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DETERMINATION OF SENSORIAL ACTIVE TRACE COMPOUNDS BY MULTI-DIMENSIONAL GAS CHROMATOGRAPHY COMBINED WITH DIFFERENT ENRICHMENT TECHNIQUES

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SUMMARY

Gas chromatographic equipment and appropriate procedures are described for the identification and determination of sensorial active compounds in complex mixtures. Different sampling systems (liquid injection, thermal desorption, dynamic headspace) were integrated in a single double-oven gas chromatograph, additionally allowing multi-dimensional separation, detection by simultaneous sniffing–mass spectrometric monitoring or micro-preparative enrichment of capillary effluents. The modular construction easily adapts to the diverse requirements of sample analysis without great modifications. The versatility and flexibility of such a combination are illustrated with different applications.

INTRODUCTION

The identification of trace components in complex mixtures is a fundamental problem in chromatography. In the field of aroma and pheromone research, for example, samples are frequently characterized only because of the presence of minor components with physiological activity[1]. As these substances are often masked by co-eluting major components, one-dimensional high-resolution gas chromatographic (GC) analysis is insufficient to resolve them. Therefore, when dealing with trace components in complex mixtures, the use of multi-dimensional techniques (*e.g.*, abbreviated to GC[2]) is necessary to increase the information derived from a chromatographic analysis. The capability to produce on-line in a single run two independent sets of retention data is a second major benefit[2].

In combination with mass spectrometry (MS), an efficient system for structure elucidation is obtained. Simultaneous "sniffing" of the column effluent with the sensitive human nose is a powerful extension for the localization of sensorial active trace compounds[3,4]. Although MS provides, in addition to molecular weight and chemical composition data, structural information which is sometimes sufficient for the characterization of a substance, additional spectroscopic data (IR, NMR, UV) are required for chemical structure assignment, especially when dealing with unknown substances. Therefore, the isolation and enrichment of individual components at trace levels is

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also necessary (micro-scale preparative GC)[5]. A modular-type construction that can easily be adapted for different instrumentation (single column, GC^2 with packed–capillary or capillary–capillary columns) is preferable[6]. Depending on the analytical problem to be solved and the amounts of substance available, facilities for both thermal desorption and solvent elution analysis of enriched column effluent are desirable.

Finally, the kind of sample material for analysis remains to be considered. The interesting and significant components occur in different homogeneous or heterogeneous gas, solid or liquid matrices, from which they have to be isolated, as most matrices are not compatible with GC. Therefore, different sampling techniques have been developed. Examples of the types of sample introduction include split–splitless and on-column liquid injection, equilibrium headspace, dynamic headspace and direct thermal desorption analysis. Such sampling procedures, combined with multidimensional GC, micro-scale preparative enrichment and "sniffing"–MS monitoring, substantially improve the identification and determination of sensorial active trace components in complex mixtures. Examples of applications to demonstrate the potential of such combinations are described in this paper.

SYSTEM DESCRIPTION AND OPERATION

A simplified schematic diagram of the combinations available with a single double-oven gas chromatograph is shown in Fig. 1. Solvent extracts can be introduced by liquid injection (split–splitless, on-column) with a programmable temperature vaporizer (PTV). Volatiles from solid or liquid samples are enriched by means of gas extraction on a cold-trap and thermally desorbed into the chromatographic column (dynamic headspace). For samples obtained by the concentration of large volumes of gases or the enrichment of selected cuts of column effluents on adsorbing traps, the introduction of the sample is achieved with a modified PTV (for focussing purposes) and a device for thermal desorption.

The separation stage consists of two columns of different polarities, coupled directly with a Live-T switching device or a total transfer system (cold trapping of a

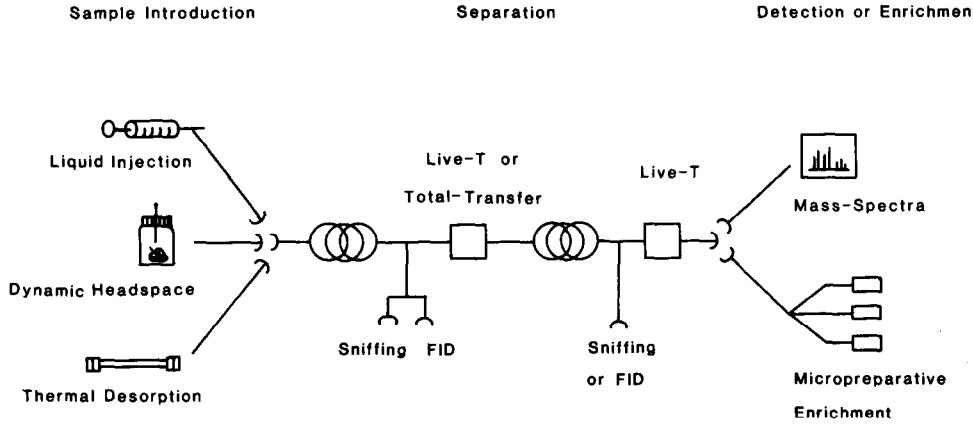

Fig. 1. Schematic diagram of the analytical system.

selected cut or cuts from the first column before transfer into the second column). Each column effluent is connected to a "sniffing mask" and a flame-ionization detector; the end of the second column is coupled to the mass spectrometer or a micro-preparative system by means of a second Live-T switching device.

