Rapid Identification of Volatile Compounds in Aromatic Plants by Automatic Thermal Desorption – GC-MS

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Key Words

Gas chromatography-mass spectrometry
Thermal desorption
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Summary

Thermal desorption is a valuable method for the fractionation of plant volatile components, which can be carried out on-line with GC analysis. The use of coupled GC-MS affords additional qualitative information, of special interest for plant species whose composition has not been previously studied. Some examples of the application of automatic thermal desorption coupled to GC-MS to the identification and characterization of volatile components of plants of different families are given.

Introduction

Many plants are currently being used in medicine, cosmetics and food. Since some of their properties are related with the presence of volatile constituents, improvement of the analytical methods used for determination of the volatile composition of plant samples is of great importance.

Volatile compounds are usually present in plants as very complex mixtures, and their qualitative and quantitative determination requires an analytical method of high resolving power. Gas chromatography (GC) is very frequently used. As the sample to be injected has to be free of non-volatile components, a fractionation step is necessary prior to gas chromatographic analysis.

This step can be carried out by several methods: liquid solvent extraction [1], supercritical fluid extraction (SFE) [2], steam distillation [2], simultaneous distillation-extraction (SDE) [3] and head space [4] or thermal desorption [5]. The first four methods involve extensive analytical manpower and are time consuming. The last method can be carried out automatically (automatic thermal desorption, ATD [6]) on-line with the gas chromatographic process, using a small (1–40 mg) sample.

A previous study [6] showed that, besides the advantages previously cited, the ATD-GC method gave better reproducibility and less artifact formation than SDE, which is usually recommended for the fractionation of plant volatiles.

In a survey of the composition of wild aromatic plants of Central Spain, more than a thousand samples of different plant species have been collected. Although the chromatographic profiles of their volatile constituents have been easily obtained by the use of ATD-GC, the qualitative information afforded by chromatographic retention was not enough in many cases to allow a positive identification of the main components. When mass spectrometry is coupled with gas chromatography (GC-MS), most components can be identified or characterized from their mass spectra.

In the present study, we report the analysis of several samples of plants belonging to different species by combined automatic thermal desorption-GC-MS and we compare the results with those obtained by the SDE method.

Experimental

Plant Samples

Plant samples were collected during 1992–1994 in the province of Madrid (Central Spain). In all cases a

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voucher specimen was deposited in the Real Jardín Botánico herbarium (Madrid). Samples were ground after drying at room temperature.

SDE

Samples – between 2 and 5 g, according to the estimated overall volatile content – were distilled using the micro simultaneous distillation-extraction apparatus (Chrompack, Middelburg, Netherlands) described in [3]. Pentane was used as solvent.

Thermal Desorption

Samples were desorbed using a commercial automatic thermal desorption system (ATD-400) (Perkin-Elmer, Norwalk, Conneticut). Instrumental details are described elsewhere [6]. The sample was placed into the desorption cartridge between two glass wool plugs and after adding internal standard was desorbed by heating the tube at 180 °C for 15 min. A flow of helium (50 mL min^{-1}) transferred the desorbed substances into a cold (–30 °C) trap, packed with Tenax TA (50 mg, 60–80 mesh, Perkin-Elmer), using a 75 mL min^{-1} inlet split. After the desorption period, the cold trap was rapidly heated (30° s^{-1}) to 320 °C (4 min) in order to inject the sample as a narrow band into the chromatographic column, using a 50 mL min^{-1} outlet split. Total split ratio was about 1:150. Sample weight depended on the estimated volatile content of the species analyzed, usually being within the 5–25 mg range.

GC-MS

GC-MS was carried out using different instrumental settings. The ATD-GC-MS configuration included the ATD-400 system previously described, connected to a Fisons GC-8000 series (Fisons, Milan, Italy) gas chromatograph coupled to a MD-800 mass detector (Fisons, VG Masslab, Manchester, England). The mass detector was used with an electron impact ion source (EI) at an interface temperature of 180 °C, scanning between 40–380 amu. A laboratory-made, fused-silica capillary column (20 m × 0.25 mm, column A) coated with methylsilicone OV-1 was kept at 70 °C for 10 min after injection and then temperature programmed to 220 °C at 4° min^{-1} and held at 220 °C for 10 min.

For GC-MS analysis of the SDE distillates, an HP-5890 GC was coupled to an HP-5971A mass detector (Hewlett-Packard, Palo Alto, CA, USA). A fused-silica capillary column (12 m × 0.22 mm I.D., column B) with OV-1 stationary phase, from Hewlett-Packard, was kept at 70 °C for 5 min and then programmed from 70 to 220 °C at 6 °C min^{-1}. Mass spectra were recorded in EI mode, at an interface temperature of 180 °C, with a 40–350 amu scan range.

A different ATD-GC-MS configuration was occasionally used, including an ATD-400 connected to an HP-5890 gas chromatograph coupled to an HP-5971A mass detector. A fused-silica capillary column (15 m × 0.22 mm I.D) with SPB-1 stationary phase, from Supelco (Supelco Inc., Supelco Park, Bellefonte, PA, USA), was kept at 70 °C for 5 min and then programmed from 70 to 180 °C at 4 °C min^{-1}. Helium was used in all configurations as carrier gas.

Methodology

All samples used in this study were obtained from a single individual plant. Between 2 and 5 g of a ground sample were used for the SDE extraction, while the ATD-GC-MS technique only required between 9 and 23 mg of the same sample, and similar or lower amounts were used in ATD-GC(FID) analysis.

