Status of TLC in Forensic Toxicology Laboratory

In forensic toxicology, the unique features of TLC are best utilized in the broad-scale screening analysis for drugs and poisons in urine or liver samples. For this application, there are equipment, dedicated software and reference libraries available from several manufacturers. Compared to HPLC or capillary electrophoresis, TLC allows the detection of even poorly UV-absorbing compounds using selective visualization reactions. Compared to GC or GC-MS, TLC allows the chromatography of polar compounds without prior derivatization. Another important application of TLC is the screening or confirmation of drugs of abuse, although the supremacy of the combination of immunoassay and GC-MS in this area has hindered the development of modern dedicated TLC methods. Immunoassay screening, however, is vulnerable to sample adulteration and high background noise. In larger, broad-service laboratories, the various techniques available today, including TLC, are considered complementary rather than exclusive.

See also: **II/Chromatography: Thin-Layer (Planar):** Modes of Development: Conventional; Modes of Development: Forced Flow, Over Pressured Layer Chromatography and Centrifugal; Spray Reagents. **III/Alcohol and Biological Markers of Alcohol Abuse: Gas Chromatography. Clinical Chemistry: Thin-Layer (Planar) Chromatography. Clinical Diagnosis: Chromatography. Forensic Sciences: Capillary Electrophoresis.**

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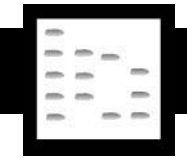
FRAGRANCES: GAS CHROMATOGRAPHY



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Introduction

It is difficult to distinguish between aromas, flavours, taints and perfumes, because to a large extent these are artificial categories that overlap. An aroma may be defined as the smell emanating naturally (possibly in the process of cooking or other method of preparation) of a foodstuff or beverage. The aroma from coffee beans on roasting is a prime example but there are many others. The main criterion is that the aroma material is essentially all in the vapour phase and the nose is responsible for sensing the aroma. A flavour is intimately related to an aroma but may contain involatile compounds that give rise to the sensation of taste but in practice it is common to have a flavour with an associated aroma. A tainted foodstuff or beverage is often unsatisfactory for consumption because there are compounds present that have an unpleasant smell or taste. Taints may arise from natural

chemical reactions such as the oxidation of the acids in an oil or fat to turn it rancid or the production of amines in fish. Other taints may occur because of leaching of material from packaging such as phenolic compounds from paper wrapping and solvents from the ink used in printing labels. Perfumes are natural or synthetic mixtures that have a pleasant smell to most people, although some of the constituents may have an unpleasant odour, a different odour or no odour when present in bulk. For example, coumarin in low concentrations has the smell of new-mown hay but this is not apparent at high concentrations. Although this article is specifically about aromas most, if not all of the techniques described are equally applicable to perfume studies. The only significant difference is that perfumes, in their final commercial form, normally exist as a solution (usually in ethanol) so that in this form they may be analysed as conventional liquid samples.

Another group of compounds that have much in common with aromas are pheromones, which cause specific behavioural effects in animals and insects. Indeed, it could be claimed that aromas and perfumes are the equivalent materials in humans (although there are true human pheromones, they are of small importance compared to those in the insect world).

Aromas frequently occur as complex, multicomponent mixtures with many of the important components (from an olfactory point of view) in very low concentrations – often at the ppm level or less.

Aroma Compounds

Since, by definition, aromas are volatile mixtures that produce an olfactory response, it follows that they should be amenable to analysis by gas chromatography (GC). This was appreciated quite early in the development of GC and Teranishi *et al.* did a considerable amount of work in the 1960s on strawberry aroma. The work was hampered by the use of packed columns and detectors of relatively low sensitivity and poor qualitative diagnostic information, but these investigations still go on today with modern equipment (see Further Reading).

Aromas contain many different types of compounds. Among the commonest are aliphatic, olefinic and aromatic hydrocarbons. A number of essential oils fall into this category but many aroma compounds have hetero-elements such as oxygen, nitrogen and sulfur in the molecule as well as a variety of functional groups such as alcohol, aldehyde, acid, phenol, ester and ether moieties. The smell of cheeses such as Camembert is due to the presence of fatty acids such as butyric acid. The smell of garlic is due to a fairly simple mixture of sulfur compounds including

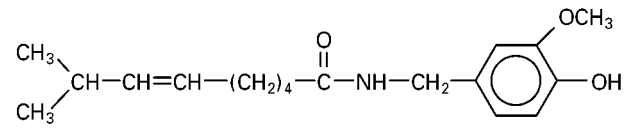


Figure 1 Structure of capsaicin, the hot agent of peppers.

diallyl disulfide, $CH_2{=}CH.CH_2.S.S.CH_2.CH{=}CH_2$, and lager owes its distinctive aroma to the presence of p.p.m. concentrations of lower mercaptans. As can be imagined, the actual amount is a vital part of quality control.

Figure 1 shows the structure of capsaicin, the hot agent in peppers. It contains a nitrogen-containing amido group, a phenolic group and an ether group as well as olefinic double bonds. Aroma compounds frequently exist as *cis* and *trans* isomers arising from such double bonds and there is also a possibility of the presence of chiral compounds.

