CHAPTER 6

SFCXGC: EXPERIMENTAL

6.1 Introduction

Complex samples require analytical methods of high resolving power to provide reliable analysis of the sample components. This cannot be achieved in a single separation step. Sample pre-separation or clean-up is required for more complex mixtures and the use of successive chromatographic methods may be necessary. The advantages of multidimensional coupled chromatographic techniques have been demonstrated in a variety of studies[1,2]. It is now not uncommon to find comprehensive multidimensional techniques such as LCxLC[3], GCxGC[4], SFCxGC[5] and fast GC time-of-flight mass spectrometry (GC-TOF-MS)[6].

Essential oils are too complex for direct analysis on a single separation method such as capillary GC. This chapter aims to show that SFC and resistively heated GC can provide comprehensive two-dimensional analysis when they are coupled together. As shown in the previous chapter, group separation of essential oil samples by SFC-FID already provides useful information on the relative percentages of terpene hydrocarbons, esters, ketones, aldehydes and alcohols. To increase the amount of data that can be obtained from essential oils, additional analysis of the SFC groups is necessary.

6.2 Instrumentation for SFCxGC

The analytical system[7] consist of a Lee Scientific (Salt Lake City, Utah, USA) model 501 SFC pump to deliver supercritical fluid CO_2 without He head pressure (SFC grade, Air Products, Sandown, South Africa) to a Chrompack silica-gel porous layer open-tubular (PLOT) column. The SFC is coupled to a Varian 3300 gas chromatograph (Varian Instrument Corporation), modified for resistive heating of a 1 m stainless steel capillary column, equipped with two FID's and a flow modulator. The SFC column exit was connected to a six-port valve (Vici, CW 6-K, Valco) flow modulator to cut small consecutive sections from the first dimension separation for transfer to the second dimension.

Fixed restrictors prepared in the laboratory[8] were used to maintain supercritical fluid pressure conditions throughout the column and the six port valve. Two integral restrictors were connected to the outlet of the flow modulator by means of a tee-junction splitter (Valco PN: ZT1C, Valco, Switzerland), one for SFC separated group quantitation directly by FID and the other to allow on-line collection of separated groups into the fast GC split/splitless injector by means of pressure drop focussing[7]. Pressure drop focussing occurs when the CO_2 density drops at the restrictor to focus the sample fraction on the head of the second column due to loss of solvation power of the CO_2 mobile phase.

A Pye-Unicam GCD gas chromatography oven maintained the isothermal SFC column temperature condition. On the Varian 3300, both FIDs for SFC and fast GC analysis were kept at 280 ^{o}C and the GC injector was kept at 250 ^{o}C. The resistively heated column was ramped from -50 ^{o}C to 300 ^{o}C at the rate of 450 ^{o}C/min (7.5 ^{o}C.s^{-1}). Liquid CO_2 (Afrox, JHB, South Africa) was used to cool the Varian GC oven temperature down to -50 ^{o}C. A tightly coiled (1 m x 0.25 mm x 0.25 μm) SE-30 stainless-steel column (Quadrex Corporation SS Ultra Alloy) was used for GC analysis. A 30 Volt power supply was used to resistively heat the GC column[7].

A thermocouple constructed from type K thermocouple wire having a diameter of 0.025 mm (25 µm) (Goodfellow, Cambridge GB) was glued to the exterior of the column with a drop of polyimide resin (Aldrich) to measure the column temperature. The temperature was controlled by Proportional Integral Derivative (PID) feedback on the thermocouple signal using a program written in LabView (version 5.1.1) Software[7].

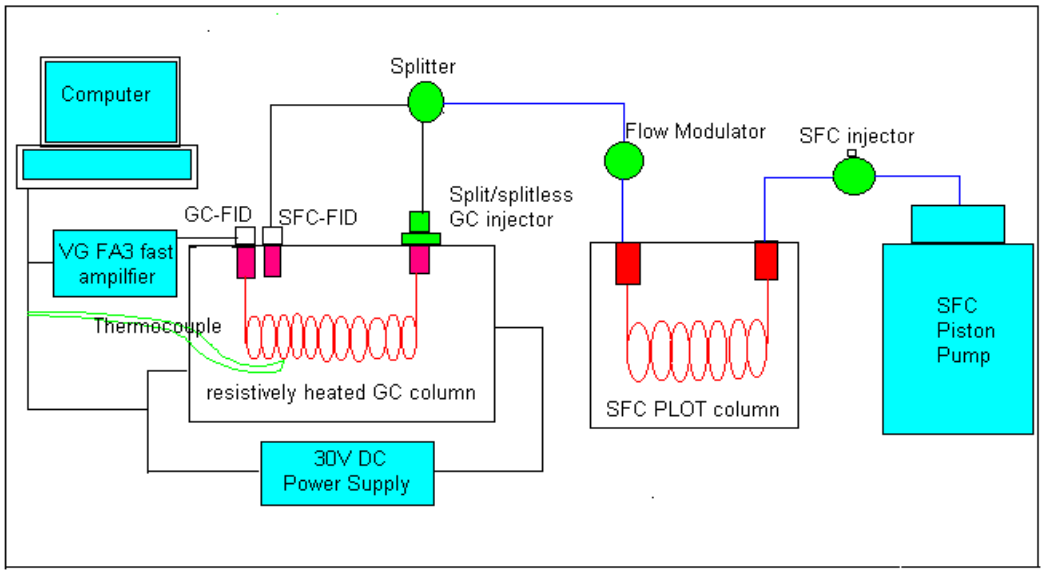

Figure 6.1. A schematic diagram of SFCxGC instrument.

6.2.1 Data Acquisition and Interpretation

All data were acquired using Chromperfect (version 3.7.4.0) Software package (Justice Innovations, California, USA). Short sections of 5 s duration were repeatedly collected from the SFC for the entire duration of the SFC run. Peak widths in the second dimension (resistively heated GC) are typically 0.5 s. Data collection rate (A/D converter) was at frequency of 100 Hz from a fast acting electrometer obtained from an old VG mass spectrometer[7]. Each GC run was recorded as a separate chromatogram. After the SFCxGC run was completed, data from the different chromatograms were compiled into a single text matrix file by a program written with LabView software (National Instruments, Texas, USA). Each 5 second cut from SFC (sum of peaks of one fast GC) constituted a data point that could be used to reconstruct the SFC

chromatogram. For visualisation, the matrix file was then imported into Transform 2D (Version 3.4, Fortner Research LLC, Sterling, VA, USA) software. Chromatograms were plotted by the use of Transform (Research Systems, Noeys Version V2.0) and MATLAB Version 6.0.0.88 (Mathworks Inc., USA) software packages[7].