Sample components were characterized from their retention times and mass spectra; when possible, they were tentatively identified by comparing their mass spectrum and chromatographic retention with published data [7–9], and, in some instances, with analytical data from standard compounds. Percent relative composition was directly calculated from reconstructed, total ion-current trace (TIC), peak areas. Total volatile yield was determined by analysing the same sample by ATD-GC(FID): 2-pentadecanone (0.5–2.5 mg, Lancaster, Eastgate, England), was added as internal standard in heptane solution to the plant sample in the desorption cartridge, and the total volatile amount was calculated from peak areas ratio.

Several blank runs were also carried out in the ATD mode to check the possibility of incomplete transfer of volatiles through the desorption system.

Results

The volatile fraction composition of samples of seven plant species, included in three botanical families: *Lamiaceae*, *Asteraceae* and *Scrophulariaceae*, was studied in order to evaluate the possibilities of the analytical method. The seven species differ in both botanic and aromatic characteristics, which suggests different volatile compositions.

The volatile compounds of plants of the *Lamiaceae* family are the most widely studied [10]. From this family, three species were analysed.

Teucrium scordium L. (water germander) is a typical species of wet places of Europe, which grows in most Spanish regions. Information about the volatile content of this species is scarce [11].

Figure 1a shows the reconstructed chromatogram of *T. scordium* L. volatiles obtained by ATD-GC-MS with column A, using the equipment and conditions above. Sample size was 19.4 mg: total volatile yield, calculated from 2-pentadecanone peak area, was 2.8 mg g^{-1} of dry plant. The chromatographic profile of an SDE extract, obtained by SDE followed by GC-MS with column B, is shown in Figure 1b.

Composition data obtained for both configurations appear in Table I. While the high and medium volatility

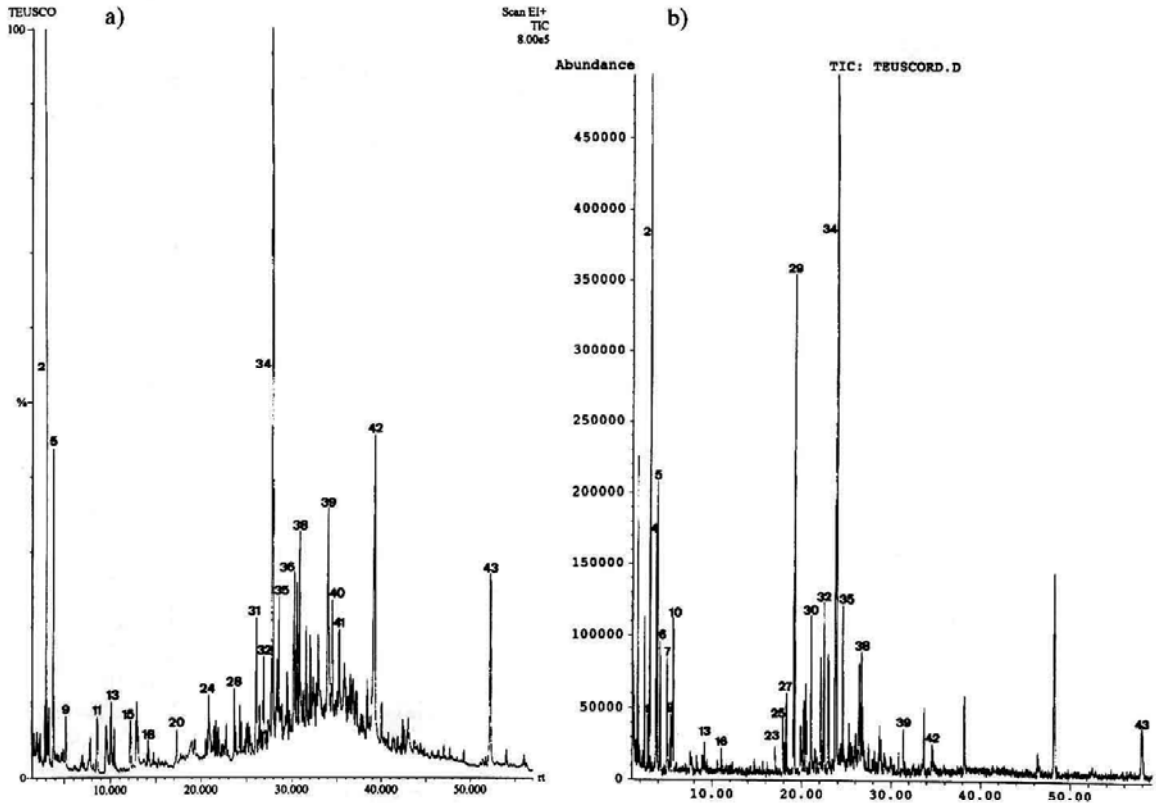

Figure 1
Total ion current profile of *Teucrium scordium* volatiles by ATD-GC-MS (a) and SDE (b). Peak number as Table I. See text for details and conditions.