Identification of geometrical and optical isomers is of great importance since the isomers often exhibit a very large variation in physiological properties, including smell. An example of this is carvone (**Figure 2**) where the D isomer has an odour of dill, whereas the L isomer has a spearmint smell. In **Figure 2** it can be seen that the centre of asymmetry is at the carbon atom at which the isopropenyl group is attached to the cyclohexene ring. The situation is also complicated by the reverse situation, i.e. it is possible to have two compounds of quite different chemical structure that smell the same. The best known examples of the latter are benzaldehyde and hydrogen cyanide, both of which have a smell of bitter almonds and both give rise to this smell in natural products.

Sampling

Since the sample is gaseous but is in contact with a liquid or a solid, all the methods of gas sampling may be used as appropriate but the most important are static and dynamic headspace sampling. Dynamic headspace sampling results in greater sensitivity since all the volatile material from a given sample is removed from the headspace but it has to be retained in a trap packed with a sorbent such as Tenax. It is difficult to adjust the purge gas conditions so that all

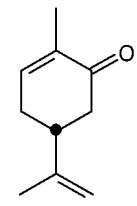


Figure 2 Structure of carvone showing the position of the chiral centre.

the compounds of low volatility are removed without losing those of high volatility in the trap and vice versa. Whilst this may not be a problem if the volatility range of the compounds is small, it may make accurate quantitative analysis in one run impossible if the range of volatility is large. One way to circumvent this problem is by closed-loop stripping but this is at the expense of considerable extra experimental complications. Quantitative analysis is much easier by static headspace sampling but this technique has a poorer lower limit of detection, especially for compounds of low volatility. When using the term volatility, it must be remembered that many samples are in an essentially aqueous medium and a nonpolar compound will have a large activity coefficient in an aqueous system and a much higher volatility than might be expected from the boiling point of the pure compound.

Transfer of the aroma sample from the sorbent trap to the GC is by thermal or solvent desorption. Both have advantages and disadvantages. Thermal desorption is a one-shot method and may cause decomposition of some of the components of the sample. It has the complication of the need for a secondary trap that can be heated very rapidly so that the sample enters the column with a plug profile. The presence of water from aqueous samples may also cause problems if steps are not taken to remove most of it. Solvent desorption has the disadvantage that the solvent may give a large peak early in the chromatogram that masks some of the volatile components of the sample. The use of CS_2 minimizes the problem if a flame ionization detector (FID) is used as the detector since this compound has a small FID response but it is toxic and highly flammable.

A more recent method of sampling is the use of solid-phase microextraction (SPME) where a quartz fibre coated with a film of stationary phase is exposed to a liquid or gaseous sample followed by thermal or solvent desorption. Although it is very convenient to use, the limit of detection is not likely be as good with SPME as with dynamic headspace sampling since SPME is essentially the same as static headspace sampling and the mass of the sorbent film on the quartz fibre is much smaller than in a conventional headspace trap. Discrimination is also possible if the correct choice of fibre coating is not made. To find a polymeric stationary-phase material that is equally selective for a broad range of compounds of different polarity is difficult and there may be memory problems with the fibre and low recoveries. In spite of this problem, SPME is becoming more popular and many recent publications use this technique with excellent results.

The concentration of the important compounds in an aroma sample may be extremely small and it may be necessary to carry out a large scale GC separation or some other enrichment procedure before GC analysis. One way of effecting preconcentration is by using supercritical fluid extraction (SFE) with CO_2 containing small amounts ($\approx$ 5–10%) of a more polar solvent such as methanol. The great virtues of SFE are that it is conducted at around ambient temperature and that it is very easy to remove the solvent (CO_2).

GC Separation Conditions

Columns

All analysis of aroma samples is now carried out on open tubular columns except if small scale preparative GC is carried out for prior enrichment.

Aroma samples consist of compounds ranging from nonpolar hydrocarbons to polar aldehydes, alcohols and acids but since they are in the gas phase under ambient conditions they will be of relatively low boiling point. These two conditions point to the use of polyglycol (Carbowax^{TM}) phases which can be operated up to about 230°C. Wax columns are particularly favoured for the analysis of fatty acid methyl esters because of their ability to separate *cis*/*trans* isomers (**Figure 3**).

Other phases such as phenyl, trifluoromethyl, cyano and hydroxy silicones have also been used. For chiral separations silicone phases containing β-cyclodextrins dissolved in the silicone have been employed. **Figure 4** shows the chiral separation of +/− 1-octen-3-ol and +/− carvone and **Figure 5** shows the separation of the chiral components of rosemary oil.

The columns should be capable of handling as large a sample as possible since some of the important aroma constituents may be present at p.p.m. level or less. In order to have a large sample capacity, the stationary-phase film should be relatively thick and films up to 5 μm have been used in large bore columns (0.53 mm i.d). Thick films cause a significant reduction in resolution so a compromise is a film of 1 μm thickness. For higher resolution and with mass spectrometric detection, standard 0.25 mm and 0.32 mm i.d columns are used with a film thickness of 0.25 μm or less. Most applications now standardize on columns 30 m long, with some samples requiring 60 m length; columns longer than this are now seldom employed in the aroma field since they carry the penalty of longer analysis time and a greater possibility of decomposition.