6.3 Experimental

6.3.1 SFCxGC analysis of essential oil standard

6.3.1.1 Method and Conditions

A standard mixture containing 0.2 µL each of selected essential oil components was prepared in 500 µL CS_2 solvent and injected into an optimized SFCxGC for analysis.

Table 6.1. Standard prepared for essential oils analysis.

Terpene Hydrocarbons	Esters	Ketones	Aldehydes	Alcohols
Monoterpenes				
α-Pinene	Linalyl acetate	Methone	Citral	Geraniol
p-Cymene		Carvone	Citronellal	Linalool
Phellandrene		Camphor		4-terpineol
Limonene				
Sesquiterpenes				
Chamazuelene				

Chromatographic method and conditions

SFC conditions

Column temperature 28°C, column flow rate 480 ml/min (7.7 cm/sec), pump pressure programming (110-200 atm at 1 atm/min)

Fast GC

Column temperature ramp (-50 °C to 300 °C) at 450°C/min, H_2 carrier gas flow rate 100 $cm.s^{-1}$.

Modulation

Cooling of the oven to –50 ^{0}C, SFC fraction collection time 5 seconds, equilibration time 5 seconds, and followed by 50 s SFC stop flow condition (during which the GC is recorded).

6.3.1.2 Results and Discussion

Figure 6.2 shows a typical polarity *vs* volatility SFCxGC chromatogram of the essential oil standard. On a two-dimensional plane, each compound forms a contour peak defined by the retention times of the two independent columns. The horizontal axis represents the retention on the polarity separation (SFC) and the vertical axis represents retention on the volatility analysis (GC). The chemical class separation of the compounds is obtained with the non-polar compounds (terpene hydrocarbons) eluting first on the first dimension axis due to their weak retention on the silica gel PLOT column phase, followed by the more polar oxygenated compounds. The oxygenated compounds are further separated into co-eluting esters and ketones followed by co-eluting aldehydes and alcohols. Terpene hydrocarbon compounds that elute together in the SFC dimension are separated into individual compounds in the second dimension based on their volatility. This group is thus separated into the closely spaced monoterpenes (C10 hydrocarbons), and the sesquiterpene (C15 hydrocarbons) eluting much later.

The first dimension axis, the SFC chromatogram, was developed in the conventional manner with pressure programming of the CO_2 mobile phase from 110 atm to 200 atm at 1 atm/min and a constant temperature of 28 ^{o}C. About 160 secondary chromatograms were generated continuously at fixed 5 seconds collection time intervals by flow modulator from the first column.

Since the separation mechanisms as well as the physical state of the mobile phases are different in each dimension, compounds that are not separated on the first column are

likely to be separated on the second column. The width of the peaks associated with the later eluting 1st dimension compounds 11-14 can be compared to the SFC chromatogram in figure 5.14. The "patched" appearance of peaks from single compounds should not be confused with multiple peaks and result from imperfect reproducibility of the fast, temperature programmed GC runs. The most important feature in the chromatogram of figure 6.2 is the orthogonal separation achieved by using the two different separation mechanisms. Table 6.2 shows the names of the essential oil standard components.

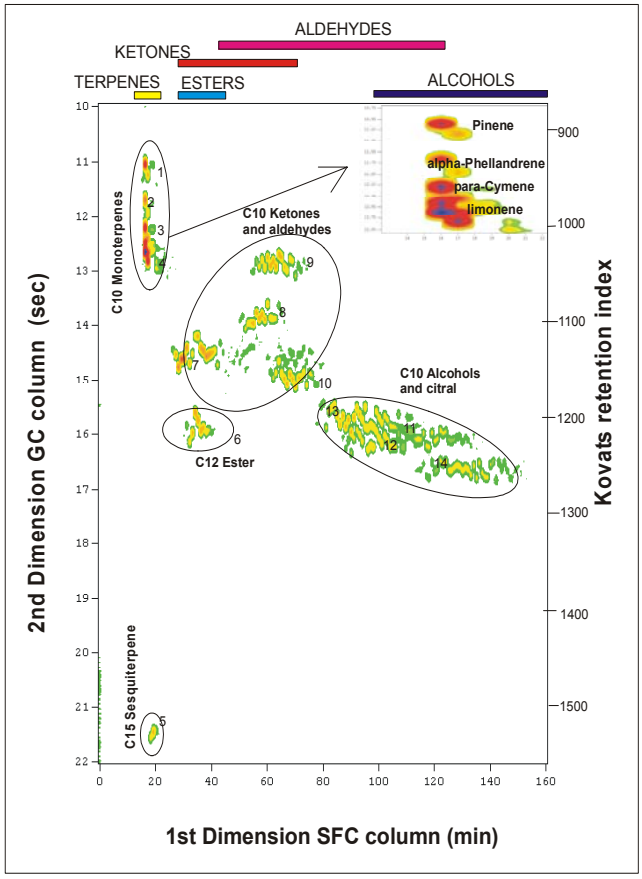

Figure 6.2. SFCxGC chromatograms of essential oil standard analysis. SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature =28 °C, Modulation: 5 s collection time and equilibration time = 5 s, Fast GC ramped -50 to 300 °C at 450 °C/min. Scale for Kovats index (1.19 sec = 100 units) calculated from peak 1 (Pinene, KI = 942) and peak 14 (Geraniol, KI =1234)

Table 6.2. Composition, chemical class, molecular weight, and retention data[14] for essential oil standard (Peak numbers refer to identified peaks in figure 6.2).