Table I. Composition of volatile fraction of *Teucrium scordium* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	α-Thujene	0.43	0.63
2	α-Pinene	7.19	7.33
3	Sabinene + $C_{10}H_{14}$	0.88	–
4	1-Octen-3-ol	< 0.1	3.03
5	β-Pinene	2.85	3.79
6	3-Octanol	–	2.36
7	Phenylacetaldehyde	–	1.81
8	*p*-Cymene	0.29	–
9	Limonene	0.59	0.92
10	*trans*-β-Ocimene	0.06	2.36
11	α-Campholene aldehyde	0.31	0.36
12	*trans*-Verbenol	1.75	0.54
13	*cis*-Verbenol	1.79	0.90
14	Pinocarvone	0.89	0.41
15	Myrtenal	0.97	0.32
16	Verbenone	1.24	0.73
17	Myrtenol	1.05	< 0.1
18	Carveol	0.69	–
19	Carvone	0.16	–
20	Bornyl acetate	0.56	< 0.1
21	$C_{12}H_{20}O_2$	1.24	–
22	2-Hydroxycineole	0.65	–
23	α-Cubebene	0.38	0.64
24	Sobrerol	2.27	–
25	α-Copaene	0.55	1.00
26	β-Bourbonene	0.69	0.64
27	β-Cubebene	0.71	1.58
28	Bergamotene	0.76	–
29	*trans*-Caryophyllene	0.39	10.42
30	α-Curcumene	0.49	3.15
31	β-Bisabolene	2.26	2.18
32	α-Bisabolene	0.65	3.17
33	Spathulenol	1.62	2.48
34	Caryophyllene oxide	19.65	37.86
35	$C_{15}H_{24}O$	2.35	2.82
36	$C_{15}H_{24}O$	4.12	0.56
37	$C_{15}H_{24}O$	3.52	2.26
38	$C_{15}H_{24}O$	3.31	2.17
39	Not identified	8.07	0.84
40	Not identified	4.40	0.56
41	Not identified	3.37	0.45
42	Hexadecanoicacid	12.47	0.56
43	n-Pentacosane	4.41	1.15

[a] Peak number in Figures 1a and 1b.

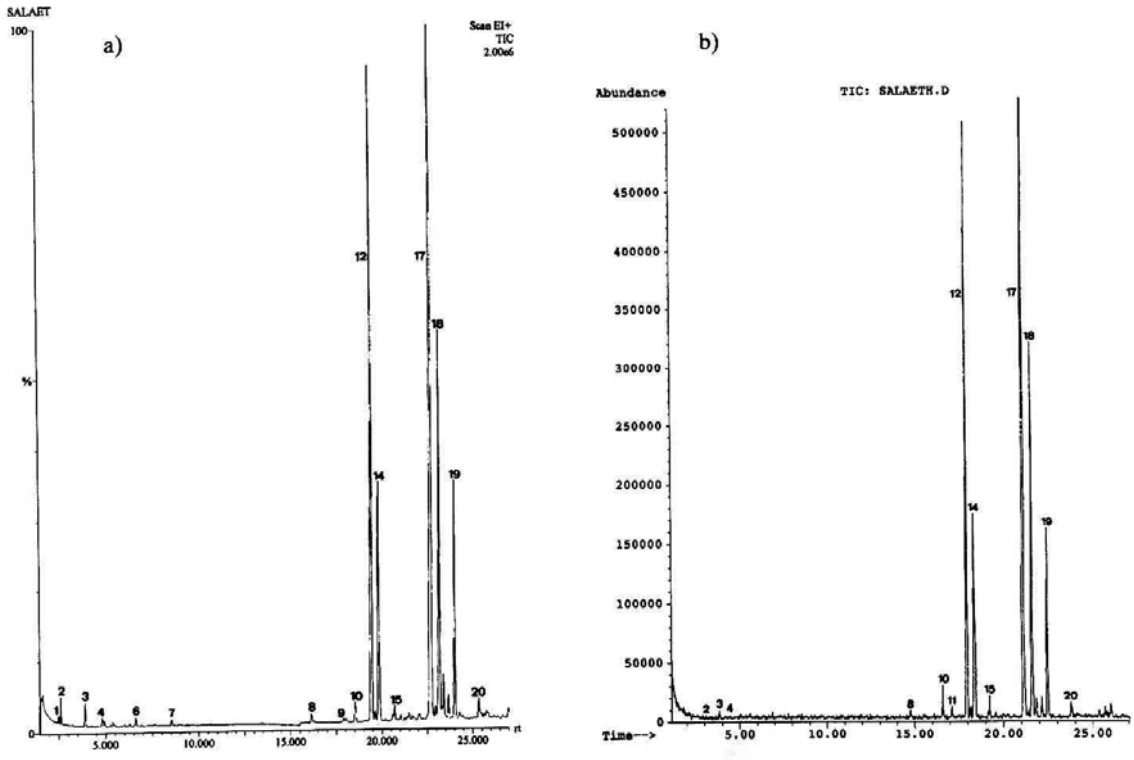

Figure 2
Total ion current profile of *Salvia aethiopis* volatiles by ATD-GC-MS (a) and SDE (b). Peak number as Table II. See text for details and conditions.

Table II. Composition of volatile fraction of *Salvia aethiopis* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	α-Thujene	0.12	–
2	α-Pinene	0.46	< 0.1
3	β-Pinene	0.52	< 0.1
4	β-Phellandrene	0.18	< 0.1
5	*p*-Cymene	0.12	–
6	Fenchone	0.20	–
7	Camphor	0.21	–
8	*p*-Vinyl-guaiacol	0.20	0.54
9	Bicycloelemene	0.15	0.19
10	α-Humulene	0.73	1.05
11	α-Cubebene	0.07	0.37
12	α-Copaene	23.19	22.52
13	β-Bourbonene	0.28	0.38
14	β-Cubebene + β-Elemene	8.46	10.15
15	Caryophyllene	0.38	1.00
16	γ-Muurolene	0.17	0.37
17	Germacrene D	37.87	40.81
18	Germacrene B	13.13	14.54
19	δ-Cadinene	7.64	7.57
20	Spathulenol	0.77	0.51

[a] Peak number in Figures 2a and 2b.

components show similar profiles, compounds which appear in the last part of the chromatogram (including some fatty acids) are present in higher relative amounts in the ATD trace. In both cases, the main component found was caryophyllene oxide. *trans*-Caryophyllene, an important component in the SDE trace, gives a very small peak in the ATD profile.

