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Note

Recoveries from an aqueous model system using a semi-micro steam distillation–solvent extraction procedure

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The dynamic composition (qualitative and quantitative) in the trace range makes it necessary to improve continuously the sample handling, isolation and gas chromatographic (GC) procedures for organic trace analysis. Moreover, once the trace substances have been extracted, isolated and analysed in an efficient and defined way, a more complex aspect of the problem must still be resolved: the relationship between the concentration of the compounds detected by the GC system and the concentration in the original matrix. Difficulties related to matrix effects, recovery efficiencies, linearity of the detector response in the trace range, etc., arise in this step and unfortunately it is not always possible to devise an adequate quantitation procedure. The complexity of organic trace analysis has been studied by many workers[1–3], but it is still necessary to improve the procedures for quantitative GC analysis of organic traces.

Simultaneous steam distillation–solvent extraction (SDE) procedures have been developed since the introduction by Likens and Nickerson[4] of a method for the isolation of volatile constituents in hop oil. In the recent procedures the original apparatus has been modified by introducing a vacuum jacket in the arm which conducts the solvent vapour to the extractor body[5] and/or more efficient cooling devices[6–8].

Recently, Godefroot *et al.*[9] reported a new design for a SDE apparatus where the amounts of both sample and solvent were reduced compared with the traditional procedure. The extracts (2 ml) were directly analysed by GC without any solvent evaporation step and almost all the components were detected. Although this apparatus is already commercially available (Alltech, Arlington Heights, IL, U.S.A.; Chrompack, The Netherlands) neither practical applications nor recovery studies in the low-ppm or sub-ppm range have been reported. Therefore, we have studied the minimum concentrations that can be efficiently extracted from the original sample and analysed by GC. This report presents recovery data obtained from an aqueous model system in the trace range (0.01–10 ppm) using the semi-micro procedure of Godefroot *et al.*

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MATERIALS AND METHODS

Eight compounds reported as fruit aroma components[10] were selected for the preparation of the model system by considering differences in polarity, volatility and water solubility (see Table I). The purity of each compound was checked chromatographically. Standard aqueous solutions of 0.01, 0.1, 1 and 10 ppm (μl/l) were prepared using deionized water. The solutions were stored at 4°C in the dark before use.

The SDE apparatus (Alltech) and the experimental procedure were as described[9], but with tap-water (15°C) as coolant. Pentane and diethyl ether (Merck, F.R.G.) were fractionally distilled and used as a 2:1 mixture. The same batch of solvent mixture was used for all the experiments. The solvent concentrates were placed in PTFE-stoppered vials and stored in the dark at −20°C before GC analysis.

An Intersmat IGC-16 gas chromatograph (France) with a splitless injection system and a flame ionization detector was used for analysis of the solvent concentrates. The injections were performed using the hot-needle technique described by Grob and Neukom[11] but with 15 sec for needle heating in the injection port. A soft-glass WCOT column coated with SE-30, 25 m × 0.25 mm I.D. (Chrompack, The Netherlands), was used. The gas flow-rates were: carrier gas (helium), 1 ml/min; make-up, 30 ml/min; hydrogen, 80 ml/min and air, 210 ml/min. The injector and detector temperatures were 200 and 250°C, respectively. The temperature program was: oven(initial), 40°C, 5 min; oven(final), 180°C, 5 min; rate of temperature rise, 6°C/min.

Reference solutions in pentane–diethyl ether (2:1) containing 0.01, 0.1, 1 or 10 ppm of the various compounds were prepared for recovery calculations. The recoveries were calculated by comparing the peak heights of the concentrate chromatograms with the peak heights of the reference solution chromatograms. The amounts of concentrate and reference solution injected into the GC system were adjusted for each concentration level so that the mass transfer from the aqueous solution to the solvent was 100%. Three injections were performed for each reference solution and concentrate and the average recoveries and standard deviations calculated.

TABLE I

SOME PHYSICAL PROPERTIES OF THE COMPOUNDS SELECTED FOR QUANTITATIVE STUDIES (IN THE 0.01–10 ppm RANGE)

Compound	*Molecular weight*	*Boiling point (°C)*	*Vapour pressure at 100°C (mmHg)**	*Water solubility*
3-Pentanone	86.1	102	356.2	Very soluble
Methyl butanoate	102.1	101	346.4	Slightly soluble
α-Pinene	136.2	156	264.1	Insoluble
D-Limonene	136.2	178	219.1	Insoluble
n-Decanal	156.3	208	76.2	Insoluble
Methyl N-methylanthranilate	151.2	256	69.3	Slightly soluble
β-Caryophyllene	204.4	$122^{13.5\ mm}$	No data	Insoluble
Geranyl butanoate**	224.3	–	10.1	Insoluble

* Calculated according to $\log p = (-0.2185\ A/K) + B$, where p = vapour pressure of the pure compound in atm, K = temperature in °K and A and B are constants. Data from ref. 15.