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determined, or "prog"if the index was determined using temperature programming[14]

Peak No.	Compound	Chemical class	MW	I[14]	T[14]
1	α-Pinene	C10 Monoterpene	136	942	100
2	α̃Phellandrene	C10 Monoterpene	136	1007	110
3	p-Cymene	C10 Monoterpene	136	1016	100
4	Limonene	C10 Monoterpene	136	1025	100
5	Chamazuelene	C15 Sesquiterpene	186		
6	Linalyl acetate	C12 Ester	196	1240	130
7	Menthone	C10 Ketone	154	1158	130
8	Camphor	C10 Ketone	154	1126	110
9	Citronellal	C10 Aldehyde	154	1017	Prog
10	Carvone	C10 Ketone	154	1228	Prog
11	Linalool	C10 Alcohol	154	1097	135
12	Terpineol	C10 Alcohol	154	1129	135
13	Citral	C10 Aldehyde	152		
14	Geraniol	C10 Alcohol	154	1234	175

Reference:[14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*

6.3.2 SFCxGC analysis of essential oil samples

6.3.2.1 Method and conditions

Real essential oil samples of *Cymbopogon citratus and C. flexuosus*, *Artemisia afra*, *Pelargonium radens X capitatum*, and *Tagetes minuta* oil were obtained from Dept. of Agricultural Conservation and Environment (Lowveld Agricultural College, Nelspruit) sourced from different farms. A 0.2 μL of each essential oil sample was injected, undiluted into an optimized SFCxGC system for group-type and individual compound separations. The same chromatographic conditions outlined in section *6.3.1.1* were used and the qualitative results of different oils were compared.

6.3.2.2 Results and conditions

Lists of possible compounds occurring in the essential oils studied were obtained prior to SFCxGC analysis. Tables A1-A5 in Appendix A list all the compounds identified in *Cymbopogon flexuosus*[9] *and citratus*[10], *Tagetes minuta*[11], *Artemisia afra*[12] and *Pelargonium captitum*[13], essential oils from literature. Figure 6.3 shows a two-dimensional separation of *C. flexuosus oil.* As shown earlier with standard terpene hydrocarbons, this group is well separated from the other chemical classes. This group consist of mono-terpenes, (C10 hydrocarbons e.g. α-pinene, limonene, p-cymene), sesquiterpenes components (C15 hydrocarbons) and diterpenes (C20 hydrocarbons). The next group consists of the carbonyls (with ester, ketones and aldehydes co-eluting) and the last group consist of alcohols co-eluting with the aldehydes (geranial and neral). Figure 6.3 of a *C. flexuosus* essential oil is characterized by two intense peaks (15,16) occurring at the usual region of alcohols in the SFC dimension. These are the two citral isomers (neral and geranial) which are the major components in the oil[9]. We suspect that they undergo keto-enol tautomerism with the enol form stabilized by stronger hydrogen bonding on the silica gel stationary phase. This could account for their added retention as is also the case in SFC silica gel data from literature[16]. We believe this is a

reversible transformation, as apposed to some permanent transformations that have been documented in the chromatographic analysis of essential oils by Sandra and Bicchi[14]: Most artefacts occur in the injector of a gas chromatograph and such reactions are difficult to detect. An example of transformation in the course of an analysis, is the isomerization of germacrene D which is an important constituent found in the essential oil of peppermint[14]. This sesquiterpene hydrocarbon undergoes several rearrangements which can be thermal, photochemical, or acid-catalyzed[14]. Another example is the transformation of linalyl acetate in the essential oil of Petitgrain (contains 80% of linalyl acetate), which at high temperature (>200 ^{o}C) converts to β-myrecene (elimination of acetate group) also to limonene, cis-β; trans-β, and allo-ocimene[14].

The use of compound parameters such as Kovats index, polarity and volatility in SFCxGC is demonstrated to identify some of the major peaks contained in the oil of *C. flexuosus.* With this information we can distinguish the different types of terpenes such as monoterpenes, sesquiterpenes and diterpenes in figure 6.3. Some of the tentatively assigned peaks are outlined in table 6.3. Jennings and Shimbato[15] pointed out that retention indices have some value as complementary criterion. It is well known that the use of Kovats indices can facilitate crucial identifications in the case of compounds with similar features such as most mono- and sesqui- terpenes, that have near-identical mass spectra.

A chromatogram of *C. citratus* oil is shown in figure 6.4. It shows very similar patterns of peaks as *Cymbopogon flexuosus* oil. Most of the compounds spread on the two-dimensional plane resemble *Cymbopogon flexuosus* oil obtained in figure 6.3. The common feature about both oils is that they contain two isomers, neral and geranial (cis and trans-citral) as the main constituents[9,10]. This is the source of citral used mainly in perfume industries. Normally neral and geranial should represent about 20 % and 40 % or more of the lemongrass composition. The absence of the unidentified compound marked X in *Cymbopogon citratus* oil also distinguishes it from *C. flexuosus*. Table 6.3 and 6.4 indicate some of the numbered peaks tentatively identified in *Cymbopogon*

flexuosus and *C. citratus* oils from the literature list of compounds for the two the oils[9,10].

Because of the structural similarities and equal molecular mass amongst the terpene hydrocarbons positive identification of these compounds is difficult in one-dimensional separation as already discussed. However, with the use of the advantages of comprehensive two-dimensional SFCxGC to spread compounds over two-dimensions it is much easier to resolve more peaks because of the enhanced peak capacity. We can distinguish the C10, C15 and C20 terpene hydrocarbons because the oxygenated compounds are separated from them. A high degree of order is obtained because of the high polar selectivity offered by silica-gel PLOT SFC separation at lower temperatures and effective volatility analysis by resistively heated GC. Compound identification can more readily be made since the two sets of retention data provide both polarity and volatility information for the sample components.

Table 6.3. Composition[10], chemical class, molecular weight, and retention data[14] for *Cymbopogon flexuosus*. (Peak numbers refer to identified peaks in figure 6.3).