Salvia aethiopis L., also from the *Lamiaceae* family, is an aromatic plant with a woolly indumentum and a distinctive candelabrum shape. It grows in the Northern Mediterranean region and in Western Asia, and has been used as antihaemorragic in popular medicine. Although several diterpenes had been previously isolated in this plant [12, 13], its volatiles composition had not been previously studied.

Figures 2a and 2b show the TIC trace of *Salvia aethiopis* volatile compounds, obtained respectively with ATD-GC-MS (column A) and SDE-GC-MS (column B) using the conditions described under Experimental. Both profiles are very similar: in table II are shown the quantitative results found using the ATD and SDE methods. Germacrene D, germacrene B, α-copaene and δ-cadinene are identified by the two techniques as the main components. The sample size for the ATD-GC-MS

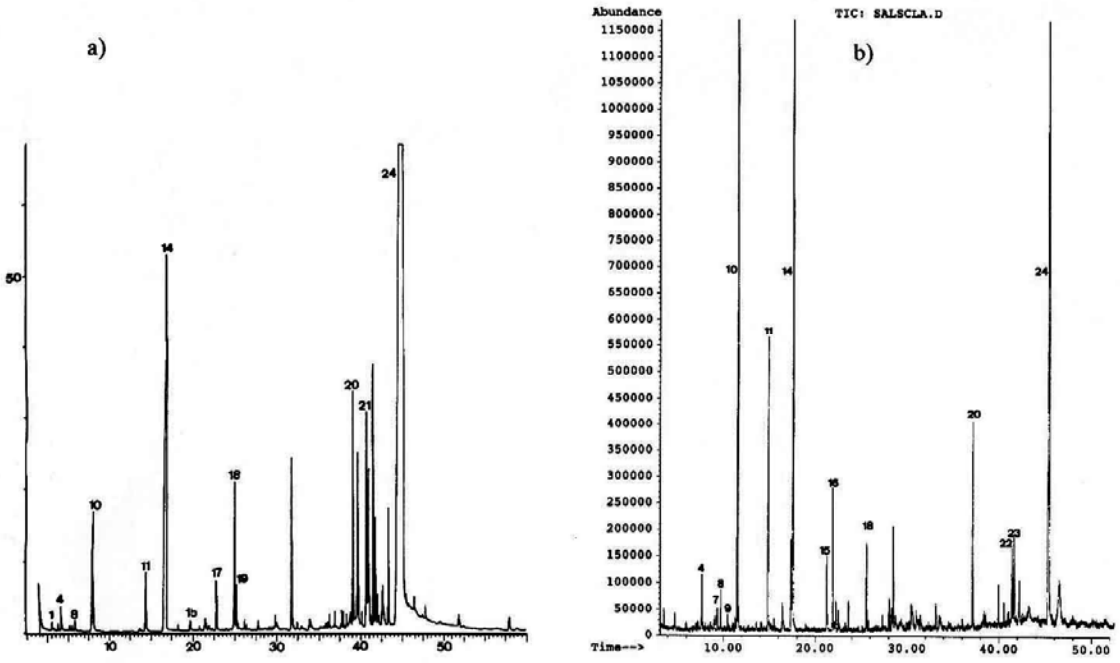

Figure 3
Total ion current profile of *Salvia sclarea* volatile components obtained by ATD-GC-MS (a) and SDE (b). Peak number as Table III. See text for details and conditions.

Table III. Composition of volatile fraction of *Salvia sclarea* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	α-Pinene	0.14	0.17
2	Camphene	0.12	0.17
3	β-Pinene	0.12	0.35
4	β-Myrcene	0.23	1.15
5	α-Phellandrene	0.11	0.35
6	3-Carene	0.11	0.70
7	*cis*-Ocimene	0.12	0.78
8	*cis*-Linalool oxide(furan)	0.14	0.87
9	*trans*-Linalool oxide(furan)	0.10	0.52
10	Linalool	3.92	16.88
11	Linalyl formate	0.99	6.46
12	Nerol	0.02	1.13
13	Geraniol	0.02	2.52
14	Linalyl acetate	15.16	25.02
15	Neryl acetate	0.07	1.62
16	Geranyl acetate	0.07	3.09
17	*trans*-Caryophyllene	0.43	1.04
18	Germacrene D	0.97	2.28
19	Valencene	0.21	0.35
20	8,13-Epoxy-15,16-dinorlab-12-ene	0.96	6.80
21	$C_{20}H_{34}O$	0.61	1.83
22	$C_{20}H_{32}$	0.39	2.49
23	$C_{20}H_{32}$	0.31	2.92
24	Sclareol	74.16	20.50

[a] Peak number in Figures 3a and 3b.

chromatographic run was 23.6 mg, and the total volatile yield, calculated from 2-pentadecanone peak area, was 3.1 mg g^{-1} of dry plant. The analysis of other samples of *Salvia aethiopis* showed that this species has a highly variable volatiles composition.

From the same genus, *Salvia sclarea* L. (clary sage) grows in all Mediterranean regions and in Western and Central Asia. Its essential oil is used in the cosmetic industry.