Liquid injection

The system description and operation need no explanation within the scope of this contribution.

Dynamic headspace

A schematic diagram of the system used for enrichment of headspace volatiles and multi-dimensional analysis is shown in Fig. 2. Extraction of volatile components is achieved by stripping the headspace vial (8-ml capacity) with a constant flow of helium (30 ml/min) followed by cryogenic concentration in a cooled trap packed with Tenax TA (−20°C) or 5% OV-101 on Chromosorb W (−130°C). The total purging volume can be adjusted by time programming solenoid valve SV_3. During this sampling period breakthrough of substances into the first column is avoided by actuating SV_1, thus maintaining the preselected column pressure (adjustment with pressure regulator PM_4) and establishing a minor gas flow through a restriction capillary into the aforementioned trap (Fig. 3). The sampling period is interrupted by closing SV_1 and SV_3. The gas flow in the trap is reversed by actuating SV_1 and enriched volatiles are introduced into column 1 by thermal desorption (Fig. 4.). After admittance into the column, the original column pressure is re-established by operating SV_1 again.

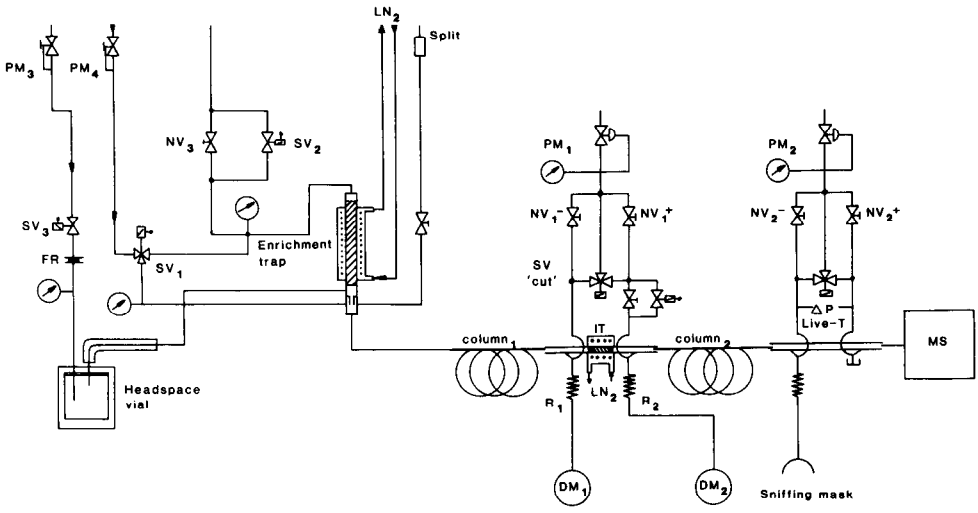

Fig. 2. Schematic diagram of dynamic headspace combined with multi-dimensional GC. PM_1–PM_4 = pressure regulators; R_1–R_3 = restriction capillaries; SV_1–SV_3 = solenoid valves; FR = flow regulator; NV_1–NV_3 = needle valves; DM_1, DM_2 = flame ionization detectors; LN_2 = liquid nitrogen; IT = intermediate trap.

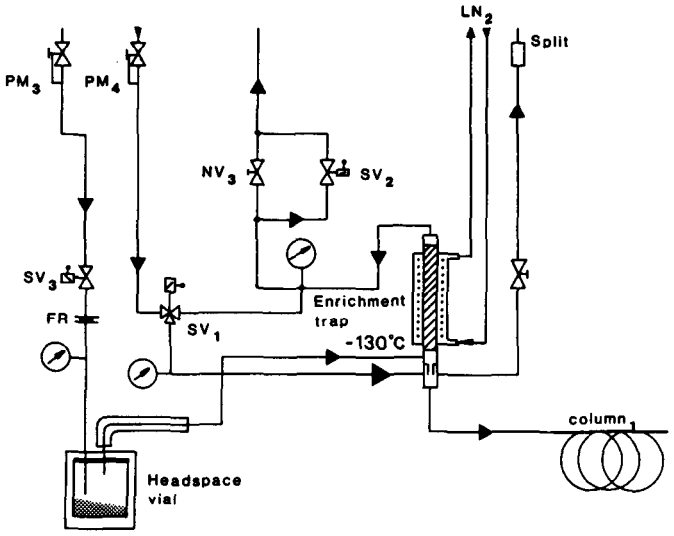

Fig. 3. Gas and substance flows during sampling.