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determine[14]

Peak No.	Compound[10]	Chemical class	MW	I[14]	T[14]	%[10]
1	α-Pinene	C10 Monoterpene	136	942	100	0.06-2.67
2	**Camphene**	C10 Monoterpene	136	953	100	**0.07-13.46**
	β-Pinene	C10 Monoterpene	136	978	100	0.1
	Myrecene	C10 Monoterpene	136	988	100	1.93-4.33
	α-Phellandrene	C10 Monoterpene	136	1007	110	0.05-0.16
3	Limonene	C10 Monoterpene	136	1024	100	0.035-.3.03
	β-Phellandrene	C10 Monoterpene	136	1034	100	0.11-0.40
	(Z)-β-Ocimene	C10 Monoterpene	136	1027	100	0.05-0.20
4	**(E)-β-Ocimene**	C10 Monoterpene	136	1042	100	**0.82-20.99**
	γ-Terpinene	C10 Monoterpene	136	1056	100	**0.21-9.91**
	Terpinolen	C10 Monoterpene	136	1074	100	0.10-0.43
14	Citronellal	C10 Aldehyde	154	1143	135	0.06-0.18
	Linalool	C10 Alcohol	154	1097	135	0.77-9.95
15	**Neral**	C10 Aldehyde	152	1227	120	**1.84-10.42**
16	**Geranial**	C10 Aldehyde	152	1260	120	**1.82-15.03**
	α-Terpineol	C10 Alcohol	154	1178	135	0.06-1.42
	Borneol	C10 Alcohol	154	1177	175	0.28-4.86
9	Geranyl acetate	C12 Ester	196	1363	135	0.62-7.74
11	Linalyl acetate	C12 Ester	196	1240	130	2.3
	Nerol	C10 Alcohol	154	1218	120	0.14-0.32
17	**Geraniol**	C10 Alcohol	154	1234	175	**3.0-74.72**

Reference: [14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*
[10] *Weiss, Essential oil Crop, CAB International, 1977 (for essential oil composition and percentage amount of each compound present)*

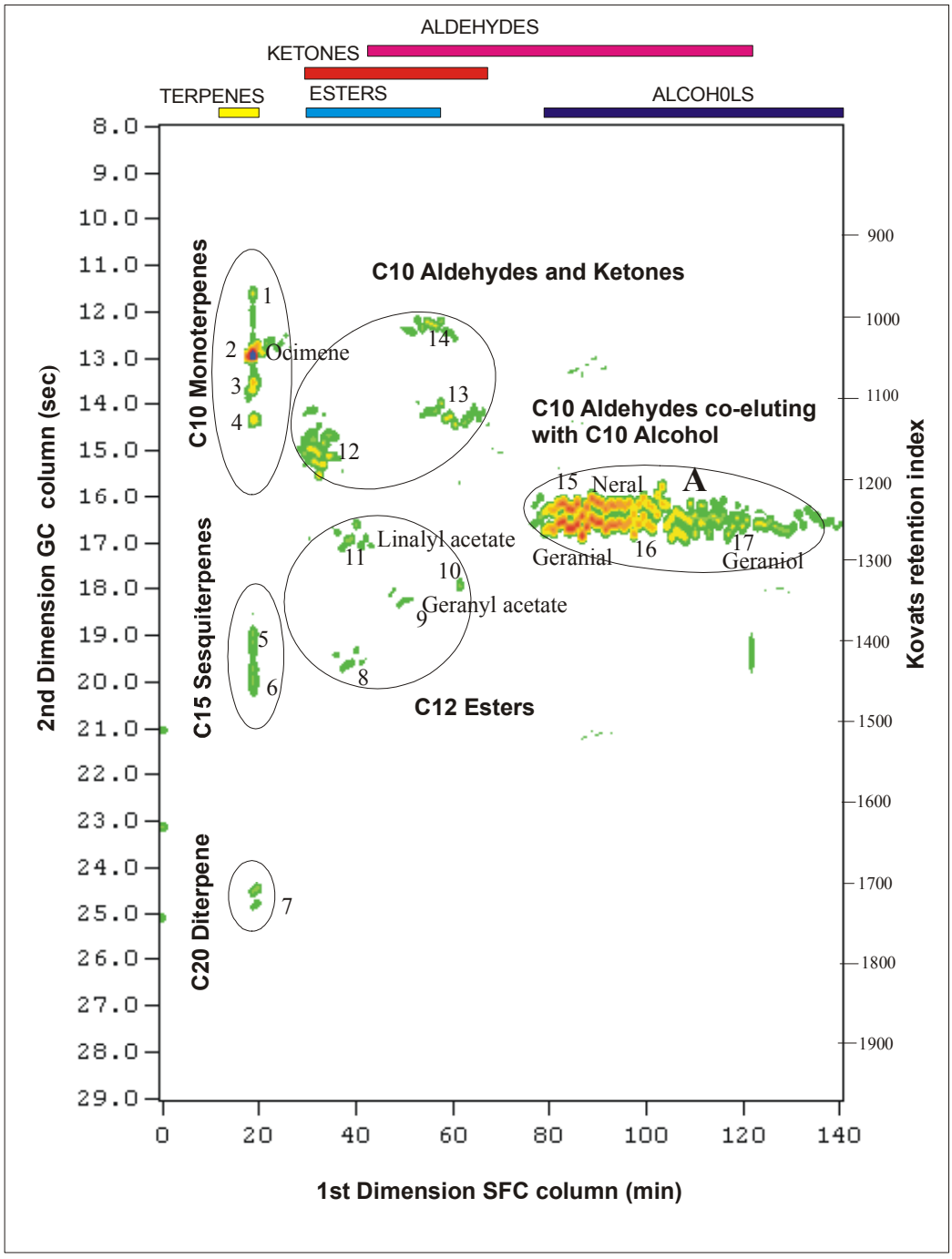

Figure 6.3. SFCxGC chromatogram of *Cymbopogon flexuosus* oil. SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature =28 ^{o}C, Modulation: 5 s collection time and equilibration time = 5 s, Fast GC ramped (-50 to 300) ^{o}C at 450 ^{o}C/min. Scale for Kovats index (1.78 sec = 100 units) calculated from peak 2 (Camphene, KI = 956) and peak 15 (Neral, KI =1227)

Table 6.4. Composition[10], chemical class, molecular weight, and retention data[14] for *Cymbopogon citratus*. (Peak numbers refer to identified peaks in figure 6.4)

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determine[14]

