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Simultaneous distillation–extraction under static vacuum: isolation of volatile compounds at room temperature

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ABSTRACT

The simultaneous distillation–extraction (SDE) system developed by Likens and Nickerson (ASBC Proceedings, 1964, p. 5) and modified by Godefroot, Sandra and Verzele [*J. Chromatogr.*, 203 (1981) 325] is one of the most popular methods currently used to isolate volatile components from a matrix prior to gas chromatographic analysis. Since it leads to a thermal generation of artefacts, the device has been improved in the present work to allow isolation between 20 and 40°C under vacuum. The system was closed and made airtight by means of a valve under static vacuum to avoid volatile losses. These conditions require the use of solvents with a boiling point close to that of water and the temperature must be electronically regulated. The absence of artefact generation was tested with linalyl acetate, honey and a Maillard model reaction. The recovery yields of classical SDE and SDE under vacuum were found to be similar.

INTRODUCTION

The analyses of flavours and fragrances require the isolation of the volatile fraction from the matrix prior to gas chromatography (GC): direct extraction with a solvent (soxhlet, liquid–liquid extraction, supercritical fluid extraction) co-solubilizes non-volatile components which contaminate the injectors and limit the possibility of concentration.

Direct vacuum distillation followed by solvent extraction and concentration is tedious because of the high volume to be handled and the different steps which are time-consuming and affect the yields.

Since Likens and Nickerson [1] published the first paper concerning simultaneous distillation–extraction (SDE), this method has become very popular in all flavour and fragrance analytical laboratories for the isolation of volatile components from a matrix. The improvement by Godefroot, Sandra and Verzele [2] allowed quantitative determination following extraction for 1 h using a microapparatus without any prior concentration before gas chromatographic analysis. Starting from a fat-containing matrix in which volatiles exhibit a high affinity, Au-Yeung and MacLeod [3] confirmed these high recovery yields (80%).

SDE has also been found to be applicable to the isolation of nitrosamines [4] and pesticides [5] from non-volatile matrices.

However, SDE suffers from a major disadvantage: because of the temperature of the water boiler (105°C), numerous artefacts are generated. The use of antioxidants and an oxygen-free atmosphere decreases oxidative degradations [6] since it is well known that steam distillation produces a lot of thermal reactions [7]. Hence the composition of SDE extracts must be compared with that of essential oils since they both involve steam distillation. On the other hand, SDE is not applicable to heat sensitive products such as food media or flower scents which undergo Maillard reactions, hydrolyses, rearrangements, etc.

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To lower the temperature of the boilers, several authors have proposed working at reduced pressure. Picardi and Issenberg [8] and Seifert and King [9] managed to maintain a sample temperature of between 45 and 50°C. However, no yields were given and volatile components were probably further lost during the continuous vacuum pumping.

Surprisingly, Schultz *et al.* [10] claimed quantitative recoveries under similar conditions (100 Torr), but 52°C in the vapour phase suggests a higher temperature in the boiler, leading to possible thermal reactions. Furthermore, our own experiences showed that significant losses of solvent would be expected when using hexane under continuous pumping.

To minimize these losses Charpentier *et al.* [11] used a three-stage condenser under 200 Torr allowing a sample temperature of 67°C but requiring the control of five different temperatures.

Therefore the aim of this work was to develop an easy-to-use device to isolate volatile components without losses, at room temperature, in a reasonable time, on a microscale and with a high concentration factor.

EXPERIMENTAL

Gas chromatography

For all chromatographic analyses, a Hewlett-Packard 5890 gas chromatograph equipped with either a flame ionization detector or a mass spectrometer was used. Two columns were used, a polar column and a non-polar column. The polar column, 60 m × 0.25 mm I.D., 0.25 μm film thickness, was a fused-silica column coated with cross-linked polyethylene glycol (Supelcowax TM10, Supelco) the non-polar column, 50 m × 0.2 mm I.D., 0.5 μm thickness, was a fused-silica column coated with cross-linked methylsilicone (Pona1, Hewlett-Packard).

The oven temperature was held at 20°C for 0.5 min, ballistically increased to 60°C, and programmed at 4°C/min, up to 250°C for the non-polar phase and 220°C for the polar phase. The programme was then held isothermally until the end of the run.