Carrier Gas

The carrier gas is not of great importance on aroma analysis; nitrogen gives the highest resolution but the

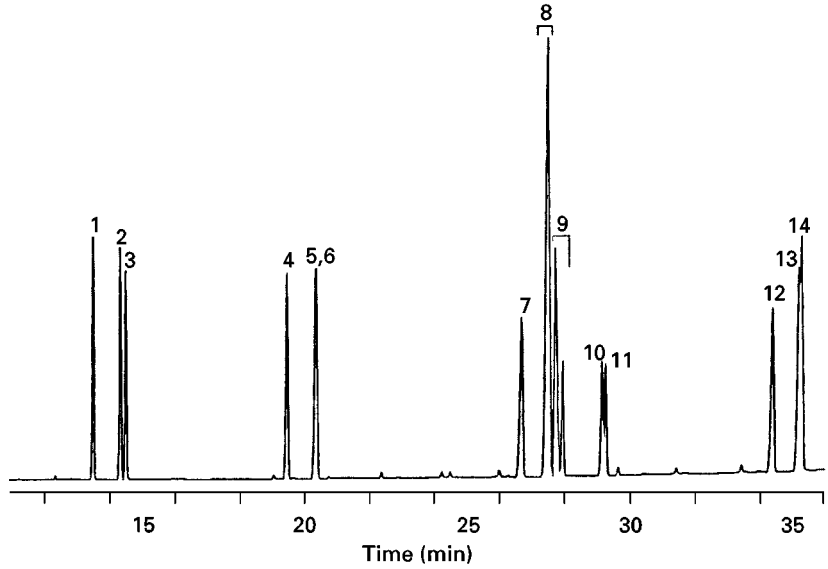


Figure 3 Separation of *cis* and *trans* isomers of fatty acids. 60 m, 0.25 mm i.d., 0.25 μm Rtx-Wax; on-column concentration 40–75 ng. Oven temperature 165–250°C at 2°C min^{-1}. Injection/detection temperature 220/250°C; carrier gas, helium. Linear velocity 20 cm s^{-1} set at 165°C; split ratio 50 : 1. Peak identification 1, C_{14} : 0; 2, C_{14} : 1 n5*cis*; 3, C_{14} : 1 n5*trans*; 4, C_{16} : 0; 5, C_{16} : 1 n7*cis*; 6, C_{16} : 1 n7*trans*; 7, C_{18} : 0; 8, C_{18} : 1 *cis* isomers (n12, n9, n7); 9, C_{18} : 1 *trans* isomers (n12, n9, n7); 10, C_{18} : 2 n6*cis*; 11, C_{18} : 2 n6*trans*; 12, C_{20} : 0; 13, C_{20} : 1 n9*cis*; 14, C_{20} : 1 n9*trans*. Reproduced by permission of Restek Corp.

slowest analysis, as may be seen from van Deemter curves for hydrogen, helium and nitrogen. Helium is the most commonly used carrier gas.

Detection

The FID is the workhorse detector for aroma analysis. It has many advantages but lacks the sensitivity of some of the selective detectors which may exhibit up to 10^3 times better lower limit of detection as well as giving qualitative information. **Figure 6** gives two headspace chromatograms of coffee aroma, one obtained with an FID and the other with a helium ionization detector with less than half the gas volume (40 μL as opposed to 100 μL for the FID) which shows the much bigger response of the latter; the tailing in the helium detector chromatogram is due to the response to a water peak. In this instance,

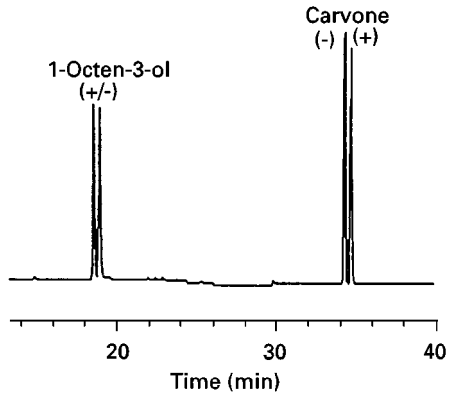


Figure 4 Chiral separation of +/− octen-3-ol and +/− carvone. 30 mm, 0.32 mm i.d., 0.25 μm, Rt-βDEXsa. Oven temperature: 40°C (hold 1 min) to 230°C at 2°C min^{-1} (hold 3 min). Carrier gas: hydrogen 80 cm s^{-1} set at 40°C. Detector: FID set at 220°C. Reproduced by permission of Restek Corp.