Peak No.	Compound[10]	Chemical class	MW	I[14]	T[14]	%[10]
1	2.6–Dimethyloctane	C10 Monoterpene	142	938	100	0.1
3	**Myrecene**	C10 Monoterpene	136	988	100	**24.3**
5	(Z)-β-Ocimene	C10 Monoterpene	136	1027	100	1.0
6	(E)-β-Ocimene	C10 Monoterpene	136	1042	100	0.7
4	p-Cymene	C10 Monoterpene	134	1016	100	0.5
	trans-Allo-Ocimene	C10 Monoterpene	136	1120	110	0.1
16	Fenchone	C10 Ketone	136	1077	105	0.2
9	β-Caryophyllene	C15 Sesquiterpene	204	1436	150	0.3
	Tetrahydrolinalool	C10 Alcohol	136	1088	90	0.2
16	Citronellal	C10 Aldehyde	154	1143	135	0.3
	β-Patchoulene	C15 Sesquiterpene		1378	120	0.2
	Linalool	C10 Alcohol	154	1097	135	0.6
14	Camphor	C10 Ketone	154	1126	110	0.1
18	**Neomenthol**	C10 Alcohol	156	1159	120	**3.3**
	Terpinen-1-ol	C10 Alcohol	154			0.4
12	**Linalyl acetate**	C12 Ester	196	1240	130	**2.3**
11	Geranyl acetate	C12 Ester	196	1363	135	
20	**Geranial**	C10 Aldehyde	152	1260	120	**33.7**
	Sabinol	C10 Alcohol	152	1224	175	0.1
	Nerol	C10 Alcohol	154	1218	120	0.8
21	Geraniol	C10 Alcohol	154	1234	175	1.9

Reference [14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*
[10] *A Weiss, Essential oil Crop, CAB International, 1977 (for essential oil composition and percentage amount of each compound present)*

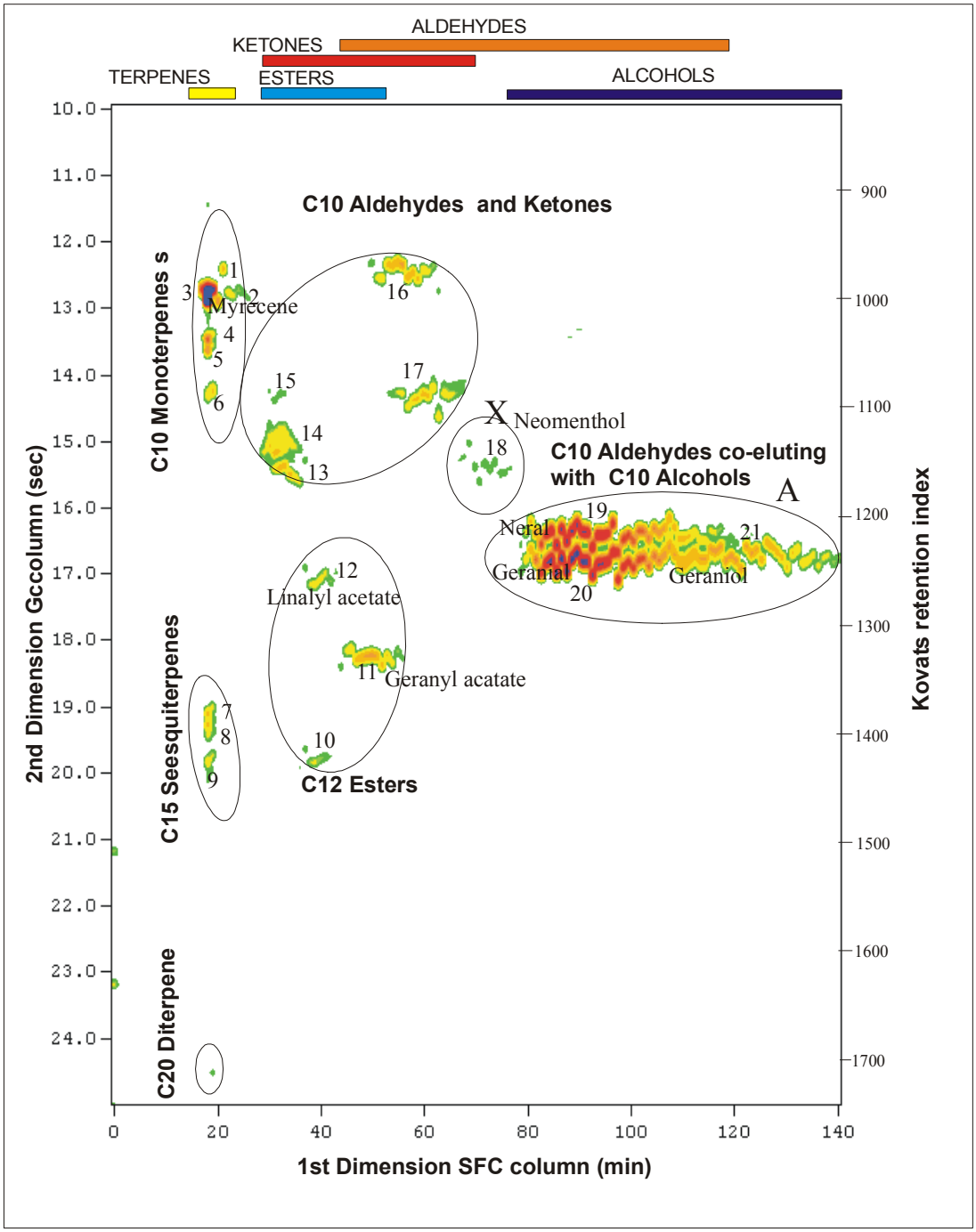

Figure 6.4. SFCxGC chromatogram of *Cymbopogon citratus* oil. SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature =28 °C, Modulation : 5 s collection time and equilibration time = 5 s, Fast GC ramped (-50 to 300 °C) at 450 °C/min. Scale for Kovats index (1.51 sec = 100 units) calculated from peak 3 (Myrecene, KI = 988) and peak 19 (Neral, KI =1227)

Figure 6.5 represents a chromatogram of a *Pelargonium capitatum* essential oil. The chemical compounds of the ester group are dominating in the oil. Some of the major oil components identified using the chemical standards and knowledge of the compound volatility and polarity are summarized in Table 6.5. Figure 6.6 illustrates a typical SFCxGC chromatogram of *Tagetes minuta* essential oil, while the chromatogram of *Artemisia afra* oil is presented by Figure 6.7. Table 6.6. presents some of the identified components of *Tagetes minuta* oil. Dihydrotagetone, (E)-tagetenone, (Z)-α-Ocimene are the main constituent components of the *Tagetes minuta* oil. Qualitatively, several differences were observed between the four essential oils (*Pelargonium radens X capitatum*, *Cymbopogon*, *Tagetes minuta* and *Artemisia afra*).

