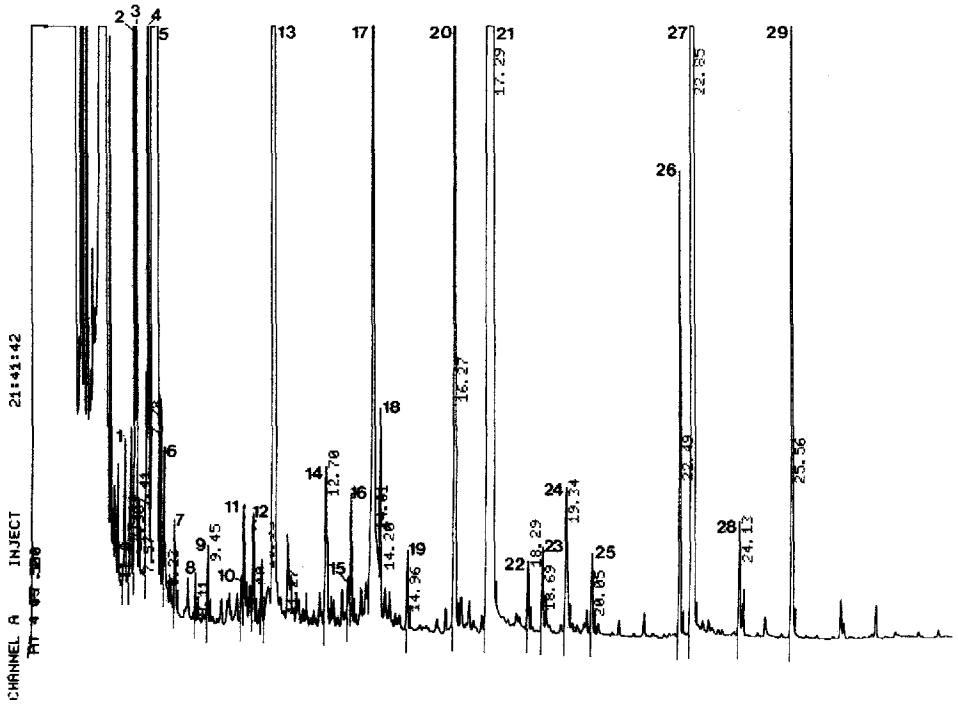

Fig. 7. Chromatogram of volatiles from 10 cm^3 of red wine sampled by the dynamic solvent effect for 10 min with a gas flow-rate of 10 cm^3 min^{-1} using *n*-hexane as solvent at 29.6°C. Peak numbers correspond to Table XI.

extremely precise for sub-nanogram amounts of a wide range of solutes[22]. Transfer of the sample from the concentrator to the column is identical for samples from solvents and from gas-borne specimens. It can be expected, therefore, that the contribution of this step to the quantitative variation will be the same for both types of specimen. From this it follows that differences in precision between the two types of specimen are due to either or both of the entrainment of volatiles from the specimen by the sampling gas and their subsequent focusing on the dynamic solvent film. The focusing mechanism is independent of the source of the volatiles (although the route by which they reach the evaporating edge of the film is different in the two cases)[7,23], so that variations in entrainment are the most likely source of variation in quantitative results.

The entrainment step is probably also why the relative sizes of the peaks in chromatograms from the solutions of carbonyl compounds and phenols (Figs. 4 and 5) do not closely reflect the relative calculated concentrations of the components of each mixture. This is most likely to be due to differences in partition coefficients between water and the hydrogen purge gas, and to differential adsorption on glass surfaces, but small differences in the purities of the standard materials may also have contributed.

The importance of precise temperature control during the sampling of gas-borne volatiles has been stressed by Jennings and Rapp[24] and by Ioffe and Viten-