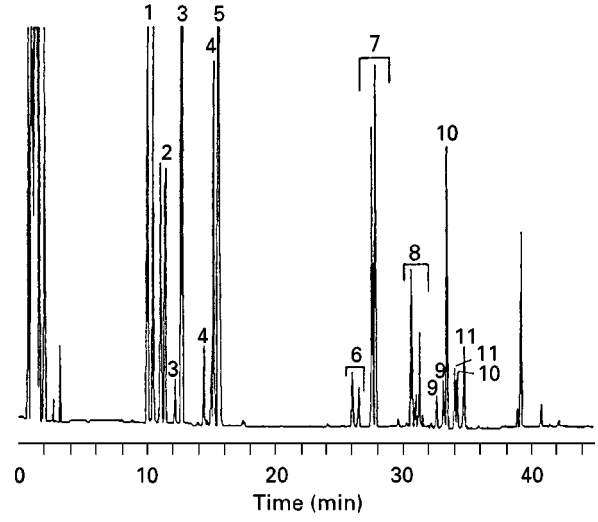


Figure 5 Chiral separation of the components of rosemary oil. 30 m, 0.32 mm i.d., 0.25 μm Rt-βDEXsm. Oven temperature 40°C (hold 1 min) to 200°C at 2°C min^{-1} (hold 3 min). Carrier gas: hydrogen 80 cm s^{-1}. Detector: FID set at 220°C. Peak identification: 1, (−/+) α-pinene; 2, (+/−) camphene; 3, (+/−) β-pinene; 4, (−/+) limonene; 5, eucalyptol (1,8-cineole); 6, (−/+) linalool; 7, (+/−) camphor; 8, (−/+) teripinen-4-ol; 9, (+/−) isoborneol; 10, (+/−) borneol; 11, (+/−) α-terpineol. Reproduced by permission of Restek Corp.

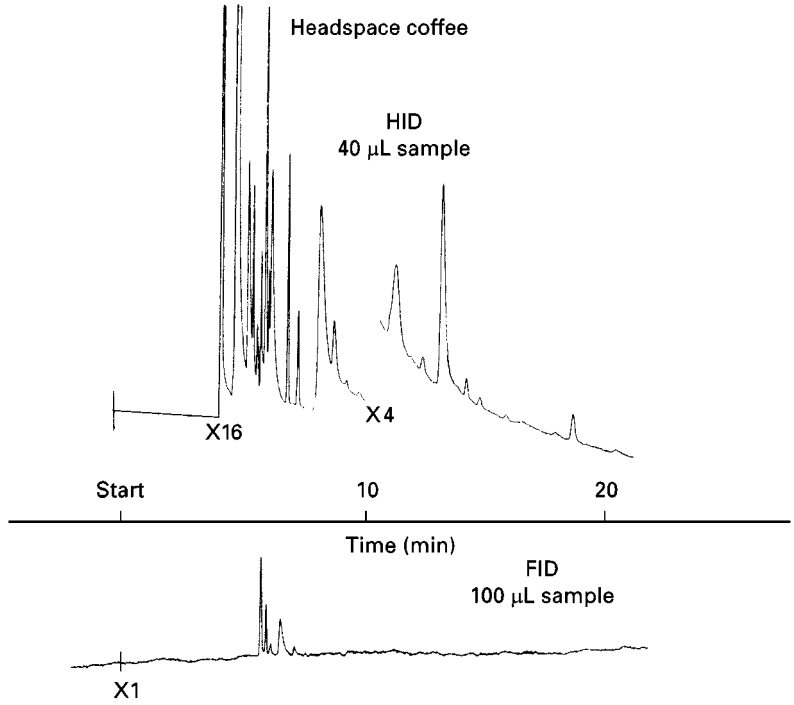


Figure 6 Helium ionization detector and FID chromatograms of coffee aroma. Column: 100 m × 0.5 mm i.d. stainless steel coated with Witconol LA-23. Column temperature 60°C isothermal. Sample introduced via a gas sampling valve. (Reproduced from Andrawes FF and Gibson EK *Journal of High Resolution Chromatography* (1982), with permisson from Wiley–VCH.)

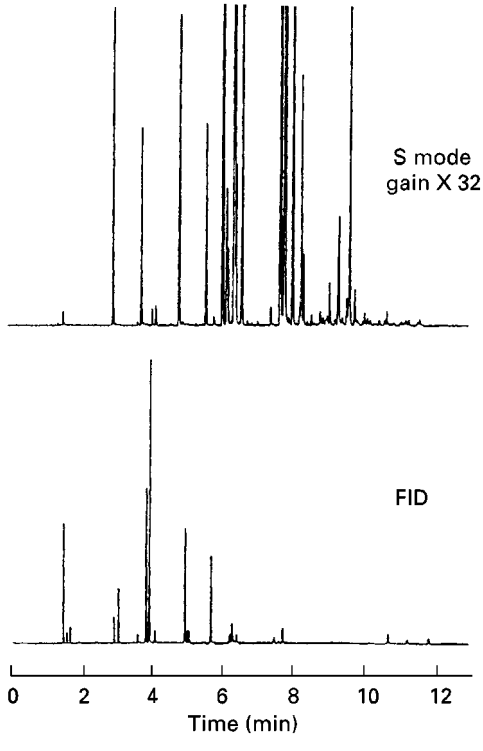


Figure 7 Pulsed flame photometric detector (in S mode) and FID chromatograms of coffee aroma. (From Varian publication 03-914625-00, 1998. By courtesy of Varian Associates.)

the helium detector clearly has great advantages over the FID.

As pointed out earlier, many aroma compounds contain hetero-elements and **Figure 7** shows headspace chromatograms of coffee aroma obtained with an FID and the pulsed flame photometric detector in the sulfur mode, which shows the presence of a large number of sulfur compounds. This detector can also be used in a nitrogen-selective mode to reveal the presence of pyrroles, pyrazines and caffeine in the coffee aroma.