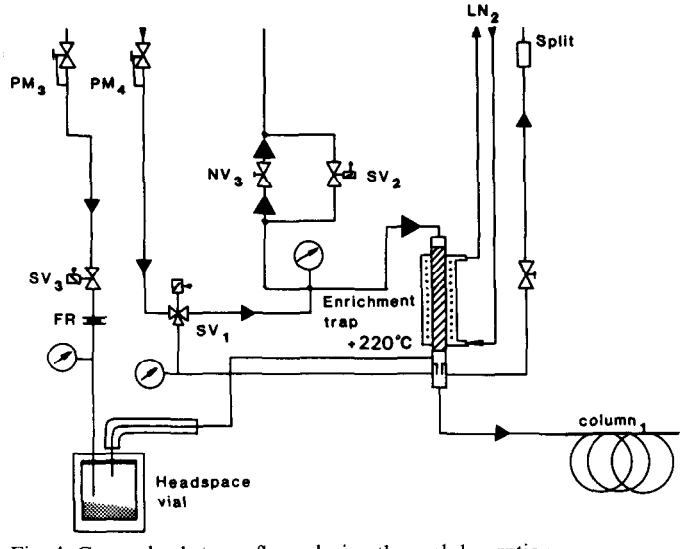

Fig. 4. Gas and substance flows during thermal desorption.

Thermal desorption system

Intermediate trap. For intermediate trapping of the desorbed volatiles, a modified programmable temperature vaporizer (Siemens PTV) was used. The following modifications were necessary. The original septum connector was replaced with a trap connector which accommodates the desorption oven and the desorption trap (Fig. 5). The capillary glass insert (4 cm longer than the original) was filled with 5% OV-101 on Chromosorb W and plugged at both ends with silanized glass-wool.

Before heating the desorption oven, the intermediate trap is cooled with liquid nitrogen to −150°C. For desorption, the carrier gas is closed and the desorption gas

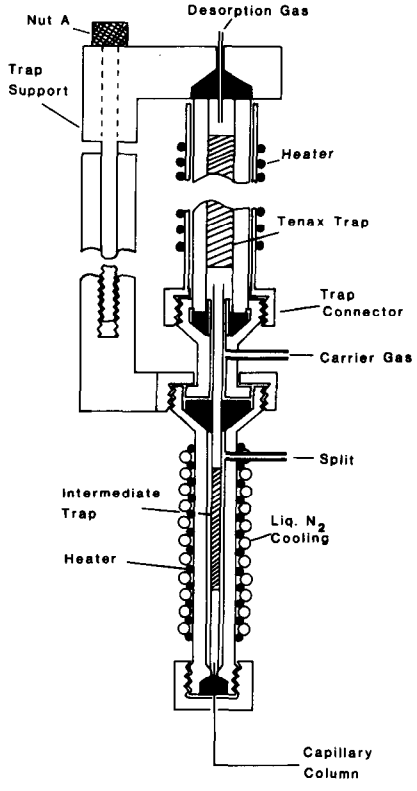

Fig. 5. Construction of thermal desorption system coupled to PTV injector for intermediate trapping.

is opened. During this step, the open split vent allows the components to desorb at high flow-rates, thus reducing the desorption time. When the condensation in the intermediate trap is complete, the split vent is closed and the trap is heated to the preset temperature within a few seconds. At the same time the desorption gas is closed and carrier gas is opened. The gas chromatograph has built-in facilities for timing the split valve open–close functions and for the carrier and desorption gas on–off functions.

Desorption device. A cross-sectional view of the desorption device is shown in Fig. 5. The traps (glass tubes, 100 mm × 5 mm O.D. × 3 mm I.D.) are held in place and sealed by pressing them against the PTFE ferrules integrated in the trap support and the trap connector. This is accomplished by tightening the nut A. An insulated cylindrical heating device with integrated thermocouple allows the temperature to be regulated up to 300°C.