TABLE XI

COEFFICIENTS OF VARIATION OF PEAK AREAS AND PERCENTAGE AREAS FOR FIVE REPLICATE SAMPLES OF WINE VOLATILES SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Peak[a]	*Approx. mass (ng)*	*Coefficient of variation (%)*		
		Area	*% Area*	*Ratio*
1	1.7	30.8	31.5	8: 9 5.9
2	2.4	21.9	21.5	11:12 4.7
3	22.0	3.0	2.9	13:27 2.3
4	8.4	36.5	37.3	19:22 3.0
5	52.0	2.7	1.0	21:27 1.1
6	1.4	34.4	35.4	23:25 1.8
7	1.4	51.2	51.1	24:28 4.7
8	0.5	5.3	3.8	26:29 9.5
9	1.0	7.9	7.7	
10	0.4	6.4	7.1	
11	1.5	9.8	8.4	
12	1.6	6.4	5.0	
13	228.0	2.7	1.1	
14	3.2	5.4	6.1	
15	0.6	18.8	19.7	
16	2.0	8.1	7.6	
17	24.5	12.9	13.4	
18	3.6	22.8	23.3	
19	1.2	3.4	2.6	
20	14.7	7.4	5.9	
21	630.0	1.4	0.4	
22	1.1	5.8	4.6	
23	1.1	1.6	1.4	
24	2.9	7.1	6.4	
25	2.3	2.2	1.4	
26	7.7	3.0	2.8	
27	112.1	0.6	1.4	
28	1.5	3.6	2.3	
29	11.2	6.8	6.2	

[a] Peak numbers correspond to Fig. 7.

berg[25], who recommend thermostating to ± 0.1°C. Slight variations in temperature may well account for some of the variability reported here. Etievant *et al.*[26] found that temperature fluctuations degraded the precision of sampling from wine.

The role of peak resolution in determining precision[27] is illustrated by the series of samples from wine (Fig. 7, Table XI). The coefficients of variation of peak areas vary from 0.63 to 51.2%. Every peak which was reported by the integrator as baseline resolved on every run had a coefficient of variation of its area of less than 8%. All peaks with coefficients of variation of their areas of more than 12% were reported as incompletely resolved in some runs. In any complex sample there are likely to be some instances of incompletely resolved peaks; if these represent significant components the chromatographic conditions may need to be adjusted to obtain precise quantitation.

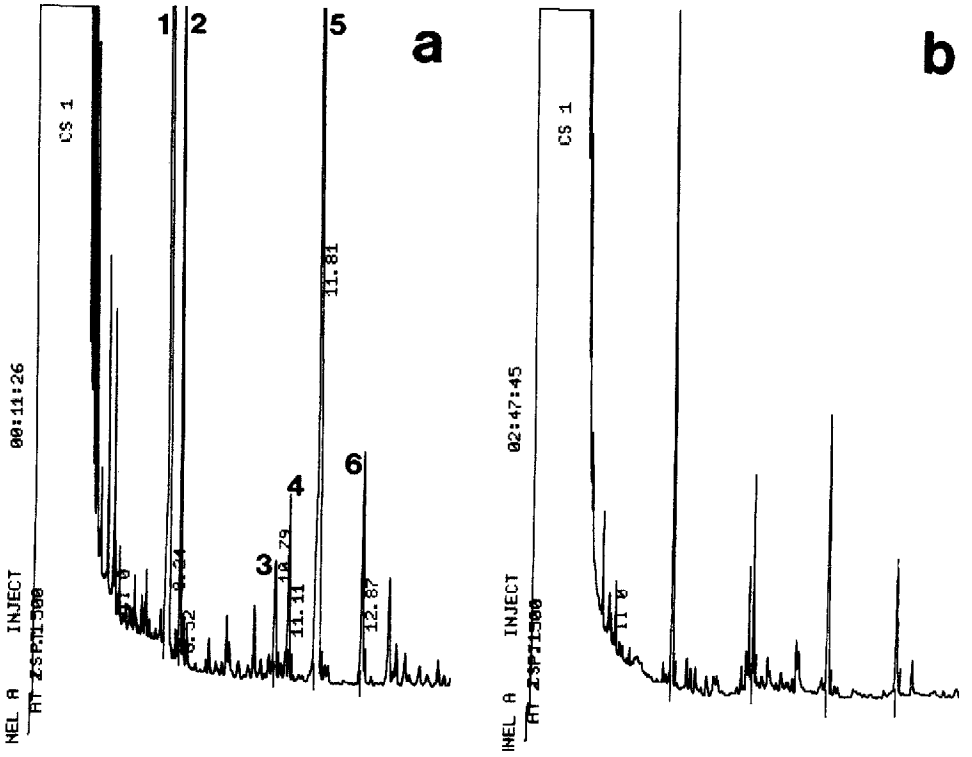

Fig. 8. Chromatograms of volatiles sampled from 10 cm^3 of human urine by the dynamic solvent effect for 10 min with a gas flow-rate of 10 cm^3 min^{-1} using *n*-hexane as solvent at 29.6°C. (a) Previous meal spiced lamb with wine; (b) previous meal sausage, egg and beans. Peak numbers correspond to Table XII.