The injector temperature was 250°C, and 1 μl was injected in splitless mode. Detector temperatures were 275°C for the flame ionization detector and 220°C for the mass spectrometer. Linear indices were calculated from the injection of *n*-alkanes (C_5–C_{28}) [12] and compared with the indices reported in the literature or with those of our own libraries determined from authentic samples.

Mass spectrometry

Electron ionization mass spectrometry was performed on a Hewlett-Packard 5995 instrument with the capillary column directly connected into the ionization source operating at 70 eV ionization energy. The mass spectra of the compounds detected were compared with those in our own libraries.

SDE at atmospheric pressure

The Godefroot–Sandra–Verzele device was used. Typical experiments were run for 1 h with 3 ml of dichloromethane (purity 99.7%) as extracting solvent and with 200 ml of sample solution. The temperature of the sample boiler and of the cooler was held at 105°C and −5°C, respectively.

For quantitation, 3 mg of 1-undecanol was added as internal standard to the solvent flask just before the SDE run. The sample flask initially contained 3 mg of each component listed in Table III.

SDE under vacuum

The Godefroot–Sandra–Verzele device was modified as shown in Fig. 1. The two flasks were connected to the extractor with spherical joint (Rotulex) and Viton toric seals. Before heating, the system was pumped with a water pump and closed under vacuum (extended-tip PTFE valve, Kontes, Switzerland) for the duration of the experiment. The cooler temperature was −5°C.

For a typical run, 200 ml of an aqueous solution or a slurry were heated in the sample flask at 37 ± 0.5°C. The solvent flask (3 ml of isooctane; purity 99.5%) was held at 20 ± 0.5°C. Both were vigorously magnetically stirred. Quantitations were performed as described for the atmospheric experiments.

Linalyl acetate extraction

A 2-mg aliquot of linalyl acetate (Fluka, Switzerland) in 200 ml of water was SDE-extracted for 1 h at atmospheric pressure and under vacuum. The organic extracts were injected into the gas chromatograph without prior concentration.

TABLE I

MAIN VOLATILES PRODUCED WITH SDE AT 105°C STARTING FROM GLUCOSE AND LEUCINE

Peak	Compound	Experimental indices	
		Polar[a]	Non-polar[b]
1	Butan-2,3-dione	968	558
2	Pentan-2,3-dione	1054	681
3	2-Methyltetrahydrofuran-3-one	1268	774
4	Hydroxypropanone	1310	625
5	4-Hydroxy-4-methyl-pentan-2-one	1366	812
6	2-Butoxyethanol	1399	887
7	Furfural	1471	804
8	2-Hydroxymethylfuran	1655	823
9	2-Acetylpyrrole	1983	1030

[a] Indices on Supelcowax TM10 (programmed temperature).
[b] Indices on Ponal (programmed temperature).