Micro-preparative system

The chromatographic and pneumatic configuration for multi-dimensional operation is shown in Fig. 6. The gas chromatograph, equipped with an automatic injection device, is operated by the integrated microprocessor. The sample is injected into the first column where a preseparation is achieved. By means of the valveless

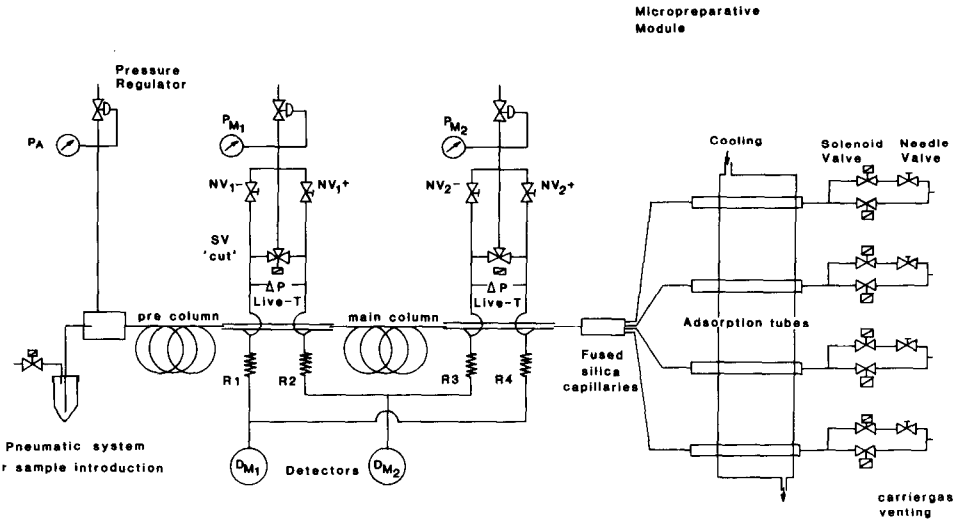

Fig. 6. Schematic diagram of multi-dimensional GC system coupled to micro-preparative system.

Live-T column-switching system, only the peak group of interest is entirely transferred to the main column without any sample loss. After final separation on the main column, the substances to be isolated are directed to the cooled traps by means of a second Live-T switching device. As can be seen in Fig. 6, the column effluent can be switched between the detector and a multiple channel manifold. Each channel of the manifold is connected to the trap, their exits being connected to solenoid valves. Manual operation of the aforementioned device is also possible. Three different flow conditions through the traps are encountered during operation:

Cut mode: (1) high flow, 10 ml/min (through the activated trap); (2) no flow, 0 ml/min (through the non-activated traps).

Stand-by mode: (3) low flow, 0.3 ml/min (through all traps to avoid back-diffusion).

In the cut mode, substances are transferred to the corresponding activated trap; in the stand-by mode, substances are diverted to the monitoring detector. The solenoid valves are controlled by means of the gas chromatograph's microprocessor, which provides high flexibility in term of sequencing. The temperature of the traps can be preset from room temperature to $-80°C$.

Apparatus

A Siemens Sichromat II double-oven gas chromatograph with autoinjector for liquid injection was used. The micropreparative module was of our own construction, as described[6]. The modfied Siemens PTV[7] and the modified Siemens headspace module[8] have been described. The mass spectrometer was a Finnigan 4021 quadrupole instrument.

APPLICATIONS

The following applications were selected to demonstrate the advantages and possibilities of the described system for the determination of sensorial active trace compounds.

Analysis of a "musty–earthy" off-flavour in wheat grains

The development of microflora on cereal grains results in the appearance of various volatiles and different odours[9,10]. We investigated wheat grains exhibiting a "musty–earthy" odour, which showed a higher bacterial and mould infection compared with sound grains[11]. A pre-chromatogram with simultaneous "sniffing" registration obtained by liquid injection of a pentane–diethyl ether (1:1) extract is shown in Fig. 7. The compounds responsible for the off-flavour are masked by major components of the sample. For identification of both substances, GC^2 with a megabore pre-column (SE-54, 5-μl injection volume) and cold trapping of selected cuts (at the retention times of the sniffing impressions marked in Fig. 7) was necessary, before separation on the analytical column (Carbowax 20M). The reconstructed ion chromatogram of the aforementioned cuts is shown in Fig. 8. Sniffing–MS monitoring and comparison with authentic reference substances showed that 2-methylisoborneol (1 ppb) and geosmine (17 ppb) are the trace compounds responsible for the off-flavour.