TABLE XII

COEFFICIENTS OF VARIATION OF PEAK AREAS AND PERCENTAGE AREAS FOR FIVE REPLICATE SAMPLES OF HUMAN URINE VOLATILES SAMPLED BY THE DYNAMIC SOLVENT EFFECT

Peak[a]	*Approx. mass (ng)*	*Coefficient of variation (%)*	
		Area	*% Area*
1	92.0	3.6	1.69
2[b]	5.5	29.6	30.63
3	1.0	17.2	15.71
4	1.2	7.2	6.36
5	11.7	0.6	2.19
6	1.7	6.9	5.46

[a] Peak numbers correspond to Fig. 8.
[b] See Fig. 10.

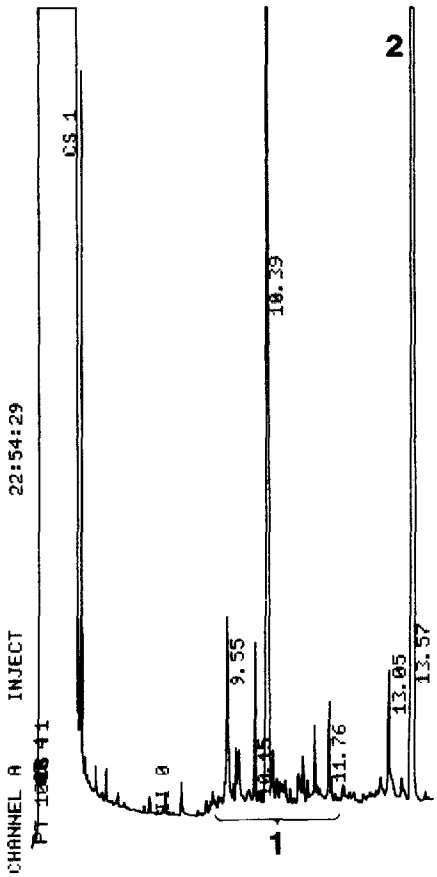

Fig. 9. Chromatogram of volatiles emitted by a Vapona Cupboard Exterminator pesticide strip, sampled by the dynamic solvent effect for 5 min with a sampling flow-rate of 5 cm^3 min^{-1} from a flow of 1 l min^{-1} over the strip. The group of peaks at 1 are hydrocarbons; peak 2 is dichlorvinphos.

The sharp, symmetrical peaks in Figs. 4–9 demonstrate that dynamic solvent-effect concentrators deactivated with silicon and ethene[28] are effectively free of adsorptive activity[22]. This contributes to high-precision analyses by preserving the resolving power of the column (above) and by simplifying the sampling procedure; for example, the test-mixture phenols were eluted as sharp, symmetrical peaks (Fig. 5) without the need for the derivatization that forms part of alternative methods[29, 30].

An additional source of variation may be the foaming which some liquids undergo during gas purging. The urine specimens in particular produced very stable foams. About 40 cm^3 of foam were produced when 100 cm^3 of gas were used for sampling, so almost half the volatiles purged from the liquid remained trapped in bubbles from which they were released only erratically when, and if, the bubbles burst. It should be mentioned that at the sensitivity levels considered here the use of silicone anti-foam agents gives rise to unacceptable levels of contamination. Differen-

ces in bubble size during purging have been identitified as a source of variability in sampling from wine[26].

Highly reproducible results cannot (indeed should not) be obtained if the specimens themselves change from sample to sample. Such variability is difficult to recognize unless its contribution to the overall variation shows some bias away from the random noise to be expected from analytical errors. A consistent trend is one such recognizable bias. Among the cases considered here, trends occurred in the area of peak 2 of the urine samples and in the emission rate of all the volatiles from the Vapona strip (Fig. 10). These trends can be confidently ascribed to changes in the specimens rather than, for example, a progressive conditioning of the apparatus, because they occurred only with these two materials whereas conditioning would have been expected to affect at least some of the others. The trend in the areas of the peaks from the Vapona strip was accompanied by a rise in the temperature of sampling. With the urine specimens the occurrence of the trend inflated the estimate of the coefficient of variation for peak 2.