The Fourier transform infrared detector (FTIR) should be extremely useful in aroma analysis because of its ability to give a response to specific functional groups in a molecule. This detector is gradually coming into use for this type of work (see Further Reading) but the sensitivity to different functional groups varies considerably and the capital cost is quite large. The mass spectrometer in the selective ion monitoring mode probably gives the best all-round sensitivity. Full scan mass spectra can give sensitivities in the pg range under favourable circumstances. **Figure 8** shows a mass chromatogram and a Gram-Schmidt FTIR chromatogram of a dynamic headspace sample of the vapour above a particular variety of strawberries. The identity of the peaks is given in **Table 1**. Although there is a general similarity between the two chromatograms, there is a considerable difference in detailed quantitative response, with

Table 1 Compounds in strawberry aroma identified by GC-MS and GC-FTIR

Peak no.[a]	*Compound*	*RRT (%SD, n = 3)*	*m/z*	λ_{max} (cm^{-1})
1	Acetic acid	0.141 (5.4%)	43 (100%), 60 (M^+, 83%)	No data
2	Methyl acetate	0.156 (5.2%)	43 (100%), 74 (M^+, 15%)	2966, 1777, 1757, 1448 1372, 1240, 1048
3	Ethyl acetate	0.255 (3.6%)	43 (100%), 61 (5%), 88 (M^+, 19%)	2992, 1769, 1755, 1373, 1238, 1093, 1052
4	Isopropyl acetate	0.310 (2.6%)	43 (100%), 59 (6%), 61 (26%), 87 (2%), 102 (M^+, 9%)	2985, 2904, 1755, 1383, 1239, 1138, 1021
5	Ethyl propionate	0.393 (1.7%)	57 (100%), 74 (12%), 102 (M^+, 33%)	2992, 2958, 1753, 1185
6	Methyl butyrate	0.415 (3.4%)	43 (100%), 55 (11%), 59 (13%), 71 (28%), 74 (33%), 87 (8%), 102 (M^+, 12%)	2968, 1760, 1444, 1359, 1297, 1256, 1184, 1103
7	4-Methyl-2-pentanone	0.440 (1.8%)	43 (100%), 58 (13%), 85 (40%), 100 (M^+, 26%)	2960, 2881, 1729, 1373, 1285, 1240, 1176
8	Ethyl isobutryate	0.499 (1.5%)	43 (100%), 71 (29%), 88 (14%), 116 (M^+, 22%)	2973, 2895, 1755, 1461, 1442, 1390, 1365, 1249, 1234, 1188, 1154, 1096 1083, 1021
9	Methyl 2-methylbutyrate	0.518 (1.7%)	41 (55%), 43 (100%), 55 (20%), 57 (58%), 59 (25%), 69 (12%), 74 (12%), 85 (20%) 88 (52%), 101 (13%), 116 (M^+, 4%)	2966, 2889, 1759, 1440, 1363, 1292, 1242, 1186, 1112, 1017
10	Methyl isobutyrate	0.518 (1.5%)	41 (40%), 43 (100%), 57 (17%), 59 (17%), 74 (22%), 85 (9%), 101 (5%), 116 (M^+, 6%)	2969, 2896, 1757, 1466, 1445, 1378, 1363, 1299, 1257, 1187, 1161, 1112, 1099, 1018
11	*n*-Hexanal	0.567 (1.1%)	41 (100%), 44 (45%), 56 (30%), 72 (7%), 82 (16%), 99 (7%), 100 (M^+, 4%)	2940, 2885, 2811, 2714, 1744
12	Ethyl butyrate	0.567 (1.4%)	43 (100%), 60 (10%), 70 (4%), 71 (45%), 88 (19%), 89 (15%), 101 (4%), 116 (M^+, 14%)	2983, 1754, 1255, 1181
13	Isobutyl acetate	0.587 (1.1%)	41 (18%), 43 (100%), 56 (14%), 61 (21%), 69 (8%), 71 (8%), 116 (M^+, 10%)	2969, 2886, 1764, 1485, 1372, 1234, 1064, 1032
14	Isopropyl isobutyrate	0.637 (1.2%)	41 (43%), 43 (100%), 71 (40%), 89 (35%), 130 (M^+, 2%)	2981, 2944, 2890, 1750, 1468, 1376, 1238, 1184, 1152, 1091, 1030
15	Ethyl 2-methylbutyrate	0.674 (0.7%)	41 (100%), 43 (50%), 57 (67%), 69 (21%), 74 (12%), 85 (11%), 115 (5%), 130 (M^+, 12%)	2979, 2948, 2891, 1750, 1466, 1377, 1248, 1182, 1149, 1088, 1033