Figures 3a and 3b show the chromatographic trace of *Salvia sclarea* volatiles, using respectively the ATD (column A) and SDE (column B) methods. Table III presents quantitative information for the 24 characterized compounds. When using the ATD method the main component found was sclareol (74.1 %), which has long since been recognized as an important component of this plant [14]: however, the SDE method yields only 20.5 % for this compound. In previous studies, sclareol was found to be the main component of *S. sclarea* when solvent extraction was used: when the volatile compounds were obtained by steam distillation the sclareol content dropped to 0.35 %, while the main components found were linalool and its derivatives. [15–18]

The sample size used in the ATD-GC-MS analysis (Figure 3a) was 9.0 mg; and the total yield, calculated from 2-pentadecanone peak area, was 32.2 mg g^{-1} dry plant.

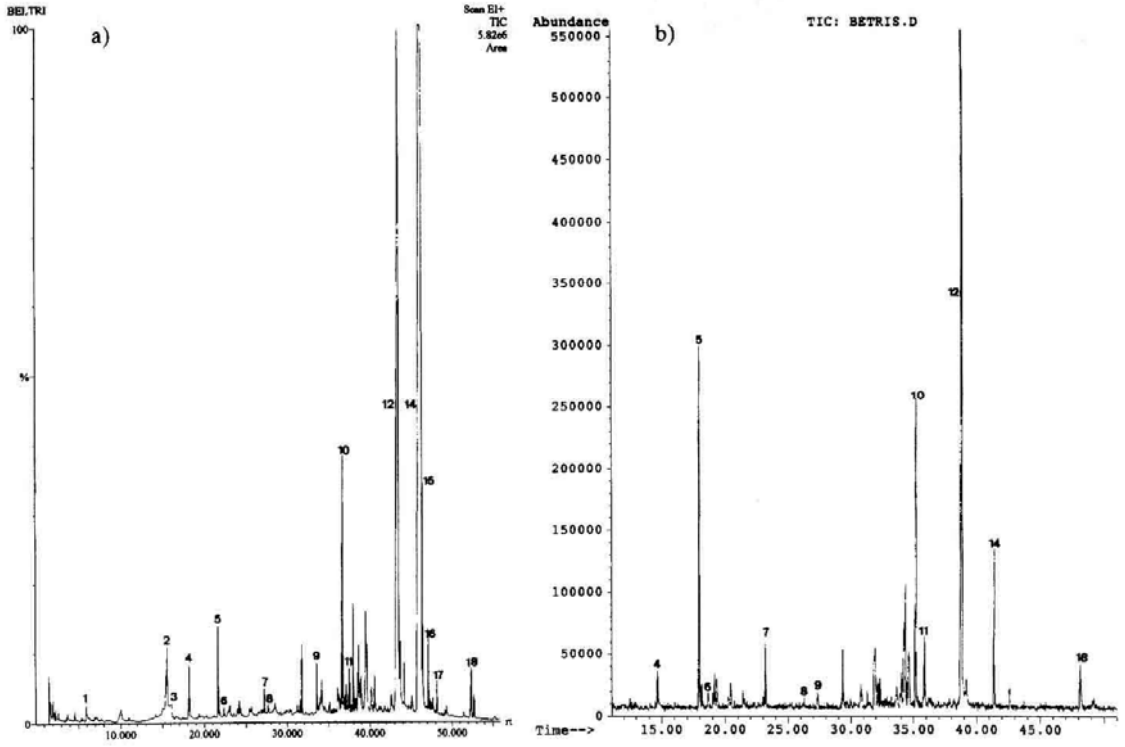

Figure 4
Total ion current profile of *Bellardia trixago* volatile components obtained by ATD-GC-MS (a) and SDE (b). Peak number as Table IV. See text for details and conditions.

Table IV. Composition of volatile fraction of *Bellardia trixago* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	Methyl benzoate	0.08	–
2	$C_{10}H_{14}O$	2.41	–
3	Benzoic acid	0.35	–
4	p-Vinyl-guaiacol	1.31	1.38
5	3,4-Dihydro-γ-ionone	1.40	14.12
6	α-Ambrinol	0.10	0.80
7	Caryophyllene oxide	0.23	2.45
8	Isomer α-Ambrinol	0.08	0.53
9	Not identified	0.96	2.64
10	Trixagoene	4.22	11.84
11	Trixagoene isomer	1.50	3.82
12	Trixagol	25.47	51.84
13	Isotrixagol	1.27	3.01
14	Trixagoyl acetate	56.17	5.99
15	Isotrixagoyl acetate	2.13	< 0.1
16	Trixagoyl acetate isomer	0.71	0.78
17	n-Tricosane	0.61	< 0.1
18	n-Pentacosane	1.00	0.78

[a] Peak number in Figures 4a and 4b.

Bellardia trixago (L.) All. belongs to the *Scrophulariaceae* family. It is an annual species which grows in stony or grassy places in Southern Europe; its aroma has been described as honey-like. Samples of this plant collected in Spain had been previously studied [19–22].

The chromatographic trace of *B. trixago* obtained with ATD and SDE techniques is shown in Figures 4a and 4b respectively. Quantitative results, shown in Table IV for both techniques, agree with published data: compounds identified possess structures related to that of trixagol, the ATD method providing higher relative values for the acetate derivatives. Sample size for the chromatographic run of Figure 4a was 20.6 mg; total volatiles yield, calculated from 2-pentadecanone peak area, was 13.0 mg g^{-1} dry plant.