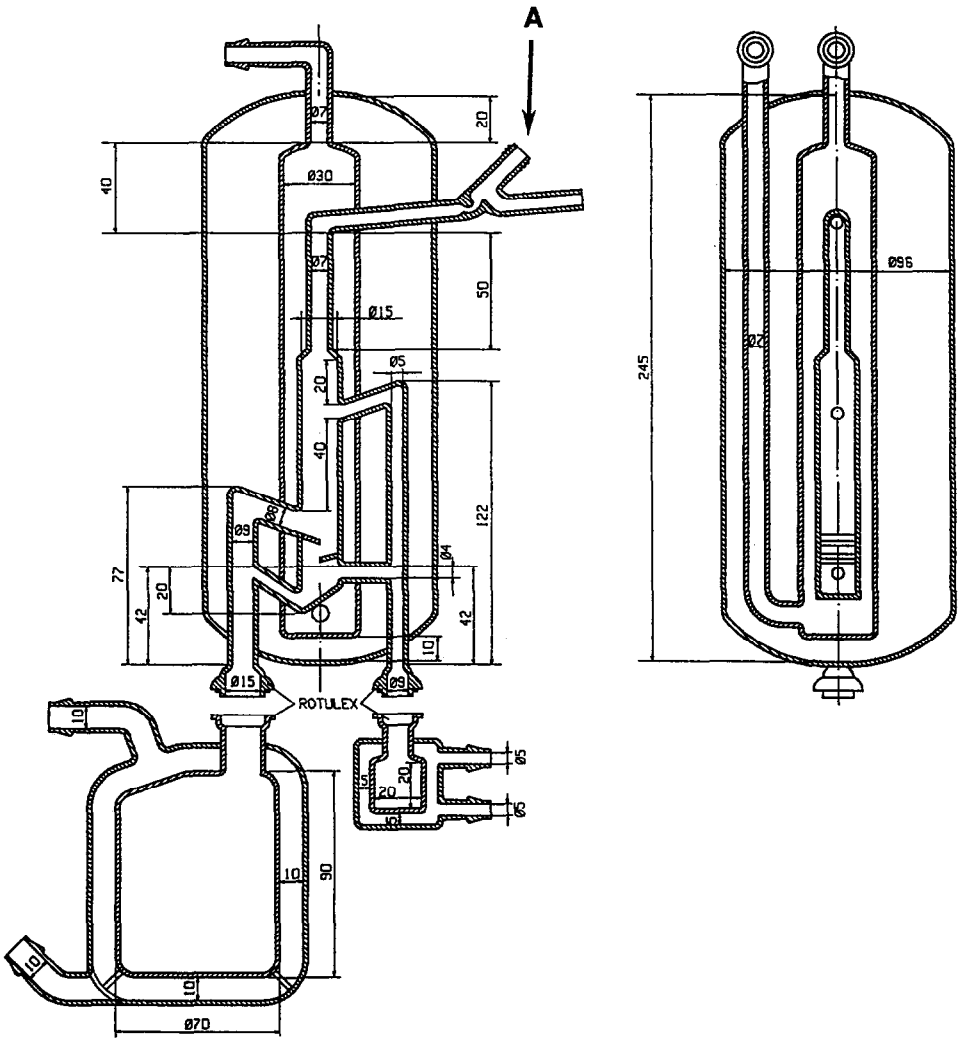

Fig. 1. Modified, static-vacuum, simultaneous steam distillation–extraction apparatus. A = PTFE valve; ∅ = diameter; all sizes in mm.

Honey extraction

An 80-g aliquot of honey diluted to 250 ml with distilled water was SDE-extracted either with dichloromethane for 2 h at atmospheric pressure, or with isooctane for 2 h under vacuum. Organic extracts were analysed by GC after a 100-fold concentration under a gentle stream of nitrogen at room temperature.

Maillard reaction

A 1-g aliquot of L-leucine (Merck, Germany) and 1 g of D-glucose (Fluka, Switzerland) diluted in 200 ml of water were extracted for 6 h for vacuum and atmospheric experiments. Organic extracts were concentrated 100-fold at room temperature under a gentle stream of nitrogen prior to GC–mass spectrometric analysis.

Quantitative assay

The sample flask contained 200 ml of water and 3 mg of each component listed in Table III. 1-Undecanol (3 mg) was used as internal standard in the solvent flask and the extraction was run as described above for vacuum and atmospheric experiments. SDE runs were performed triplicate, and each organic extract was analysed by GC in duplicate without prior concentration, using an autosampler.

RESULTS AND DISCUSSION

Vacuum conditions

In order to avoid losses of volatiles mentioned in the Introduction as well as sophisticated cooler systems, the extractor was equipped with a PTFE valve to maintain a static vacuum during the run. A blank experiment indicated that the Viton rings of the flasks allowed a stable pressure for more than 6 h without giving rise to artefacts.

Device modifications

Compared with the Godefroot–Sandra–Verzele device the main modifications were as follows:

(1) Enlargement of the steam arm diameter from 4 to 8 mm to increase the distillation rate of water. This enables extractions of up to 200 ml of sample and only 1–3 ml of solvent.

(2) An external double-jacket cooler which gives a higher condensation area than the cold finger of the original apparatus and avoids vaporization of the liquids in the phase separator.

(3) A third jacket to insulate the distillation arms and to avoid condensation of the atmospheric moisture on the cold parts while the strength of the glassware was enhanced.

(4) Double-jacketed flasks and electronic control of the thermostatic fluid.

(5) Rotulex joints and a PTFE valve allowing maintenance of a stable static vacuum.

The modified device is commercially available from Trabold (Bern, Switzerland).

Solvent choice

Low-boiling point solvents (*e.g.* dichloromethane, pentane and diethyl ether) are commonly used for atmospheric SDE because they possess good extractive properties, they elute rapidly in GC and they are easily evaporated to concentrate the extract. Their condensation under vacuum requires a very low cooler temperature (about −40°C) with subsequent ice formation in the system. A compromise was found by choosing solvents with boiling points close to that of water and with low retention indices.