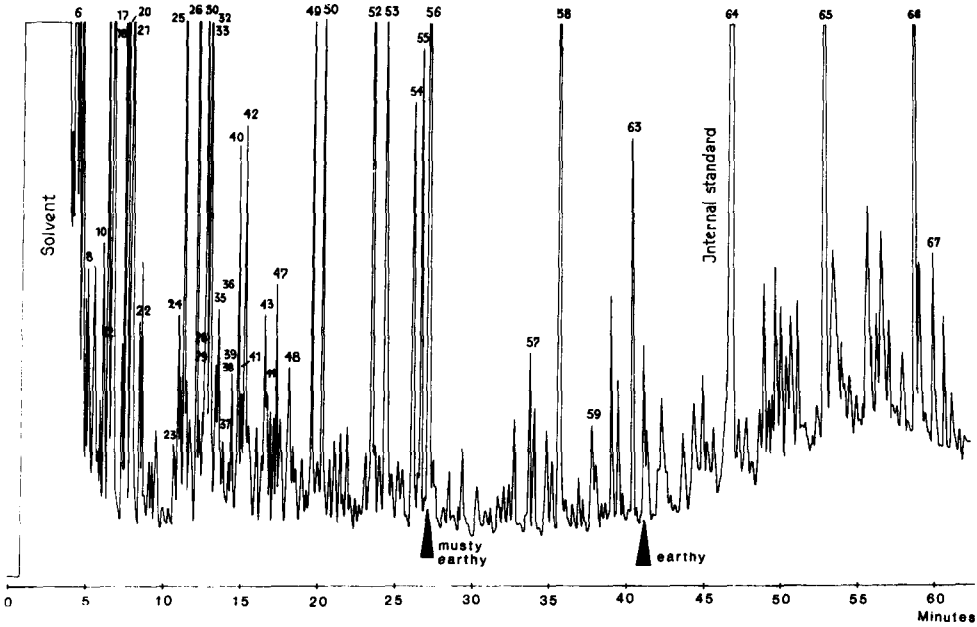

Fig. 7. Gas chromatogram of volatiles from wheat grain with "musty–earthy" odour (arrows show the retention ranges of sensorial interest).

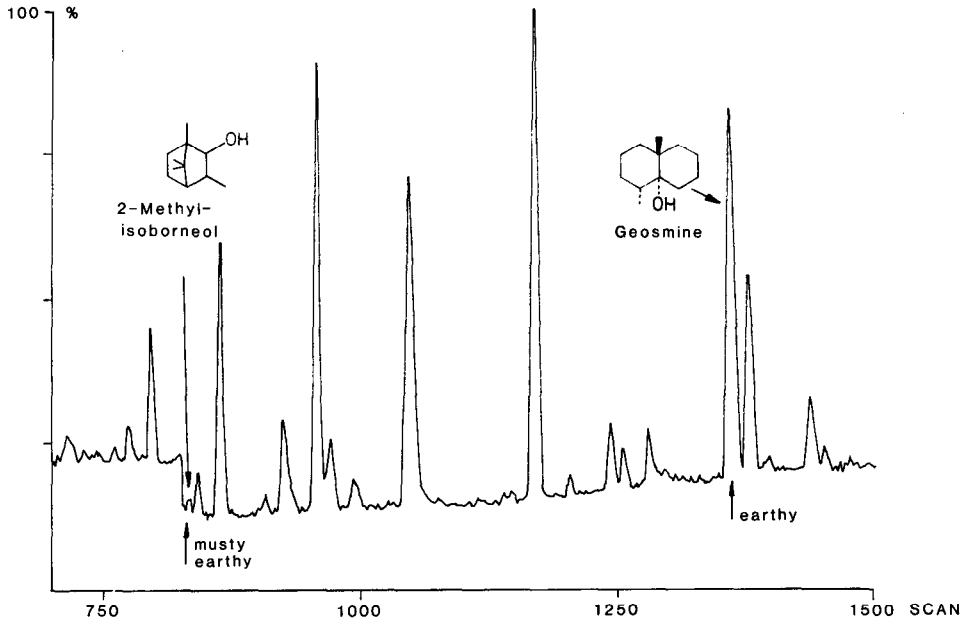

Fig. 8. Sniffing–MS trace of selected cuts of volatiles of wheat grain with "musty–earthy" odour from pre-column 1 (SE-54, two cuts) separated on the main column 2 (Carbowax 20M).

Analysis of a "peasy" off-flavour in coffee beans

Coffee defects derived from moulds, overfermentation, insects or other factors often considerably impair the flavour of the roasted end product. Certain African coffee beans with an off-flavour, known among experts as "peasy", were investigated[12].

In this instance the major advantages of the system are exploited to a large extent: determination of trace volatiles in a complex matrix without clean-up procedures, small sample amounts (only three coffee beans), adjustable enrichment effect due to continuous gas extraction conditions and cryofocusing, heart-cutting of off-flavour-related peaks by means of GC[2] and identification of the responsible compounds by simultaneous sniffing–MS.

The aforementioned analytical steps are summarized in Fig. 9. The enriched coffee headspace and the heart-cuts at the retention times corresponding to the elution of the "peasy" compounds are shown in Fig. 9A. The chromatograms of the cuts from column 1 (Carbowax 20M) on column 2 (SE-54) for three normal and three defective coffee beans are shown in Fig. 9B and C, respectively. Whereas 2-methoxy-3-isobutylpyrazine is present in nearly equal amounts in both samples, 2-methoxy-3-isopropylpyrazine is markedly increased (10-fold) in the defective beans, and has to be considered as responsible for the off-flavour. Overloading of the first column in this instance is of secondary importance, as the main separation is performed on column 2 with a preselected cut.