Matricaria matricarioides Porter ex Britton (pineapple weed), from the *Asteraceae* family, is a wild chamomile probably of North American origin, which grows in Europe in roadsides and disturbed grounds. In Spain it is frequent in the North and in mountain areas. In an analysis of its essential oil [23] the main components

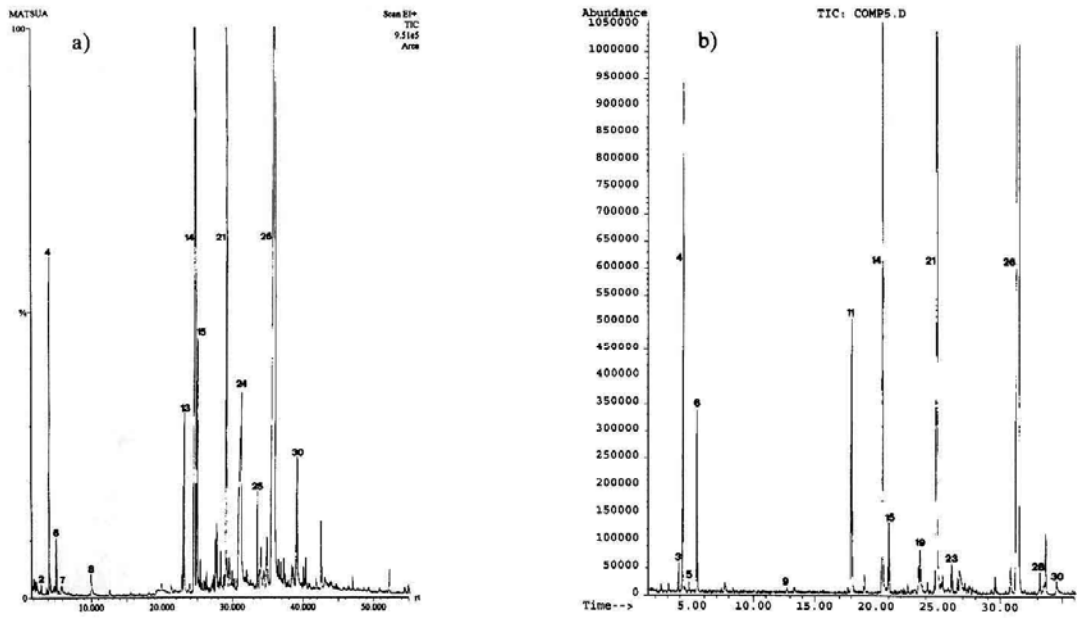

Figure 5
Total ion current profile of *Matricaria matricarioides* volatile components obtained by ATD-GC-MS (a) and SDE (b). Peak number as Table V. See text for details and conditions.

Table V. Composition of volatile fraction of *Matricaria matricarioides* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	α-Thujene	0.02	< 0.1
2	α-Pinene	0.10	0.07
3	β-Pinene	0.07	0.27
4	β-Myrcene	2.98	4.68
5	n-Decane	0.14	0.12
6	Limonene	0.67	1.97
7	Artemisia alcohol	0.07	–
8	1-Acetyl-pyrrolidine	0.61	–
9	Nerol	0.05	0.12
10	Geraniol	0.04	0.09
11	Methyleugenol	–	3.43
12	$C_{15}H_{24}$	0.29	< 0.1
13	2-Methyl-1-naphtalenol	2.75	–
14	β-Farnesene	22.73	8.12
15	Bicyclosesquiphellandrene	6.14	0.97
16	Germacrene B	0.42	< 0.1
17	δ-Cadinene	0.23	–
18	α-Bisabolol	0.87	< 0.1
19	Geranyl isopentanoate	0.82	0.76
20	Neryl pentanoate	0.22	0.10
21	Geranyl pentanoate	8.71	17.38
22	Not identified	0.60	0.27
23	$C_{15}H_{24}O$	0.37	0.44
24	Ayapanin(7-Metoxicoumarin)	8.91	0.72
25	Not identified	0.50	–
26	*cis*-Dicycloether MW 200	37.18	59.83
27	*trans*-Dicycloether MW 200	1.04	< 0.1
28	*cis*-Dicycloether MW 214	0.28	0.39
29	*trans*-Dicycloether MW 214	0.17	< 0.1
30	Hexadecanoic acid	3.04	0.27

[a] Peak number in Figures 5a and 5b.

found were *trans*-β-farnesene and germacrene D. However, the study of the plant extract [24] afforded 7-methoxi-coumarin (ayapanin) and a labile sesquiterpene hydrocarbon.

Figures 5a and 5b show the TIC trace of *M. matricarioides* with ATD (column A) and SDE (column B) techniques. The main volatile components were found to be a *cis*-dicycloether, β-farnesene, geranyl pentanoate and, in the ATD run, ayapanin: quantitative results are reported in table V. Sample size for the chromatographic run of Figure 5a was 12.4 mg: total volatiles yield, calculated from the 2-pentadecanone peak area, was 18.6 mg g^{-1} dry plant.

Helichrysum italicum subsp. *serotinum* (Boiss.) P. Fourn, also from the *Asteraceae* family, grows only in dry places of South Western Europe, being extensively used in popular medicine. Studies on its volatile composition have been previously reviewed [25]: α-pinene, camphene, β-pinene, neryl acetate, nerol, flavonoids and four C-10-β-diketones appeared to be the most important constituents. More recently [26], 3(*iso*penten-2-yl) acetophenone has been described as the major phenolic constituent in *H. italicum* extracts: this compound had previously been found in the aerial parts of *H. argyrophyllum* [27] and in other *Helichrysum* species [28].