Table 1 *Continued*

Peak no.[a]	*Compound*	*RRT (%SD, n = 3)*	*m/z*	λ_{max} (cm^{-1})
16	Ethyl isovalerate	0.678 (0.8%)	43 (100%), 57, (59%) 69 (10%), 87 (20%), 130 (M^+, 10%)	2971, 2884, 1753, 1468, 1374, 1295, 1250, 1184, 1115, 1039
17	Hex-2(*Z*)-enal	0.681 (0.9%)	41 (100%), 55 (41%), 69 (18%), 83 (11%), 98 (M^+, 14%)	2972, 2949, 2885, 2814, 2727, 1715, 1634, 1151, 1091, 1037, 981
18	Isoamyl acetate	0.712 (1.5%)	43 (100%), 55 (24%), 61 (7%), 70 (17%), 87 (4%), 130 (M^+, 4%)	2969, 2887, 1761, 1468 1371, 1234, 1038
19	2-Methylbutyl acetate	0.72 (1.6%)	43 (100%), 55 (13%), 61 (6%), 70 (12%), 87 (1%)	2972, 2899, 1762, 1468 1373, 1233, 1039
20	3-Methyl-2-heptanol[b]	0.747 (0.6%)	45 (100%), 55 (26%), 57 (23%), 69 (8%), 83 (7%), 92 (7%), 112 (1%)	No data
21	Amyl acetate	0.798 (0.3%)	43 (100%), 55 (13%), 61 (25%), 70 (11%), 130 (M^+, 2%)	2963, 2944, 1767, 1361, 1234, 1143, 1048
22	Methyl caproate	0.816 (0.5%)	43 (100%), 55 (24%), 59 (21%), 69 (13%), 74 (40%), 87 (13%), 99 (11%), 130 (M^+, 13%)	2963, 2879, 1760, 1440, 1241, 1215, 1173, 1110
23	Ethyl 3-methyl-2-butenoate	0.849 (1.5%)	43 (20%), 55 (100%), 83 (40%), 100 (13%), 113 (27%), 128 (M^+, 5%)	2987, 2948, 2936, 2904, 1731, 1652, 1268, 1138, 1081, 1053
24	2,5-Dimethyl-4-methoxy-3(2*H*)-furanone	0.933 (0.8%)	43 (100%), 55 (30%), 69 (10%), 85 (13%), 101 (5%), 127 (4%), 142 (M^+, 2%)	No data
25	Ethyl caproate	0.966 (1.6%)	43 (100%), 55 (27%), 60 (72%), 73 (40%), 88 (42%), 99 (37%), 101 (18%), 115 (10%), 144 (M^+, 8%)	2969, 2943, 2882, 1754, 1464, 1375, 1241, 1172, 1109, 1041
26	2,5-Dimethyl-3-hydroxy-4-methoxy-2,3-dihydrofuran[a]	0.990 (0.1%)	43 (70%), 67 (45%), 83 (100%), 112 (6%), 129 (2%), 128 (3%), 144 (M^+, 6%)	No data
27	Hexyl acetate	0.994 (0.1%)	43 (100%), 55 (13%), 56 (22%), 61 (14%), 69 (7%), 84 (5%), 144 (4%)	2966, 2942, 2871, 1762, 1369, 1234, 1060, 1030
28	Hex-2-(*E*)-enyl acetate	0.997 (0.1%)	43 (100%), 55 (17%), 67 (48%), 82 (31%), 142 (M^+,3%)	2969, 2942, 2883, 1762 1675, 1455, 1358, 1230 1081 1024, 968
29	Cyclohexyl acetate	1	43 (100%), 55 (13%), 67 (19%), 82 (35%), 83 (44%), 142 (M^+, 4%)	3018, 2970, 2947, 2908, 2886, 1752, 1465, 1375, 1233, 1042

Table 1 *Continued*

Peak no.[a]	*Compound*	*RRT (%SD, n = 3)*	*m/z*	λ_{max} (cm^{-1})
30	2-Ethyl hexenoate (isomer)	1.028 (1.1%)	41 (55), 55 (100%), 68 (18%), 69 (18%), 73 (22%), 97 (31%), 142 (M^+, 16%)	2975, 2939, 1743, 1650, 1528, 1312, 1252, 1176, 1047, 991
31	Amyl butyrate	1.054 (0.5%)	43 (100%), 55 (29%), 60 (6%), 70 (27%), 71 (52%), 89 (10%), 158 (M^+, 3%)	2969, 2908, 2887, 1752, 1460, 1353, 1238, 1177, 1096
32	Unidentified unsaturated aldehyde	1.073 (0.6%)	41 (100%), 55 (78%), 69 (42%), 83 (32%), 93 (7%), 109 (57%), 128 (48%), 144 (50%),	2966, 2934, 2882, 2817, 2738, 1787, 1716, 1623
33	Nona-2,4-dienal (isomer)	1.155 (0.9%)	43 (39%), 81 (100%), 95 (19%), 138 (M^+, 9%)	2749, 1745, 1673
34	Non-2-en-1-ol (isomer)	1.175 (0.8%)	57 (100%), 67 (36%), 68 (18%), 69 (67%) 70 (34%), 81 (39%), 83 (36%), 95 (13%) 96 (10%), 124 (7%), 142 (M^+, 2%)	No data
35	Methyl caprylate	1.194 (0.9%)	43 (100%), 55 (40%), 69 (11%), 74 (65%), 87 (25%), 101 (9%), 115 (8%), 127 (11%), 158 (M^+, 7%)	2937, 2867, 1758, 1443, 1353, 1238, 1191, 1113, 1045
36	Benzyl acetate	1.244 (1.3%)	43 (100%), 51 (9%), 69 (9%), 77 (14%), 79 (29%), 91 (87%), 108 (54%), 150 (M^+, 4%)	No data
37	Ethyl benzoate	1.267 (1.2%)	43 (33%), 51 (15%), 69 (10%), 77 (53%), 105 (100%), 122 (19%) 150 (M^+, 10%)	No data
38	*n*-Hexyl butyrate	1.316 (1.2%)	43 (100%), 56 (30%), 71 (54%), 84 (7%), 89 (52%), 117 (16%), 172 (M^+, 15%)	2943, 2895, 2877, 1754, 1263, 1176, 1097
39	Hexyl isobutyrate	1.319 (1.1%)	43 (97%), 55 (48%), 71 (89%), 84 (100%), 89 (16%), 101 (9%), 172 (M^+, 26%)	2971, 2943, 1754, 1265, 1173, 1095, 1053, 976
40	Ethyl caprylate	1.322 (1.2%)	43 (100%), 57 (35%), 60 (29%), 61 (20%), 69 (28%), 81 (17%), 88 (26%), 101 (13%), 115 (5%), 127 (8%), 172 (M^+, 10%)	2967, 2938, 2678, 1753, 1465, 1366, 1342, 1263, 1188, 1167, 1107, 1042
41	Decanal	1.339 (1.2%)	43 (100%), 57 (78%), 69 (37%), 70 (28%), 83 (75%), 95 (28%), 109 (9%), 156 (M^+, 2%)	2934, 2865, 2780, 2765, 1746