The role of adsorption onto sampling glassware in degrading quantitative performance is clear from the improvement in precision achieved by simply rinsing the glassware used for the carbonyl standard (Tables I and IV). Adsorption is also indicated by the tendency of the precision to be better for lower molecular weight carbonyl compounds and for compounds present in higher concentrations. The extent to which the variability seen when the glassware had been rinsed was due to residual adsorptive activity is uncertain. When variable adsorption was eliminated, as with the hydrocarbon and phenol standards, the precision was higher, even though phenols are less tractable, and the hydrocarbon standard was more complex, than the carbonyls. The

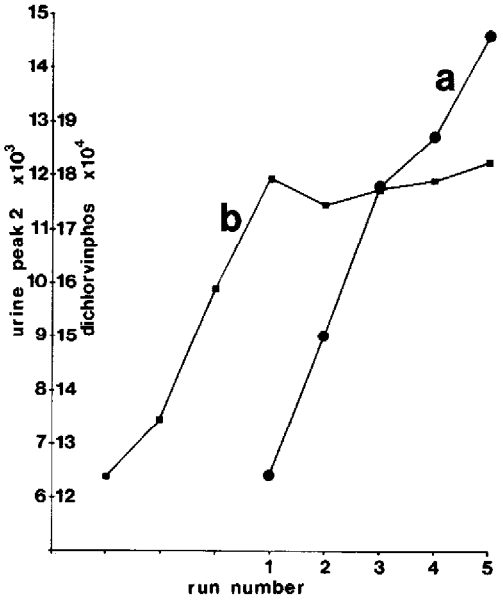

Fig. 10. Trends in peak area with sample number (and time) for (a) peak 2 from the urine sample (Fig. 8), (b) the dichlorvinphos peak in Fig. 9. Precision was calculated from the numbered samples.

TABLE XIII

QUANTITATIVE PERFORMANCE OF A VARIETY OF SAMPLING METHODS FOR GAS CHROMATOGRAPHIC ANALYSIS OF GAS-BORNE VOLATILES

Techniques are abbreviated as follows: ads = adsorption; ct = cold-trapping; d = derivatization; di = distillation; e = extraction; ECD = electron-capture detection; FID = flame ionization detection; hs = headspace; HPLC = high-performance liquid chromatography; MS = mass spectral detection; Nafion = water subtraction by porous polymer; NPD = nitrogen–phosphorus detection; oc = on-column injection; p = purging; ptv = programmed-temperature vaporizer; SIM = specific-ion monitoring; sl = splitless injection; sp = splitting; TEA = thermal energy analyser detection; TIM = total ion monitoring; vi = valve inlet.