** Geranyl butanoate is actually a mixture of two *cis–trans* isomers: geranyl and neryl butanoates.

TABLE II

RECOVERIES OBTAINED IN THE ppm- AND SUB-ppm-RANGE (0.01–10 ppm) FROM AN AQUEOUS MODEL SYSTEM USING AN ATMOSPHERIC SDE METHOD REPORTED BY GODEFROOT *et al.*[9]

Component	*Recovery ± S.E. (%)*			
	10 ppm	*1 ppm*	*0.1 ppm*	*0.01 ppm*
3-Pentanone	90.0 ± 5.7	110.0 ± 6.1	–	–
Methyl butanoate	90.8 ± 4.8	113.0 ± 5.0	–	–
α-Pinene	64.3 ± 6.6	34.8 ± 9.7	43.8 ± 6.5	25.0 ± 4.7
D-Limonene	74.6 ± 8.2	54.6 ± 8.9	45.9 ± 7.2	29.0 ± 5.1
n-Decanal	92.8 ± 2.3	95.6 ± 1.6	75.0 ± 1.1	60.7 ± 0.8
Methyl N-methylanthranilate	93.5 ± 1.8	113.1 ± 2.3	92.2 ± 0.8	59.4 ± 0.5
β-Caryophyllene	97.3 ± 3.6	95.2 ± 4.1	82.5 ± 1.6	79.0 ± 1.1
Geranyl butanoate	125.9 ± 2.1	102.7 ± 2.8	84.1 ± 1.1	52.4 ± 0.8

RESULTS AND DISCUSSION

One of the advantages of the SDE apparatus used in this study is that the solvent extract can be analysed directly by GC without any prior solvent evaporation. However, the results reported in the original work were obtained for a synthetic mixture containing solutes at 50 ppm (μg/ml), which is a rather high level; no results were reported for lower concentration levels. In many cases, as in food aroma research, the analytes are present at the low- or sub-ppm range, thus evaporation of the solvent is imperative for this procedure, but not so critical as in the conventional methods. We have found that a four-fold reduction of the solvent extract (from 2 ml to 500 μl) is sufficient for detecting minor amounts of aroma components present at > 1 ppb in grapefruit juice[12].

The recoveries for each model compound are shown in Table II. Except for α-pinene and D-limonene, the recoveries are over 90% for the 10-ppm and 1-ppm samples, with relatively low standard errors. The recoveries of the most volatile components (3-pentanone and methyl butanoate) could not be measured at 0.1 and 0.01 ppm owing to interference from the solvent peak. The chromatograms of the concentrates are shown in Fig. 1. No large impurity peaks were detected in the blank run even at the maximum sensitivity.

The efficiency of this method for the sub-ppm levels is rather low. Such low recoveries have also been obtained from aqueous model systems at 50 ppb, but with a modified Likens–Nickerson apparatus[13]. In work with actual systems (grapefruit juice)[12] we could improve the efficiency by 5% using methanol (1°C) as coolant, but this is still not sufficient and more improvements are needed.

Recent work of Demole *et al.*[14] demonstrates that it is necessary to use larger volumes of sample for the analysis of trace components at the sub-ppm or lower concentration levels. In this way, a sulphur compound at the sub-ppb level was identified for the first time in grapefruit juice by extracting 100 l of juice in a reduced-pressure SDE apparatus.

The semi-micro procedure seems to be able to extract components above 1

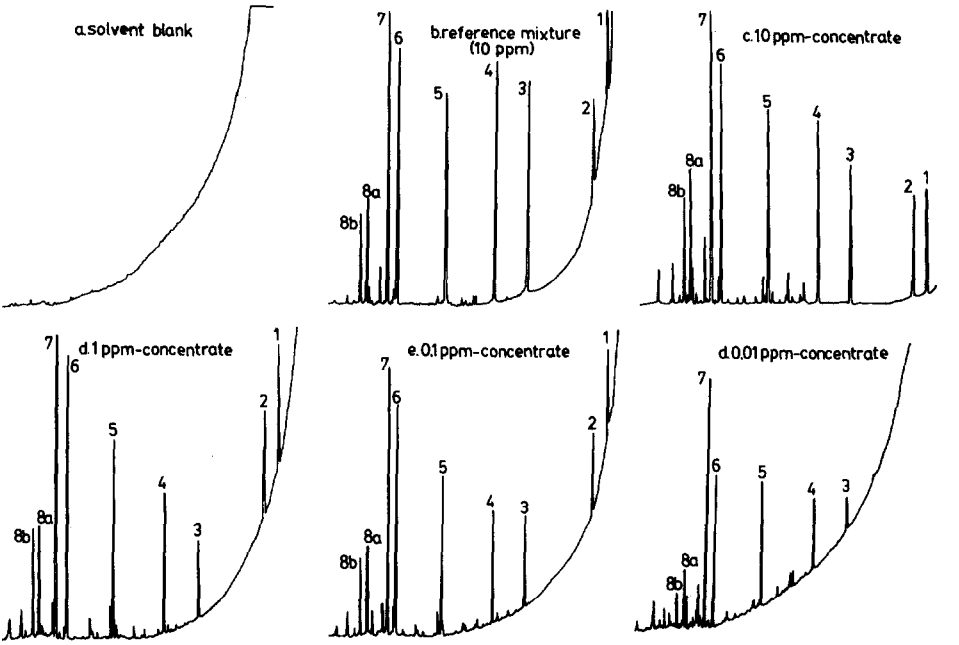

Fig. 1. Chromatograms of the solvent blank (a), 10-ppm reference solution (b), 10-ppm concentrate (c), 1-ppm concentrate (d), 0.1-ppm concentrate (e) and 0.01-ppm concentrate (f). Peaks: 1 = 3-pentanone; 2 = methyl butanoate; 3 = α-pinene; 4 = D-limonene; 5 = *n*-decanal; 6 = methyl N-methylanthranilate; 7 = β-caryophyllene; 8a = geranyl butanoate; 8b = neryl butanoate. See text for chromatographic conditions.

ppm, but larger amounts of sample or other improvements are still necessary when components in the sub-ppm range are to be analysed.

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