Table 1 *Continued*

Peak no.[a]	*Compound*	*RRT (%SD, n = 3)*	*m/z*	λ_{max} (cm^{-1})
42	Octyl acetate	1.354 (0.1%)	43 (100%), 55 (30%), 83 (12%), 56 (17%), 57 (30%), 61 (37%), 69 (28%), 70 (15%), 71 (42%), 112 (23%), 172 (M^+, 12%)	2937, 2866, 1760, 1462, 1369, 1233, 1038, 1018
43	Amyl caproate	1.427 (1.5%)	43 (100%), 55 (32%), 60 (8%), 70 (30%), 71 (31%), 99 (8%), 117 (16%), 186 (M^+, 1%)	2967, 2907, 2880, 1753, 1466, 1369, 1239, 1194 1163, 1113
44	Nonyl acetate	1.511 (1.6%)	43 (100%), 55 (31%), 61 (13%), 69 (22%), 83 (11%), 97 (9%), 186 (M^+, 1%)	No data
45	*n*-Decyl acetate	1.657 (4.4%)	43 (100%), 55 (31%), 69 (28%), 83 (40%), 97 (16%), 200 (M^+, 2%)	2936, 2982, 2865, 2846, 1755, 1456, 1272, 1175 1094
46	Hex-3(*Z*)-en-1-ol	[c]	41 (100%), 55 (19%), 67 (38%), 69 (8%), 81 (11%), 82 (15%), 100 (M^+, 3%)	No data

[a]Numbering 1–45 from **Figure 1**.
[b]Tentative identification.
[c]Reproduced with permission from Marco *et al., Journal of High Resolution Chromatography* (1997), 20: 276–278.

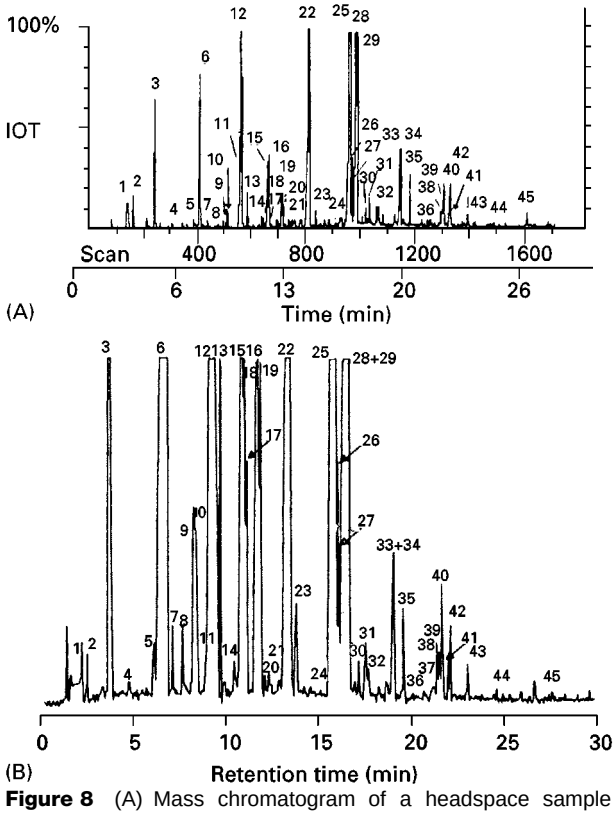


Figure 8 (A) Mass chromatogram of a headspace sample above strawberries; (B) FTIR chromatogram of the same sample. For peak identities see **Table 1**. (By courtesy of the *Journal of High Resolution Chromatography* (1997), 20: 279.)

some minor peaks in the mass chromatogram giving a large FTIR response.