No.	*Matrix*	*Solute*	*Technique*	*Concentration*	*Coefficient of variation (%)*	*Ref.*
1	Air	C_{14}–C_{16} acetate	hs ads e sl FID	4.8 ± 3.5 ng/600 cm^3	10–19	34
2	Air	Solvents	vi FID	7–529 ppm, 28 μl	2.2–11.3	35
3	Air	Hydrocarbons	ct FID	32–6680 ppt, 300 cm^3	1.5–8.4	36
4	Air	Pesticides	ads NPD/ECD	0.5 ng/5 l	3.4–12.4	37
		Amphetamine	ads/d SIM	50 ng	13.3	37
5	Air	Halocarbons	ads[a] e sp ECD	0.5–0.05 ng	0.4–3.9	19
6	Aqueous	Wine flavour	p ct sp FID	400 ppm	2–12	26
			p e sp FID	400 ppm	4–14	26
			hs sp FID	400 ppm	1–35	26
7	Water	Hydrocarbons	hs ct sl FID	0.1–15 μg/50 cm^3	8.6–27	38
8	Water	Alcohols/ carbonyls	e ads ct FID	0.13 ng/100 μl	4.5–9.8	39
9	Water	Pollutants	p ads[a] FID	640 ng/40 cm^3	1.4–18	40
10	Water	Aromatics	p Nafion ct FID	27–100 ng/5 cm^3	0.4–2.6	41
			p Nafion ct FID	260–500 ng/5 cm^3	2–2.6	41
11	Water	Chlorophenols	d e sl ECD	1–100 ng/100 cm^3	1–8.9	30
			d e sl ECD	0.1–0.5 ng/100 cm^3	10–20	30
12	Water	Halocarbons	hs sp ECD	0.5–3.0 μg/l	5.5–7.1	42
13	Water	Wide range	ads e FID	100 μg/l	2–22	43
14	Water	Phenols	di/e sp FID	20 ppb	2.4–12	44
15	Water	Pesticides	HPLC vi ECD	0.1 ng/1 cm^3	1.9–32	45
			HPLC vi ECD	2.5 ng/1 cm^3	1.1–48	45
16	Water	Range	p Nafion ct FID	100 ng/8 cm^3	1.3–26	46
17	Water	Non-polar	p ads[a] ct FID	100 ng/l	3–250	15
18	Water	Pyridines	di e SIM	1 μg/500 cm^3	2–3.9	47
19	Water	Halomethanes	p ads[a] FID	100–120 ng/20 cm^3	4–23	48
20	Water	Hydrocarbons	p ads[a] FID	0.15–15 μg/15 cm^3	6.4–14.3	49
21	Urine/ plasma	Drugs	e d spl SIM	2–500 ng/1 cm^3	2–5	50
22	Water	Pollutants	p ads SIM	200 ng/5 cm^3	0.8–31	51
23	Water	Flavour test	p ads[a] ct FID	100 ng	1.7–6.8	31
24	Solid	Aromatics	p ads[a] ct FID	800–935 ng	0.8–2.8	52
25	Sediment	PCBs	e oc/sl ECD	250–375 μg/l	1.8–9	53
26	Meat	Nitrosamines	di/e TEA	5–20 ppb	3.3–25	54

[a] The adsorbent used was Tenax.

adsorptive activity of containers can be neutralized by rinsing until the surface is in equilibrium with the specimen solution but then, during sampling, adsorbed material will bleed back into the specimen when its concentration falls as volatiles are purged from it.

When the coefficients of variation from pooled data and from individual concentrators are compared (Tables I and II *vs.* Table III, Tables V and VI *vs.* Table VII and Tables VIII and IX *vs.* Table X), it can be seen that using a single concentrator for replicate samples provides higher precision than if three are used. If the highest precision is required, the serial use of one concentrator is the method of choice; failing this it may be possible to select matched sets of concentrators.

Sampling was carried out at room temperature (26–27°C) or just above (29.6–30.6°C). This is in contrast to the elevated temperatures in some other sampling techniques. For example, Werkhoff and Bretschneider[31] heated their standard to 80°C, Belkin and Eposito[32] to 70°C and Kolb *et al.*[33] to 150°C. Elevated temperatures distort the volatile profile in ways which may be unacceptable in investigations of semiochemicals or flavours and may hasten the degradation of the specimen.

The literature was surveyed for reports on the precision of various methods of sampling from specimens similar to those investigated here. Table XIII is a compilation of those reports which included sufficient information on the composition, concentration and size of their test specimens for a meaningful comparison with the results for the dynamic solvent effect.

During the compilation of Table XIII, it became apparent that quantitative precision is not an aspect of analytical performance that has received general attention[55]. Indeed, it was the exception, rather than the rule, that data on quantitative performance accompanied, or even followed, descriptions of sampling techniques. For example, the series of papers by Grob and co-workers[56–59] on their closed-loop stripping apparatus contains no figures for precision. Neither Reece and Scott[27] nor Jennings and Rapp[11] provided any figures for the precision of the sampling and separation systems which they discussed. Even where precision was reported, its interpretation was confounded by a lack of information on the composition of the test specimens. The works by Schomburg *et al.*[60], Haynes and Steimie[61], Grob[62] and Yang *et al.*[63] are examples of (otherwise detailed) reports of high-precision results from which the quantitative compositions of the test specimens were omitted, and which, as a consequence, have had to be omitted from the present discussion.

Of the five studies of airborne volatiles in Table XIII, four were as precise as dynamic solvent-effect sampling. Two of these four used specific detectors. Schmidbauer and Oehme[36] (3) cold-trapped light hydrocarbons, to which their method's range of application is limited by the use of a potassium carbonate drying tube which would remove fatty acids and phenols as well as water. Radell and Rea[35] (2) sampled difficult solvent vapours in a process-monitoring application, but their method is restricted to small sample volumes, and therefore high concentrations, by the use of a valve inlet with no focusing step.