Table II summarizes the parameters obtained with *n*-hexane, *n*-heptane, 2,2,4-trimethylpentane, *n*-octane, toluene and 3-pentanone.

The high value of the retention indices on the polar column of toluene and 2-pentanone (1050 and 920, respectively) could mask the early-eluting peaks of the extract during GC. However, their polarity and/or their polarizability [14] offered better extractive properties than the non-polar alkanes. Since the indices on the apolar column are low, they were a viable compromise between the SDE and GC requirements.

The use of *n*-octane (and other higher-boiling-point solvents) provides for a reduction of the sample temperature to 19°C. On the other hand, the solvent flask must be held at 26°C, which decreases the suitability of this solvent unless a lower pressure can be used.

In spite of their higher boiling points than hexane or dichloromethane, the solvents used for vacuum SDE were easily removed at room temperature under a gentle stream of pure nitrogen within less than 30 min. Alkanes were more suitable for sensory evaluation of the extract because of a much weaker

TABLE II

PARAMETERS OF SEVERAL SOLVENTS FOR VACUUM SDE

Solvent	B.p. (γ)	Retention Index		Vacuum (mbar)	Sample temperature	Solvent temperature	Solvent odour
		Polar[a]	Non-polar[b]				
Iso-octane	99	698	691	49	37	20	Weak
Heptane	99	700	700	35	30	17	Weak
2-Pentanone	102	920	677	31	25	17	Unpleasant
Toluene	111	1050	744	37	32	26	Unpleasant
Hexane	69	600	600	115	50	22	Weak
Octane	125	800	800	20	19	26	Weak

[a] Indices on Supelcowax TM10 (programmed temperature).
[b] Indices on Pona1 (programmed temperature).

odour than toluene and 2-pentanone. They quickly evaporated when the extract was sniffed on paper strips.

Artefact decrease

Linalyl acetate is known to be hydrolysed during the steam distillation of various plants [7]. A comparative experiment was run (Fig. 2): almost half of the ester was transformed under the atmospheric SDE conditions, whereas it was not modified under vacuum.

Maillard reactions. Since food media contain amino acids and reducing sugars which are subject to Maillard reactions, it is compulsory to reduce this reaction if one wants to identify the components which are really responsible for the authentic taste. For this purpose, a model Maillard mixture of leucine and glucose was used [13].