One detector specific to aroma/perfumery studies is the human nose. The effluent from the GC is split between a conventional detector such as the FID and a sniffing port which is purged with humidified nitrogen. Because the ability to recognize the presence of an odour varies considerably from one individual to another it is necessary to select a panel from people who have been shown to possess a keen sense of smell and to train them to recognize the odour of particular compounds. Although it is always stated how insensitive the human nose is compared to those of animals, nevertheless it is still a highly sensitive organ. It is possible, apparently, for trained panellists to indicate the emergence of an odoriferous compound from a GC column in parts of the chromatogram where no signal is obtained from conventional detectors. Under these circumstances the procedure is to use small scale preparative GC and to collect fractions at the points indicated by the panel; these fractions are then re-run under analytical GC conditions.

Conclusion

The study of aromas is intimately connected to the study of flavours, taints and perfumes in that they all make extensive use of GC with a variety of detectors,

of which the mass spectrometer is the most important. Advances in this type of work will depend on advances in the instrumentation, particularly in the sensitivity of the mass spectrometer and on general advances in knowledge of food components under various circumstances.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **III/Natural Products:** Liquid Chromatography. **Solid Phase Micro-Extraction:** Environmental Applications; Food Technology Applications. **Tobacco Volatiles: Gas Chromatography.**

Further Reading

There do not seem to be any modern texts which deal specifically with aroma analysis but the literature contains numerous references. The following is a partial list of papers published from 1997 to 1999 showing the wide variety of foodstuffs and drinks covered, ranging from wine, yogurt and tomato juice to strawberries and alligator meat. Although mass spectrometry is the main method of detection, other techniques are covered, together with a variety of methods for extraction prior to analysis.

Ahn DU, Jo C and Olson DG (1999) Volatility profiles of raw and cooked turkey thigh as affected by purge temperature and holding time before purge. *Journal of Food Science* 64(2): 230–233.

Baek HH and Cadwallader KR (1997) Aroma volatiles in cooked alligator meat. *Journal of Food Science* 62(2): 321–325.

Clarke RJ and Macrae R (1985) *Coffee*, Vol. 1 Chemistry. Amsterdam: Elsevier.

De la Calle-Garcia, Reichenbacher D, Danzer M *et al.* (1997) Investigations on wine bouquet components by solid-phase micro-extraction-capillary gas chromatography (SPME-CGC) using different fibres. *Journal of High Resolution Chromatography* 20(12): 665–668.

De la Calle-Garcia, Reichenbacher D, Danzer M *et al.* (1998) Analysis of wine bouquet components using headspace solid phase micro-extraction-capillary gas chromatography. *Journal of High Resolution Chromatography* 21(7): 373–377.

Gomes da Silva MDR and Chaves das Neves HJ (1997) Differentiation of strawberry varieties through purge-and-trap HRGC-MS, HRGC-FTIR and principal components analysis. *Journal of High Resolution Chromatography* 20(5): 275–283.

Guadayol JM, Caixach J, Ribe J *et al.* (1997) Extraction and identification of volatile organic compounds from paprika oleoresin (Spanish type). *Journal of Agriculture and Food Chemistry* 45(5): 1868–1872.

Kralj Cigic I and Zupancic-Krajl L (1999) Changes in odour of Bartlett pear brandy influenced by sunlight irradiation. *Chemosphere* 38(6): 1299–1303.

Morales, MT Berry AJ, McIntyre PS and Aparicio R (1998) Tentative analysis of virgin olive oil aroma by supercritical fluid extraction–high resolution gas chromatography–mass spectrometry. *Journal of Chromatography A* 819(1–2): 267–275.

Ott A, Fay LB and Chaintreau A (1997) Determination and origin of the aroma impact compounds of yogurt flavour. *Journal of Agriculture and Food Chemistry* 45(3): 850–858.

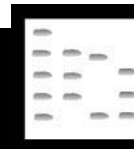
Pinnel V and Vandegans J (1997) Study of the aroma profile of gherkin by purge-and-trap followed by GC-MS. *Journal of High Resolution Chromatography* 20(6): 343–346.

Rapior S, Breheret S, Talou T and Bessiere J-M (1997) Volatile flavour constituents of fresh *Marasmius alleaceus* (garlic *Marasmius*). *Journal of Agricultural and Food Chemistry* 45(3): 820–825.

Sucan MK and Russell GF (1997) A novel system for purge and trap with thermal desorption: optimization using tomato juice volatile compounds. *Journal of High Resolution Chromatography* 20(6): 310–314.

Tarantilis PA and Polissiou MG (1997) Isolation and identification of the aroma components from saffron (*Crocus sativus*). *Journal of Agricultural and Food Chemistry* 45(2): 459–462.

FUELS AND LUBRICANTS: SUPERCRITICAL FLUID CHROMATOGRAPHY



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Supercritical fluid chromatography (SFC) has a number of advantages over gas chromatography (GC) and high performance liquid chromatography (HPLC) for mixtures such as polyaromatic hydrocarbons (PAH). SFC operates with diffusivities that are more gas-like, viscosities that are lower than liquids, and densities that are more liquid-like. The resulting mass transfer coefficients lead to more rapid analysis in SFC than in HPLC. The diffusion and viscosity range available in SFC allows GC-like separations on capillary columns but at much lower temperatures.