By using the distinctive peak patterns observed in all four essential oil chromatograms (Figure 6.3 to 6.7), some of the components such as linalool and geraniol identified in C. *citratus & flexuosus* are detectable in *Pelargonium radens X capitatum* oil. A striking differences among these oils is that *Pelargonium capitatum* is rich with sesquiterpenes, alcohols, C11, C12, and C14 esters and C*ymbopogon* oils contain terpenes and aldehydes. *Tagetes minuta* and *Artemisia afra* are both rich with carbonyls and *Tagetes minuta* oil (Figure 6.6) shows a detectable alcohol component. Furthermore, more carbonyl compounds are detectable in *Pelargonium capitamum* and *Tagetes minuta* oils than the other oils. These characteristic features provide a means of differentiating the four oils and useful conclusions can readily be drawn about the type of oil based on these analytical measurements.

Comparison of figure 6.5 to 6.3 and 6.4 immediately reveals some of the advantages of two-dimensional plane chromatograms developed by SFCxGC analysis. *Pelargonium* oil (figure 6.5) shows the presence of the most ester compounds compared to the *Cymbopogon flexuosus* and *citratus* oils (figure 6.3 and 6.4). The presence of the peaks marked XZ in *Pelargonium* oil at the alcohols region in SFC dimension and esters (C13 & C14) in fast GC scale differentiate *Pelargonium* from *Cymbopogon*. The peaks marked XZ can be assumed to be C15 alcohols.

Table 6.5. Composition[10], chemical class, molecular weight, and retention data[14] for *Pelargonium capitatum* l. (Peak numbers refer to identified peaks in figure 6.5).

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determined, or "prog"if the index was determined using temperature programming[14]

Peak No.	Compound	Chemical class	MW	I[14]	T[14]	%[10]
1	α-Pinene	C10 Monoterpene	136	942	100	1.00
2	Myrecene	C10 Monoterpene	136	988	100	0.30
3	Cis-β-Ocimene	C10 Monoterpene	136	1027	100	0.30
4	Cis-Rose oxide	C10 Oxygenate		1087	prog	0.20
5	trans-Rose oxide	C10 Oxygenate		1100	prog	Ng
36	**Linalool**	C10 Alcohol	154	1097	135	**4.60**
	Menthone	C10 Ketone	154	1158	130	0.40
35	**Isomenthone**	C10 Ketone		1156	130	**7.80**
	α-Terpineol	C10 Alcohol	154	1178	135	0.30
37	**Citronellol**	C10 Alcohol	156	1224	175	**19.00**
38	**Geraniol**	C10 Alcohol	154	1234	175	**21.50**
	Geranial	C10 Aldehyde	152	1260	120	Ng
	Citronellyl formate	C11 Ester	184	1261	prog	**8.50**
	Geranyl formate	C11 Ester	182	1282	prog	**9.50**
26	Geranyl acetate	C12 Ester	196	1363	prog	
27	Citrinellyl acetate	C12 Ester	196	1335	135	0.50
10	β-Bourbonene	C15 Sesquiterpene		1406	prog	0.70
11	β-Caryophyllene	C15 Sesquiterpene	204	1428	prog	0.80
	Citronellyl propionate	C13 Ester		1427	prog	0.20
12	**Guaiadiene 6.9**	C15 Sesquiterpene				**7.20**
	Geranyl propionate	C13 Ester				1.60
13	Germacene D	C15 Sesquiterpene	204	1488	150	2.30
	Citronellyl butyrate	C14 Ester	226	1511	prog	1.00
	Geranyl butyrate	C14 Ester	224	1532	prog	1.20
	Phenylethyl tiglate	C14 Ester				0.70
	Citronellyl tiglate	C14 Ester				0.10
	Geranyl tiglate	C14 Ester				1.30

Reference [14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*

[10] *A Weiss, Essential oil Crop, CAB International, 1977 (for essential oil composition and percentage amount of each compound present)*