The figures obtained here for the precision of the dynamic solvent effect are similar to those from the eighteen investigations of sampling from aqueous specimens in Table XIII. Eleven of these studies (Nos. 6, 7, 9, 10, 12, 16, 17, 19, 20, 22 and 23) can be directly compared with the performance of the dynamic solvent effect in that they employed a purging or headspace step, which would be necessary if the techniques were to be used for quantitative semiochemistry or work on flavours. Two of these eleven (10 and 16) included cold trapping, and a drying step which removed medium-polarity solutes and hydrocarbons above *n*-decane[46]. A further five used only hydrocarbon and halocarbon test compounds, which throw little light on the performance to be expected with less tractable substances.

Etievant *et al.*[26] (6) tested five different sampling techniques, four of which involved gas-phase volatiles, with synthetic wine flavour specimens. Unfortunately, the concentration of their specimens ($400:10^6$) was 2–3 orders of magnitude higher than that of the phenol standard, and four orders higher than that of the aqueous solution of carbonyls used in this study, making meaningful comparisons difficult.

Gas purging and trapping on Tenax were used for sampling an aqueous mixture of pollutants, including some ketones and an alcohol, by Otson and Williams[40] (9). Although their test mixture covered a wide range of volatilities and included some difficult compounds, each component was present in amounts too large to provide a realistically challenging test for a technique as sensitive as capillary gas chromatography.

From 5-cm^3 specimens, Lopez-Avila *et al.*[51] (22) purged and trapped 250 ng of pollutant test compounds, including some ketones. The precision of their method was good, although variable, but its dependence on single-ion mass spectrometric monitoring is likely to restrict its applicability.

Werkhoff and Bretschneider[31] (23) investigated a purge–adsorb–thermally desorb–cold trap system for flavour compounds in water. Their adsorbent was Tenax and cold trapping was at an elevated flow-rate to overcome the incompatability between analytical and desorption flow-rates. The high precisions reported, and the quality of the chromatograms presented, suggest that a similar system might be useful for some types of semiochemical and clinical analysis, provided that the precision could be maintained if the specimen size and solute abundance were to be reduced by two orders of magnitude.

Of the three studies on sampling from solids, only that by Venema[52] (24) involved a non-specific detector, and the amounts and types of solutes do not suggest that the method would be particularly versatile.

Surprisingly few workers have tested their methods with standards that take advantage of the sensitivity of capillary column analyses. For example, Liebich and Al-Babbili[64] employed 150 ng per component in a urine test mixture and Schomburg *et al.*[60] injected micrograms of some of their test compounds. Bertsch *et al.*[29] used 500 ng of pure compounds or 2.8 μg of gasoline per sample. In only eight of the 26 studies in Table XIII (Nos. 1, 3, 4, 5, 8, 11, 15 and 26) were the amounts of solute as small as, or smaller than, those used to test the dynamic solvent effect. In four of these cases (4, 5, 11 and 15) an electron-capture detector, which is both selective and two to three orders of magnitude more sensitive than a flame ionization detector, was used.

The work by Du *et al.*[34] (1) on acetate moth pheromones closely approaches the performance of the dynamic solvent effect in terms of high precision with small amounts of test compounds. If their method of adsorption on glass-wool is adaptable to a wider range of compounds, it will probably prove to be more than adequately precise for most work.

Lee *et al.*[39] (8) are the only group in Table XIII to report coefficients of variation from a sampling system designed, like the dynamic solvent effect, to handle small amounts of biological materials. Their transevaporator operates in two stages. First the lighter volatiles are purged from the specimen and collected on Tenax, then the less volatile and more polar solutes are collected on glass beads by an extraction–readsorption process. It is a pity that precision was not reported for the Tenax mode, as this would be the more useful for work on semiochemicals and flavours. The

adsorption mode yielded excellent results with very small amounts of intractable solutes. Even to elute 0.13 ng of butanol as a recognizable peak requires, apart from anything else, an uncommonly well deactivated column. In view of the use of very active silica adsorbents and a dynamically coated stainless-steel capillary column, this performance must be regarded as extremely good.

No single study in Table XIII covers such a diversity of specimen types as the present one on the dynamic solvent effect. Only adsorption–desorption appears to match the dynamic solvent effect in terms of demonstrated versatility.