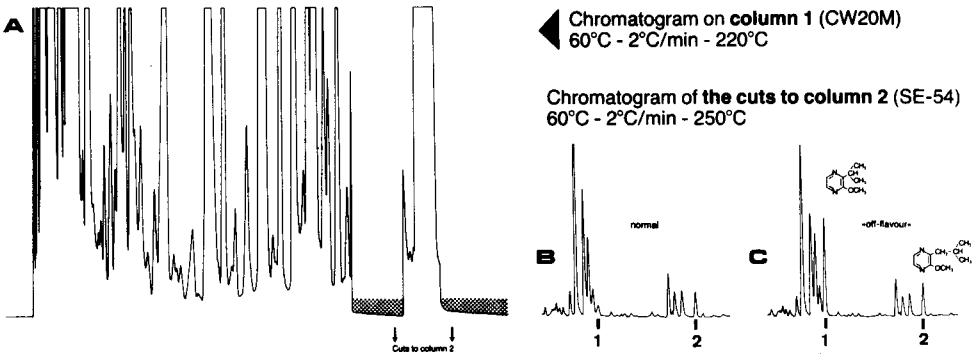

Fig. 9. (A) Headspace of defective coffee beans on column 1; (B) selected cut of a normal coffee bean on column 2; (C) selected cut of a defective coffee bean on column 2. Odorous compounds identified (simultaneous sniffing–MS): 1 = 2-methoxy-3-isopropylpyrazine ("peasy" odour); 2 = 2-methoxy-3-isobutylpyrazine ("bellpepper" odour). Compound 1 was identified as responsible for the "peasy" off-flavour. Sample, 3 coffee beans ($\approx$ 300 mg); sampling, 5 min at 80°C.

Analysis of an "obnoxious rotten" odour of a car mat

The automobile industry is often confronted with customer claims concerning offensive and pungent odours in new cars. The source of the substances responsible for the odour is plastics and synthetic materials. These compounds are present in very low concentrations and their release is markedly increased in summer owing to sun irradiation. The identification of the substances is also of interest in the fields of toxicology and environmental chemistry, as they constitute an additional source of human exposure to abiotic and possibly noxious chemicals.

In the present instance we investigated a felt-coated rubber mat principally to identify the odourous trace components. The chromatogram obtained after direct thermal desorption of 500 ml of enriched headspace (from 10 g of mat at 80°C) on Tenax TA is shown in Fig. 10. The usefullness of smell analysis is illustrated by the

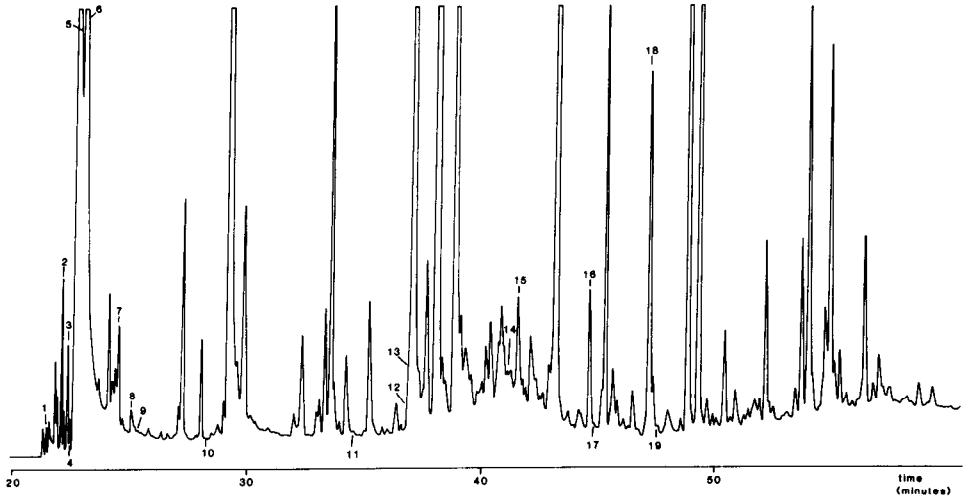

Fig. 10. Gas chromatogram of volatiles from a car mat on column 1 (SE-54).