Figure 6 shows the TIC profile of *H. italicum*, obtained by ATD-GC-MS using column A and conditions described under Experimental. The qualitative and quantitative data obtained are listed in table VI. The main compound determined by ATD-GC-MS is 3(*iso*penten-2-yl) acetophenone, present at 65.2 %,

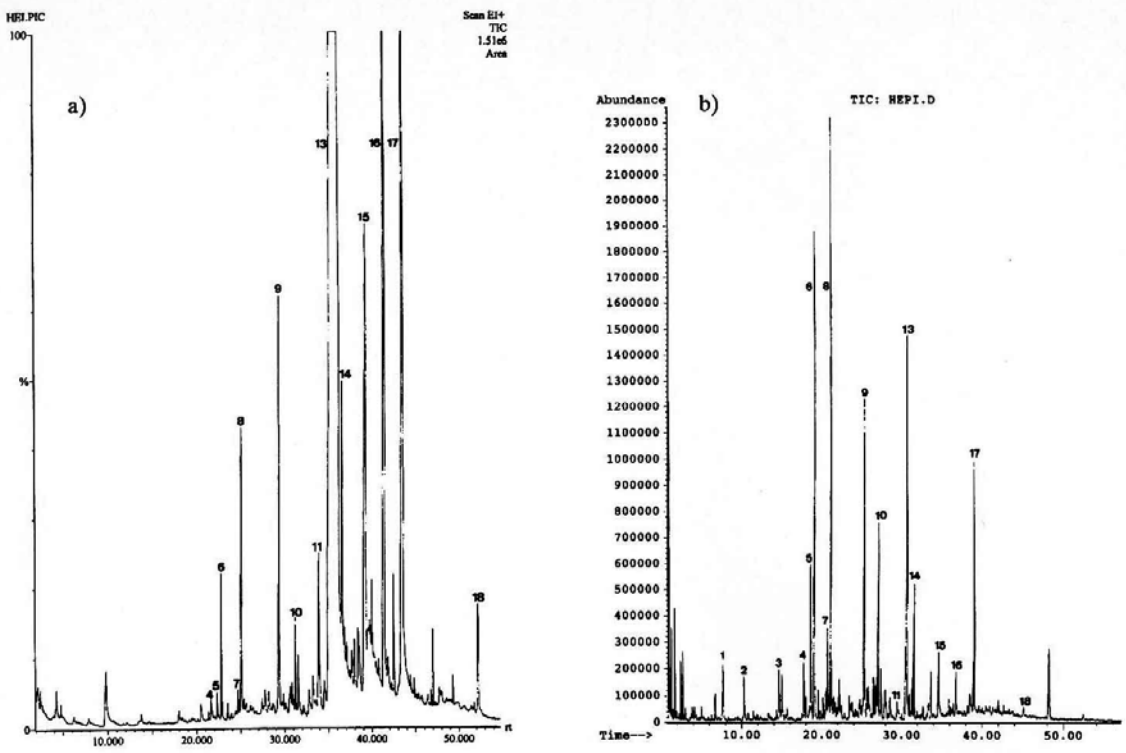

Figure 6
Total ion current profile of *Helichrysum italicum* subsp. *serotinum* volatile components by ATD-GC-MS (a) and SDE (b). Peak number as Table VI. See text for details and conditions.

Table VI. Composition of volatile fraction of *Helichrysum italicum* subsp. *serotinum* by SDE and ATD.

Peak no.[a]	Compound	ATD (%)	SDE (%)
1	Linalool	–	1.92
2	α-Citronellol	–	1.43
3	Not identified	–	1.40
4	α-Cedrene	0.17	1.50
5	β-Cedrene	0.14	4.50
6	Caryophyllene	0.58	15.45
7	$C_8H_{14}O_2$	0.14	3.33
8	α-Bergamotene	1.55	16.82
9	1-(4-Cyclohexylphenyl)etanone	2.47	11.59
10	Monoterpeneacetate	0.57	5.67
11	Tetradecanoicacid	1.14	0.70
12	Not identified	0.22	0.45
13	3(*iso*Penten-2-yl)acetophenone	65.24	18.89
14	$C_{17}H_{24}O_2$	1.85	3.82
15	Hexadecanoicacid	5.65	2.45
16	$C_{17}H_{24}O_2$	8.22	1.56
17	$C_{18}H_{26}O_2$	10.94	8.46
18	Phytol	1.13	0.05

[a] Peak number in Figures 6a and 6b.

while the concentration for the same compound was found to be, using the SDE-GC-MS method (Figure 6b), only 18.9 %. The sample size for the chromatographic run was 10.2 mg; the total volatile yield, calculated from 2-pentadecanone peak area, was 14.9 mg g^{-1} dry plant.

Asteriscus aquaticus (L.) Less also belongs to the *Asteraceae* family. It grows in poorly drained grounds in the Mediterranean area, and has a very pleasant smell. This plant is characterized by the presence of lactones with the humulane skeleton (asteriscunolides and asteriscanolides, [29–32]).

In Figure 7 appears the TIC trace obtained from ATD-GC-MS with column A using conditions described under Experimental. Most peaks in the last part of the chromatogram seem to correspond to asteriscunolides, asteriscanolides or related compounds. Some of them were tentatively identified by comparison with published mass spectra: the quantitative composition is given in Table VII. Sample size was 22.2 mg; total volatile yield, calculated from 2-pentadecanone peak area, was 24.0 mg g^{-1} dry plant.