The high precision of dynamic solvent-effect sampling has already found application in semiochemical analyses[65–68] and, as an example, changes in human urine volatiles due to a change in diet are readily detectable (Fig. 8).

CONCLUSION

Provided that other potential sources of variation are adequately controlled, dynamic solvent-effect sampling allows low- and sub-nanogram amounts of a wide range of solutes to be determined with coefficients of variation of less than 10%. In terms of precision, the dynamic solvent effect is at least as good as other sampling techniques and in terms of the amounts with which the precision is achieved it is substantially better than most.

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REFERENCES

1 F. Pavelka, *Mikrochim. Acta*, 6 (1964) 1121.
2 K. Grob, *Chromatographia*, 8 (1975) 423.
3 W. G. Jennings, *J. High. Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 221.
4 J. Roerade and S. Blomberg, *Chromatographia*, 17 (1983) 387.
5 V. Pretorius and W. Bertsch, *J. High Resolut. Chromatogr. Commun.*, 6 (1983) 567.
6 V. Pretorius and K. Lawson, *S. Afr. J. Chem.*, 40 (1987) 169.
7 P. J. Apps and V. Pretorius, *J. Chromatogr.*, 471 (1989) 81.
8 P. J. Apps, V. Pretorius, K. H. Lawson, E. R. Rohwer, M. R. Centner, H. W. Viljoen and G. Hulse, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 122.
9 P. J. Apps, *Ph.D. Thesis*, University of Pretoria, Pretoria, 1988, pp. 98–108.
10 P. J. Apps, *Ph.D. Thesis*, University of Pretoria, Pretoria, 1988, p. 18.
11 W. G. Jennings and A. Rapp, *Sample Preparation for Gas Chromatographic Analysis*, Hüthig, Heidelberg, 1983.
12 R. S. Barret, *Analyst (London)*, 106 (1981) 817.
13 B. V. Ioffe and A. G. Vitenberg, *Head Space Analysis and Related Methods in Gas Chromatography*, Wiley, New York, 1983, p. 234.
14 C. K. Huynh and T. Vu Duc, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 198.
15 W. E. Hammers and H. F. P. M. Bosman, *J. Chromatogr.*, 360 (1986) 425.
16 J. Namiesnik, L. Torres, E. Kozlowski and J. Mathieu, *J. Chromatogr.*, 208 (1981) 239.
17 G. Bertoni, F. Bruner, A. Liberti and C. Perrino, *J. Chromatogr.*, 203 (1981) 263.
18 S. Crisp, in E. Reid (Editor), *Trace Organic Sample Handling*, Ellis Horwood, Chichester, 1980, pp. 39–42.