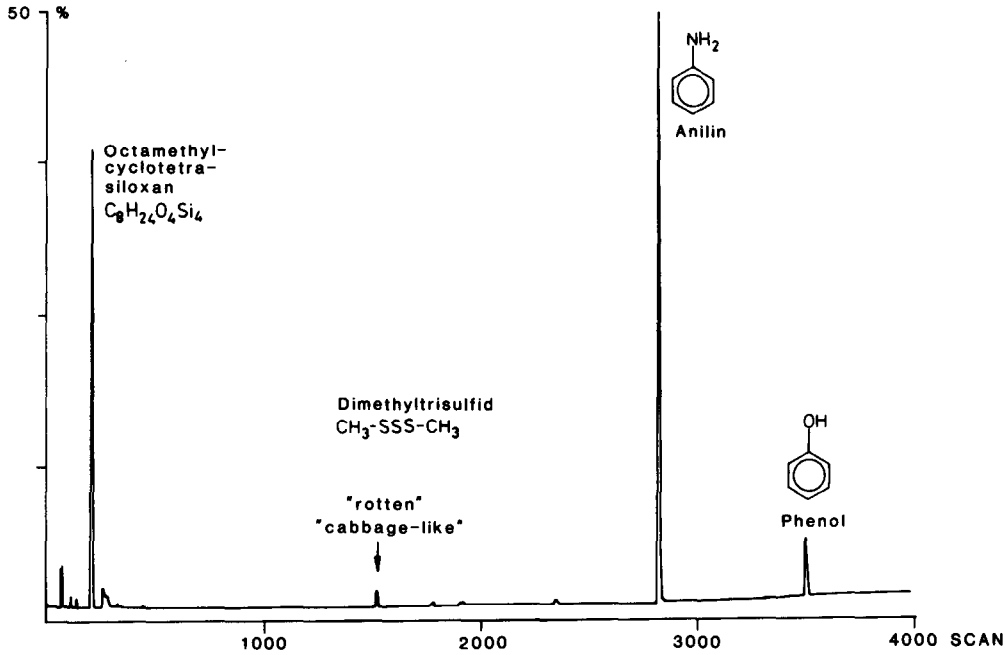

Fig. 11. Sniffing–MS trace of a selected cut (peak 13) of volatiles from a car mat on column 2 (Carbowax 20M).

fact that compounds present in small amounts, totally or partially masked by major components, can be characterized by this technique. It can be seen from Fig. 10 also that reliable identification without additional separation is impossible. Therefore, dynamic headspace (which allows on-line repetitive sampling) combined with GC[2] and sniffing–MS monitoring was applied for identification purposes. The analysis of a composite peak consisting of several components is discussed as an example.

A smell resembling the general odour impression of the car mat was observed at peak 13 (see Fig. 10). The MS analysis revealed that at least four components were present in that peak, the major one being aniline. It was clear that the rotten cabbage-like odour could not be attributed to this substance. The chromatogram of a selected cut of this peak from column 1 on column 2 is shown in Fig. 11. Smell analysis and MS monitoring revealed that the minor component (dimethyl trisulphide) was responsible for the perceived odour; the siloxane, aniline and phenol were odourless at these concentrations. The results obtained by performing selected heart-cuts at the corresponding retention times of elution of sensorial active compounds, separation on a second analytical column and subsequent smell asessment and MS registration are summarized in Table I.

The results indicate that the composite smell of the car mat can be principally attributed to sulphur compounds and alkyl isocyanides. Especially methanethiol and dimethyl trisulphide have low odour threshold values and have been reported to possess a pheromone character for polecat, mink and skunk[13].

TABLE I

ODOROUS COMPONENTS IN A CAR-MAT

Peak No.[a]	*Compound*[b]	*Odour description*
1	Methanethiol	Putrid
2	Dimethyl sulphide	Cabbage, cooked vegetable
3	Carbon disulphide	Putrid, pungent, vegetable
4	2-Methylpropanal	Milky, chocolate
5	2,3-Butanedione	Butter-like
6	Diethylamine	Fish-like
7	n.i.	Chocolate
8	Propyl? isocyanide	Repulsive, obnoxious
9	Ethyl acrylate	Like plastic
10	n.i.	Like plastic
11	n.i.	Sweet, nutty
12	1-Octen-3-one?	Fungal
13	Dimethyl trisulphide	Typical mat, cabbage-like
14	n.i.	Burnt, nutty
15	n.i.	Soap-like
16	n.i.	Plastic
17	Tetralin	Burnt vegetable
18	Benzothiazole	Typical mat, plastic
19	n.i.	Herbaceous

[a] Peak Nos. according to Fig. 10.
[b] n.i. = Not identified.

Isolation of components from parsley oil

1,3,8-*p*-Menthatriene and the corresponding aromatized terpene (α,*p*-dimethylstyrene) were isolated from parsley oil (Fig. 12), applying GC[2] combined with automated liquid injection and micro-preparative enrichment of the capillary GC effluents[6]. The first component was required for photochemical studies[14] and the second for unequivocal identification by NMR. Although the Kováts retention index of 1092 (100°C) on SE-54 is consistent with that (1080 on OV-101) of α-*p*-dimethylstyrene reported by Jennings and Shibamoto[15], the retention index was different to the value of 1277 (100°C) on OV-101 found by Swigar and Silverstein[16]. The same was observed for the Kováts retention index on Carbowax 20M of 1491 (100°C) when compared with the value given by Jennings and Shibamoto (1278 on Carbowax 20M). The mass and ^{1}H NMR spectra obtained from the enriched substance confirmed the postulated structure; the retention data reported in the literature have to be corrected.