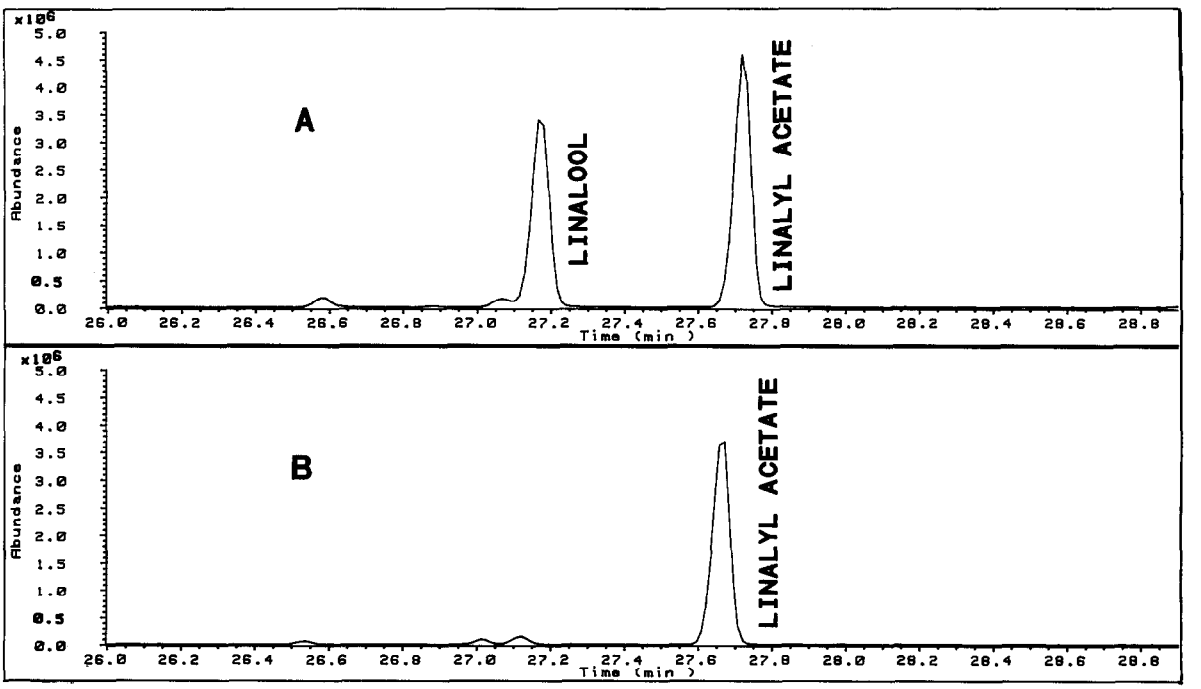

Fig. 2. (A) Distillation–extraction of linalyl acetate at 105°C. (B) Distillation–extraction of linalyl acetate at 37°C. Polar column.

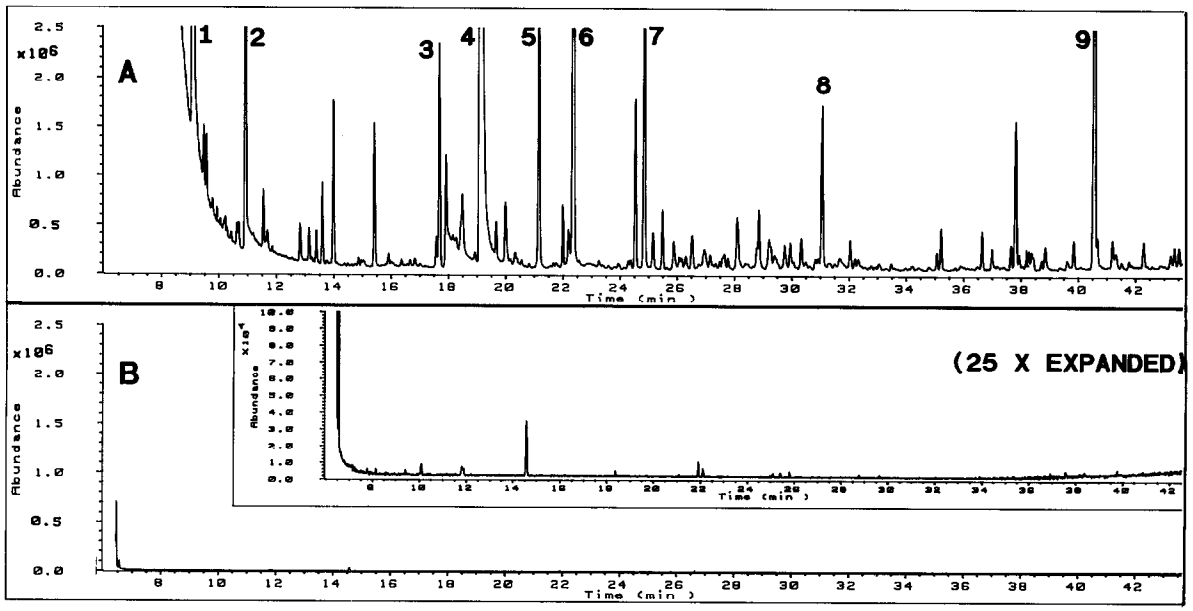

Fig. 3. (A) Distillation–extraction of glucose and leucine at 105°C. (B) Distillation–extraction of glucose and leucine at 37°C. Polar column. For peak identification see Table I.