Figure 7
Total ion current profile of *Asteriscus aquaticus* volatile components by ATD-GC-MS. Peak number as Table VII. See text for details and conditions.

Table VII. Composition of volatile fraction of *Asteriscus aquaticus* by ATD/GC/MS.

Peak no.[a]	Compound	ATD (%)
1	α-Thujene	0.04
2	α-Pinene	1.34
3	Sabinene	0.10
4	β-Pinene	0.74
5	β-Myrcene	0.23
6	α-Terpinene+Limonene	0.50
7	γ-Terpinene	0.03
8	Sylvestrene	0.07
9	*trans*-Pinocarveol	0.07
10	Not identified	0.07
11	Not identified	0.39
12	α-Humulene	0.16
13	α-Amorphene	0.51
14	α-Muurolene	0.34
15	Cadina-1,4-diene	0.16
16	Nerolidol	2.24
17	$C_{15}H_{24}$	7.23
18	Asteriscunolide mixture	16.95
19	Not identified	0.81
20	$C_{15}H_{18}O_3$	6.19
21	$C_{15}H_{18}O_3$	2.90
22	$C_{15}H_{18}O_3$	6.08
23	Asteriscunolide+Asteriscanolide	26.93
24	Asteriscanolide	5.25
25	Asteriscunolide A+Asteriscanolide	11.37
26	Asteriscunolide D	7.70
27	Phytol	0.87
28	$C_{20}H_{30}O_2$	0.74

[a] Peak number in Figure 7.

Discussion

The main advantages which ATD presents in the analysis of plant volatiles by gas chromatography are the elimination of the extraction or fractionation step and the small amount of sample required. Quantitative aspects of the ATD technique have been addressed in previous work: in spite of possible sampling errors caused by the small sample size, the average coefficient of variation for a plant with a complex volatile composition is only 5.1 % [6]. The recovery of plant components was checked by desorbing the cartridge twice, and depends strongly on both operating conditions and compounds volatility. Using the ATD instrumental parameters described under Experimental, compounds with MW lower than 270 are completely recovered while those with MW between 280–330 remain partially in the desorption cartridge and compounds of higher MW hardly desorb at all. The recovery when using SDE is lower, specially for low-volatility polar compounds [6].

From our results with two different instrumental settings, the ATD advantages are also held by on-line coupling ATD-GC-MS: the mass spectra of the volatile components of a sample can be recorded in one hour, from 1–40 mg dry plant.

The determination of common volatile compounds in well known species can usually be carried out from chromatographic data, although mass spectra should be used to confirm identification, as in *Salvia sclarea*: also, (as in *Matricaria matricarioides*), the presence in a given sample of "unexpected" components cannot be ruled out. *Teucrium scordium* and *Salvia aethiopis* are species whose volatile composition had not been previously studied, but their components are simple mono and sesquiterpenes whose structure has now been determined, using ATD-GC-MS, from their chromatographic retention and mass spectra.

Other species, such as *Asteriscus aquaticus*, *Bellardia trixago* and *Helichrysum italicum* give volatile constituents of a less common structure: mass spectral library data is insufficient to identify these compounds. However, when previous studies on these species include mass spectral data for the most important components isolated, the information can be enough to allow their structural determination by ATD-GC-MS, although in most cases (see for instance compounds 8, 11 and 16 in *Bellardia trixago*, or the asteriscanolide and asteriscunolide isomers in *Asteriscus aquaticus*) differentiation between isomers is very difficult when standard compounds are not available.

When the composition of the species studied is not well known, as in *Teucrium scordium* and *Salvia aethiopis*, mass spectral data can be insufficient to allow identifica-

tion of all constituents. However, they can provide useful information about their structure: for instance, although compounds 35–38 in *T. scordium* (Table I) cannot be identified, their MWs can be deduced from mass spectral data. Retention data and mass spectra characterize almost unequivocally each component and can be used to detect its presence in other plants or to compare it with available standards.

The use of heat in the fractionation of volatile components of a plant always implies the possibility of artifact formation, whose extent depends on the chemical structure of the compounds present in the plant: also, compounds of too low or too high volatility are incompletely or not at all recovered. The ATD operating conditions listed in Experimental were chosen in order to minimize thermal decomposition, while maintaining good quantitative desorption of volatile components [6], but they can be modified according to special sample requirements. When comparing ATD and SDE quantitative results, some species afforded similar results (see for instance *S. aethiopis* (Figure 2) and *M. matricarioides* (Figure 5)) while other presented clearly differentiated profiles (*T. scordium* in Figure 1 and *B. trixago* in Figure 4).

The largest differences between ATD and SDE profiles correspond to species containing thermally labile volatile components. The concentration of sclareol in *Salvia sclarea* depends clearly on the technique used. The distillation required in the SDE method decomposes sclareol partially, reducing its observed concentration. The same thermal decomposition was observed in *B. trixago* and in *H. italicum* with trixagol acetate and 3(*iso*penten-2-yl) acetophenone, respectively. A similar result has been described for labile compounds in *Chamaecyparis lawsoniana* [6]. Although thermal desorption uses a relatively high temperature to desorb sample volatiles, its effect on labile compounds seems to be smaller than in distillation.

The low artifact formation, the small sample amount required, the easy sample pretreatment and the possibility of on-line coupling with GC-MS are the main advantages of thermal desorption in the qualitative analysis of plant volatiles.

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