The chromatographic parameters, column load and amount and purity of the isolated components are given in Table II as an example.

As can be seen from Table I, the purity of the isolated compounds is fairly good, although the column load selected was near the overloading limit of wide-bore capillaries. These operating conditions were deliberately chosen in order to achieve maximum efficiency (maximum throughput per unit time) and to demonstrate that in this extreme situation, the resolution losses can be overcompensated by means of multi-dimensional operation (in this instance the combination of two columns with different polarities).

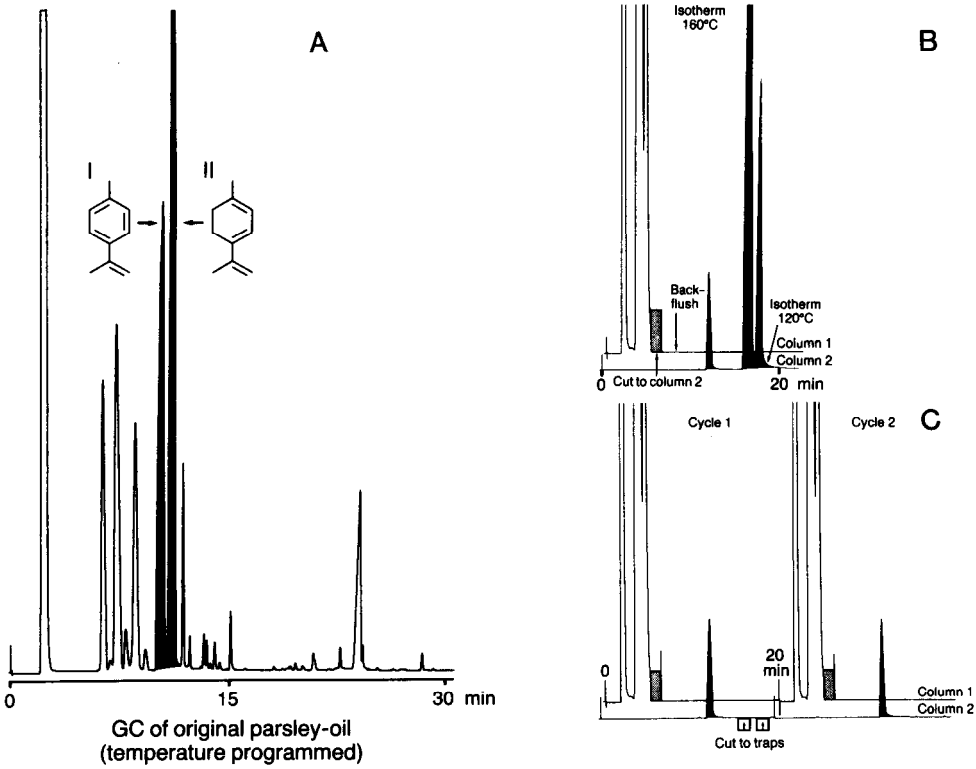

Fig. 12. (A) Chromatogram of parsley oil, temperature programmed. (B) Chromatogram of parsley oil on column 1 (isothermal) and cut chromatogram on column 2 (isothermal). (C) Same as (B), but with cut of eluents from column 2 into traps. Peak 1 = α-*p*-dimethylstyrene; peak 2 = 1,3,8-*p*-menthatriene.

TABLE II

ANALYTICAL PARAMETERS AND YIELDS OF COMPOUNDS ISOLATED FROM PARSLEY OIL

GC parameters:

Column 1: 30 m × 0.4 mm I.D., SE-54, film thickness 2 μm. Carrier gas: helium at 25 cm/s. Temperature: 160°C.

Column 2: 30 m × 0.53 mm I.D., Carbowax 20M, film thickness 2 μm. Carrier gas: helium at 23 cm/s. Temperature: 120°C.

Concentration of components in undiluted sample to be isolated: I, 18,2%; II, 32,8%.

Sample volume per cycle: 1 μl [25% parsley oil in pentane–diethyl ether (1:1)].

No. of cycles: 40.

Separation time per cycle: 20 min.

Total analysis time: 13.3 h.

Trap material: Tenax TA.

Trap temperature: −28°C.

Yields of isolated compounds:

I: 1.6 mg (purity 98.5%)

II: 3 mg (purity 99.2%)

Recovery:

>90% for both substances

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