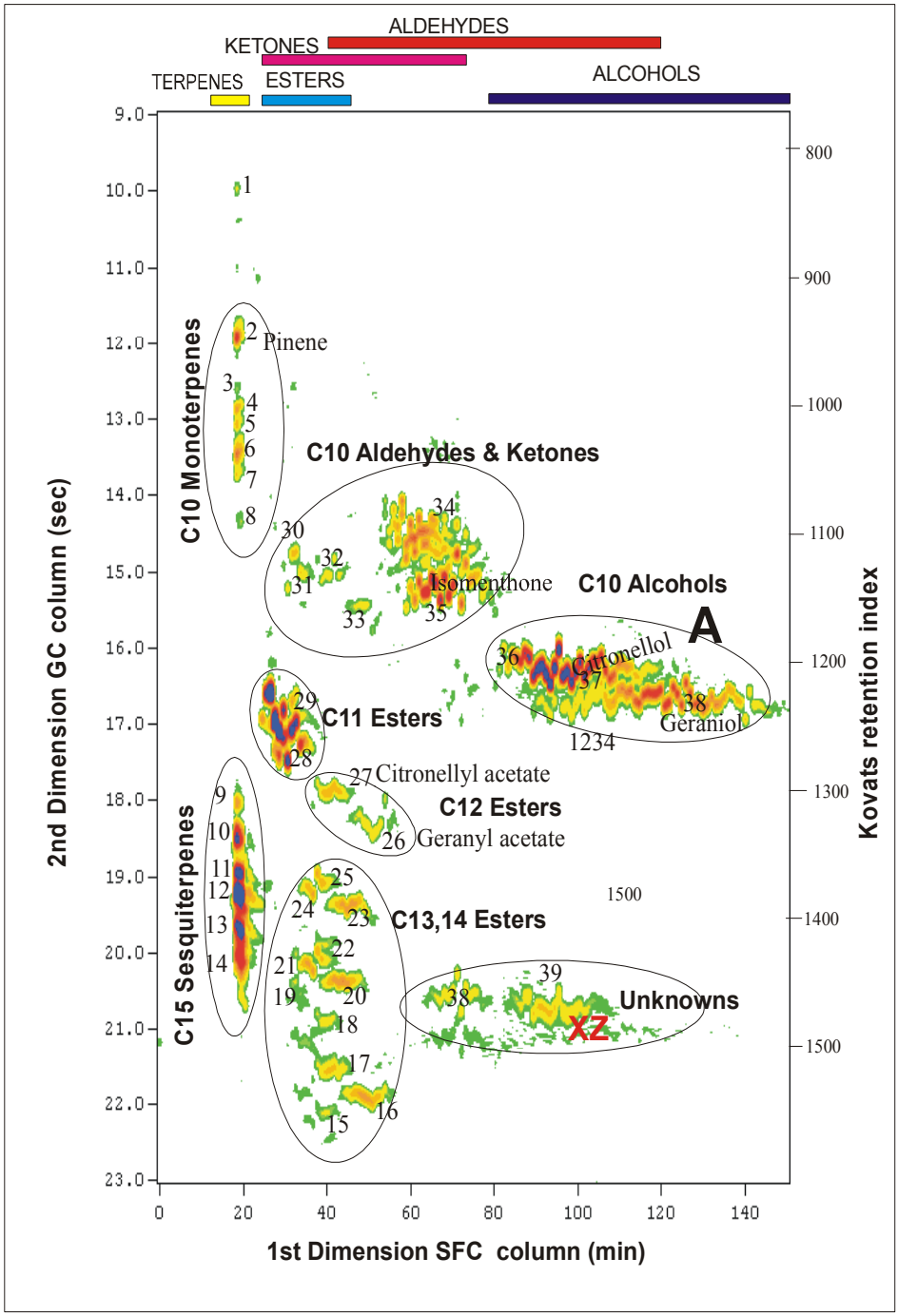

Figure 6.5. SFCxGC chromatogram of *Pelargonium* essential oil SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature =28 ^{o}C, Modulation: 5 s collection time and 5 s equilibration, Fast GC ramped (-50 to 300) ^{o}C at 450 ^{o}C/min. Scale for Kovats index (1.64 sec = 100 units) calculated from peak 2 (Pinene, KI = 942) and peak 38 (Geraniol, KI =1234).

Table 6.6. Composition[11], chemical class, molecular weight, and retention data[14] for *Tagetes minuta*. (Peak numbers refer to identified peaks in figure 6.6)

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determined, or "prog"if the index was determined using temperature programming[14]

Peak No.	Compound[11]	Chemical class	MW	I[14]	T[14]	%[11]
1	α-Pinene	C10 Monoterpene	136	942	100	0.06
	Ethyl-2-methylbutyrate	C10 Monoterpene	136			0.08
	Sabinene	C10 Monoterpene	136	972	100	0.96
	Myrecene	C10 Monoterpene	136	988	100	0.1
	α-Phellandrene	C10 Monoterpene	136	1007	110	0.09
	α-Terpinene	C10 Monoterpene	136	1016	100	0.02
3	Limonene	C10 Monoterpene	136	1025	100	7.24
	β-Phellandrene	C10 Monoterpene	136	1007	110	0.07
	(E)-2-hexanal	C6 Aldehyde				0.06
6	**(Z)-β-Ocimene**	C10 Monoterpene	136	1027	100	**28.49**
	γ-Terpinene	C10 Monoterpene	136	1056	100	0.05
	(E)-β-Ocimene	C10 Monoterpene	136	1042	100	0.39
	allo-Ocimene	C10 Monoterpene	136	1132	Prog	0.32
15	β-Caryophyllene	C15 Sesquiterpene	204	1428	Prog	0.47
16	Bicyclogermacrene	C15 Sesquiterpene	204	1490	Prog	0.1
18	**Dihydrotagetone**	C10 Ketone				**30.3**
	(Z)-Tagetone	C10 Ketone				0.25
	Decanal	C10 Aldehyde				0.12
23	(E)-Tagetone	C10 Ketone				4.8
	(Z)-Tagetonone	C10 Ketone				1.87
20	**(E)-Tagetonone**	C10 Ketone				**15.35**
	Iso-piperitenone	C10 Ketone				0.26

Reference [14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*

[11] *J. Chalchat, R.P. Granny, A. Muhayima, J. Essent. Oil Re., 7(1995)375-386 (for essential oil composition and percentage amount of each compound present)*