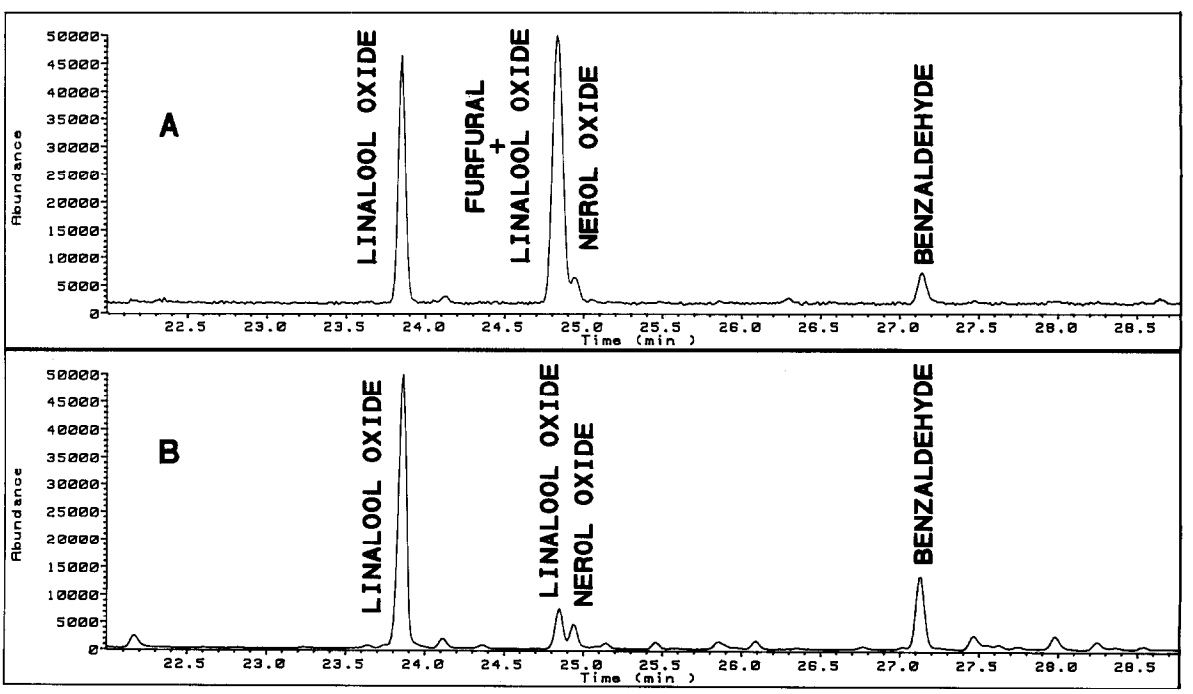

Fig. 4. (A) Distillation–extraction of honey at 105°C. (B) Distillation–extraction of honey at 37°C. Polar column.

TABLE III

RECOVERY YIELDS FOR DIFFERENT COMPOUNDS

Compound	Atmospheric SDE		Vacuum SDE	
	Recovery (%)	Confidence[a]	Recovery (%)	Confidence[a]
Ethyl butyrate	118	13	92	13
Limonene	109	2	103	2
2-Hexenal (*E*)	91	3	76	4
Pyrazine	55	2	44	2
Anethol (*E*)	102	2	92	2
Dodecanol	97	2	92	2

[a] 95% confidence range.

Fig. 3 indicates that the reaction did not occur in our modified system whereas the classical conditions generated numerous reaction products (Table I). Furthermore, the browning reaction observed during the atmospheric extraction was suppressed under vacuum.

Honey extraction. Atmospheric SDE produces a cooked honey flavour, similar to the taste of honey candies. The main components formed during the extraction were observed to be furfural and 5-methylfurfural.

The vacuum experiment gave a furfural-free extract (Fig. 4) with a fresh honey note. The corresponding chromatogram exhibits a small peak of linalool oxide, which was completely hidden by the furfural in the atmospheric experiments. No colour change was noticed under vacuum, unlike with classical SDE.

Quantitative trial

The modified system was tested for its quantitative recovery using six pure synthetic compounds commonly found as flavourings and representing different functional groups. The results summarized in Table III indicate that similar recoveries can be expected when quantitative yields are obtained at atmospheric pressure, whereas they are slightly lower for the less SDE-extractable components. The recovery should be improved by increasing the run time since the sample is not subjected to thermal degradations, unlike atmospheric experiments.

CONCLUSIONS

Classical SDE offers a good selectivity based on the volatility of the molecules responsible for the odour and flavour, however, its applicability is limited because of the formation of thermal artefacts.

On the other hand, dynamic headspace is rather dedicated to the very volatile fraction and gives high concentration factors without allowing easy quantitative measurements.

Thus vacuum SDE would appear to be a valuable method which avoids thermal degradations as well as heavy-component extraction, while it remains quantitative. However, very volatile molecules are masked by the solvent.

Hence vacuum SDE and dynamic headspace appear to be complementary methods for sample preparation in gas chromatography.

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