19 M. A. Leiber and H. C. Berk, *Anal. Chem.*, 56 (1984) 2134.
20 M. L. Lee, F. J. Yang and K. D. Bartle, *Open Tubular Column Gas Chromatography: Theory and Practice*, Wiley, New York, 1984, p. 224.
21 M. L. Lee, F. J. Yang and K. D. Bartle, *Open Tubular Column Gas Chromatography: Theory and Practice*, Wiley, New York, 1984, pp. 222–223.
22 P. J. Apps, *J. Chromatogr.*, submitted for publication.
23 P. J. Apps, *Ph.D. Thesis*, University of Pretoria, Pretoria, 1988, p. 147.
24 W. G. Jennings and A. Rapp, *Sample Preparation for Gas Chromatographic Analysis*, Hüthig, Heidelberg, 1983, pp. 19–21.
25 B. V. Ioffe and A. G. Vitenberg, *Head Space Analysis and Related Methods in Gas Chromatography*, Wiley, New York, 1983, p. 15.
26 P. Etievant, H. Maarse and F. Van den Berg, *Chromatographia*, 21 (1986) 379.
27 C. E. Reece and R. P. W. Scott, in E. Katz (Editor), *Quantitative Analysis Using Chromatographic Techniques*, Wiley, Chichester, 1987, pp. 157–191.
28 P. J. Apps, *Ph.D. Thesis*, University of Pretoria, Pretoria, 1988, pp. 75–76.
29 W. Bertsch, E. Anderson and G. Holzer, *J. Chromatogr.*, 112 (1975) 701.
30 K. Abrahamson and T. M. Xie, *J. Chromatogr.*, 279 (1983) 199.
31 P. Werkhoff and W. Bretschneider, *J. Chromatogr.*, 405 (1987) 99.
32 F. Belkin and G. G. Eposito, *J. Chromatogr. Sci.*, 24 (1986) 216.
33 B. Kolb, B. Liebhardt and L. S. Ettre, *Chromatographia*, 21 (1986) 305.
34 J.-W. Du, C. Löfstedt and J. Löfqvist, *J. Chem. Ecol.*, 13 (1983) 1431.
35 E. A. Radell and D. F. Rea, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 189.
36 N. Schmidbauer and M. Oehme, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 502.
37 A. H. Lawrence, *J. Chromatogr.*, 395 (1987) 531.
38 J. Drozd, J. Novak and J. A. Rijks, *J. Chromatogr.*, 158 (1978) 471.
39 K. Y. Lee, D. Nurok and A. Zlatkis, *J. Chromatogr.*, 158 (1978) 377.
40 R. Otson and D. Y. Williams, *Anal. Chem.*, 54 (1982) 942.
41 J. W. Cochran, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 573.
42 B. Kolb, M. Auer and P. Pospisil, *J. Chromatogr.*, 279 (1983) 341.
43 M. Giabbai, L. Roland, M. Ghosal, J. H. Reuter and E. S. K. Chian, *J. Chromatogr.*, 279 (1983) 373.
44 J. Rijks, J. Carvers, T. Noy and C. Cramers, *J. Chromatogr.*, 279 (1983) 395.
45 E. Noroozian, F. A. Maris, M. W. F. Nielen, R. W. Frei, G. J. De Jong and U. A. T. Brinkman, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 17.
46 T. Noij, A. van Es, C. Cramers, J. Rijks and R. Dooper, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 60.
47 T. Tsukioka and T. Murakami, *J. Chromatogr.*, 396 (1987) 319.
48 A. Cailleux, A. Turcant, P. Allain, D. Toussaint, J. Gaste and A. Roux, *J. Chromatogr.*, 391 (1987) 280
49 F. Belkin and G. G. Eposito, *J. Chromatogr. Sci.*, 24 (1986) 216.
50 T. Marunaka, Y. Umeno, Y. Minami, E. Matsushima, M. Maniwa, K. Yoshida and M. Nagamachi, *J. Chromatogr.*, 420 (1987) 43.
51 V. Lopez-Avila, R. Wood, M. Flanagan and R. Scott, *J. Chromatogr. Sci.*, 25 (1987) 286.
52 A. Venema, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 637.
53 F. I. Onuska, K. J. Kominar and K. A. Terry, *J. Chromatogr.*, 279 (1983) 111.
54 M. Gavinelli, L. Airoldi and R. Fanelli, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 257.
55 P. Werkhoff and W. Bretschneider, *J. Chromatogr.*, 405 (1987) 87.
56 K. Grob, *J. Chromatogr.*, 84 (1973) 255.
57 K. Grob and G. Grob, *J. Chromatogr.*, 90 (1974) 303.
58 K. Grob, K. Grob and G. Grob, *J. Chromatogr.*, 106 (1975) 299.
59 K. Grob and F. Zürcher, *J. Chromatogr.*, 117 (1976) 285.
60 G. Schomburg, H. Husmann and R. Rittmann, *J. Chromatogr.*, 204 (1981) 85.
61 L. V. Haynes and A. R. Steimie, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 441.
62 K. Grob, *Classical Split and Splitless Injection in Capillary Gas Chromatography*, Hüthig, Heidelberg, 1985, pp. 206 and 249.
63 F. J. Yang, A. C. Brown and S. P. Cram, *J. Chromatogr.*, 158 (1978) 91.
64 H. M. Liebich and O. Al-Babbili, *J. Chromatogr.*, 112 (1975) 539.
65 P. J. Apps, H. W. Viljoen and V. Pretorius, *Onderstepoort J. Vet. Res.*, 55 (1988) 135.
66 P. J. Apps, A. Rasa and H. W. Viljoen, *Aggress. Behav.*, 14 (1988) 451.
67 D. Jacobs, P. J. Apps and H. W. Viljoen, *Comp. Biochem. Physiol. B*, 93 (1989) 459.
68 P. J. Apps, *Ph.D. Thesis*, University of Pretoria, Pretoria, 1988, pp. 186–297.