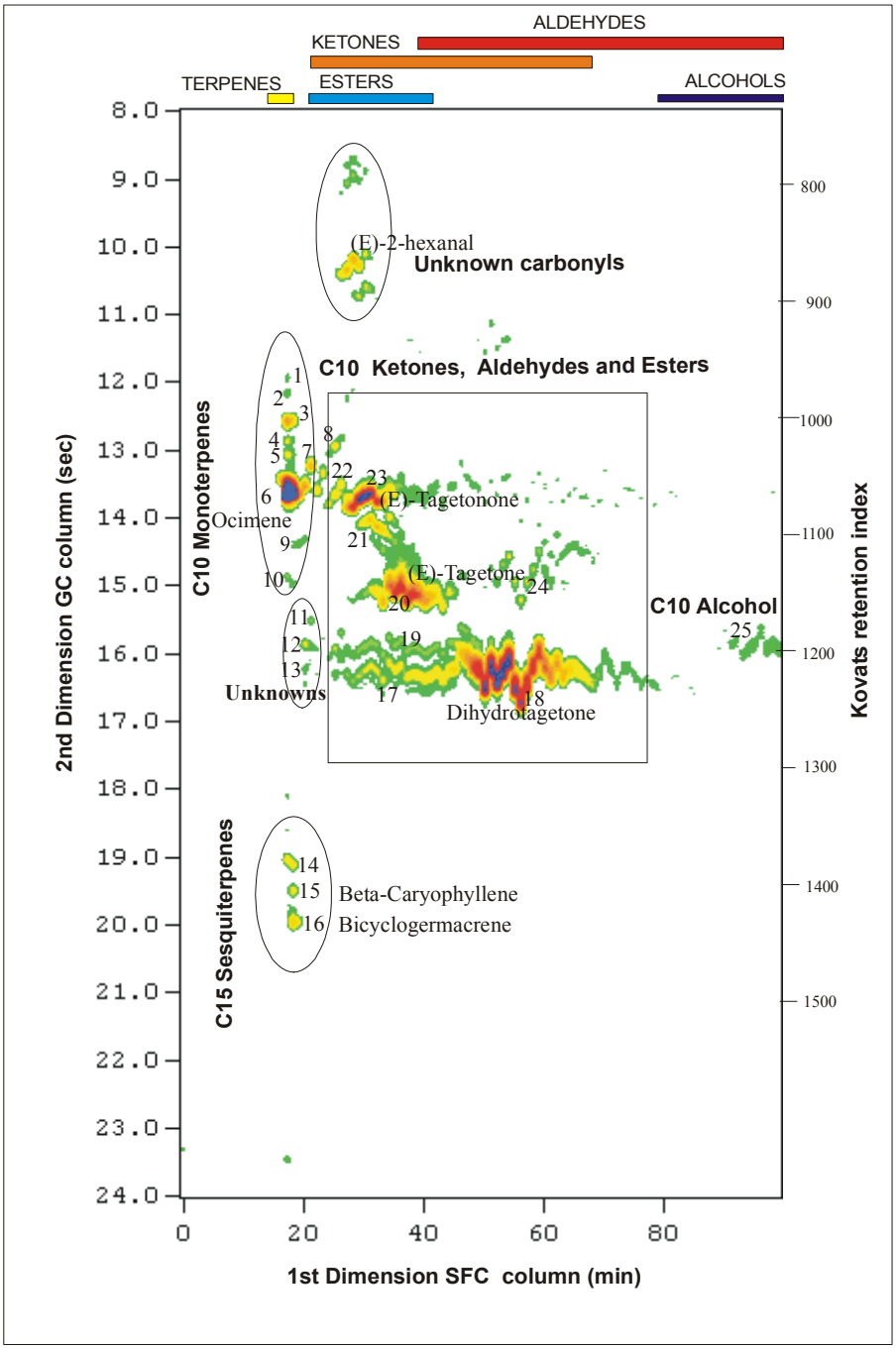

Figure 6.6. SFCxGC chromatogram of *Tagetes minuta* essential oil. SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature = 28°C, Modulation, 5 s collection time and equilibration time = 5 s, Fast GC ramped -50 to 300 °C at 450 °C/min. Scale for Kovats index based on retention time scale of figure 6.5 (*Pelargonium* oil)

Table 6.7. Composition[12], chemical class, molecular weight, and retention data[14] for *Artemisia afra* oil. (Peak numbers refer to identified peaks in figure 6.7)

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determined, or "prog"if the index was determined using temperature programming[14]

Peak No.	Compound[12]	Chemical class	MW	I[14]	T[14]	%[12]
3	Tricyclene	C10 Cycloalkane		928	100	0.1-0.2
4	α-Pinene	C10 Monoterpene	136	939	100	0.4-1.1
6	α-Fenchene	C10 Monoterpene	136	957	110	0.1-1.0
5	**Camphene**	C10 Monoterpene	136	956	100	**0.3-3.9**
7	β-Pinene	C10 Monoterpene	136	978	100	0.1-0.7
8	Sabinene	C10 Monoterpene	136	976	Prog	0.1-1.1
9	Myrecene	C10 Monoterpene	136	984	100	0.1-1.1
	α-Terpinene	C10 Monoterpene	136	1016	100	0.1-1.1
	Dehydro-1,8-cineol	C10 Alcohol				0.1-0.2
	Limonene	C10 Monoterpene	136	1025	100	0.1-0.2
	1.8-Cineol	C10 Alcohol	136	1025	100	**0.1-27.9**
	(E)-β-Ocimene	C10 Monoterpene	136	1027	100	0.1-0.3
	γ-Terpinene	C10 Monoterpene	136	1056	100	0.3-1.9
	p-Cymene	C10 Monoterpene	136	1018	100	0.3-2.0
	Terpinolene	C10 Monoterpene	136	1081	100	0.1-0.5
	β-Caryophyllene	C15 Sesquiterpene	204	1432	150	0.5-0.2.3
27	**Artemisia ketone**	C10 Ketone	152	1153	Prog	**6.3-41.9**
	Santolina alcohol	C10 Alcohol				**3.1-10.1**
	α-Thujone	C10 Ketone		1100	110	1.0-2.9
	Artemisyl acetate	C12Ester				0.1
	β-Thujone	C10 Ketone				Trace
	Artemisia alcohol	C10 Alcohol				0.1
	cis-Sabinene hydrate					0.2-0.6
17	**α-Copaene**	C15 Sesquiterpene		1398	Prog	**8.5-27.1**
	trans-Sabinene hydrate					1.8-4.4
	cis-p-Mentha-2-en-1-ol	C10 Alcohol		1111	Prog	0.2-0.4
	Bornyl acetate	C12Ester		1278	135	0.3-1.5
	Terpinen-4-ol	C10 Alcohol		1129	135	0.1
	Myrtenal	C10 Aldehyde		1173	120	0.1
	trans-p-Mentha-2-en-1-ol	C10 Alcohol		1128	Prog	0.2-0.3
	Borneol	C10 Alcohol		1154	110	**0.6-3.4**
	α-Terpineol	C10 Alcohol		1178	135	0.1-.2.5

Reference [14] *N.W. Davies, J. Chromatogr., 503(1990) 1-24, for Kovats retention indices.*
[12] *JS Chagonda, C. Makanda, J. Claude Chalchat, Flavour and Frag. J., 14(1999) 140-142 (for essential oil composition and percentage amount of each compound present)*

Table 6.7. Composition[12], chemical class, molecular weight, and retention data[14] for *Artemisia afra* oil. (Peak numbers refer to identified peaks in figure 6.7)

I=Kovats index on methyl silicone, T= isothermal temperature at which the index was determined, or "prog"if the index was determined using temperature programming[14]

Peak No.	Compounds	Chemical class	MW	I[14]	T[14]	%[12]
11	Bicyclogermacrene	C15 Sesquiterpene	204	1490	Prog	0.2-0.5
	Piperitol	C10 alcohol				0.1-.07
13	δ-Cadinene	C15 Sesquiterpene	204	1507	130	0.5-0.8
	Cumminaldehyde	C10 Aldehyde				0.5
	Myrtenol	C10 Alcohol	152	1281	120	0.1
12	Calamenene	C15 Sesquiterpene	204	1502	Prog	0.1-0.9
	cis-Carveol	C10 Alcohol		1215	120	0.1
	trans-Caryophyllene oxide	C15 ether		1576	Prog	0.1
	Methyl linolenate					0.1
	Germacene-D-4-ol	C15 Alcohol				0.1
	p-Cymen-8-ol	C10 Alcohol	152	1167	115	0.1
	Spathulenol	C10 Alcohol				0.1
	T-muurolol	C10 Alcohol				0.5
	Intermomedol	C10 Alcohol				0.4

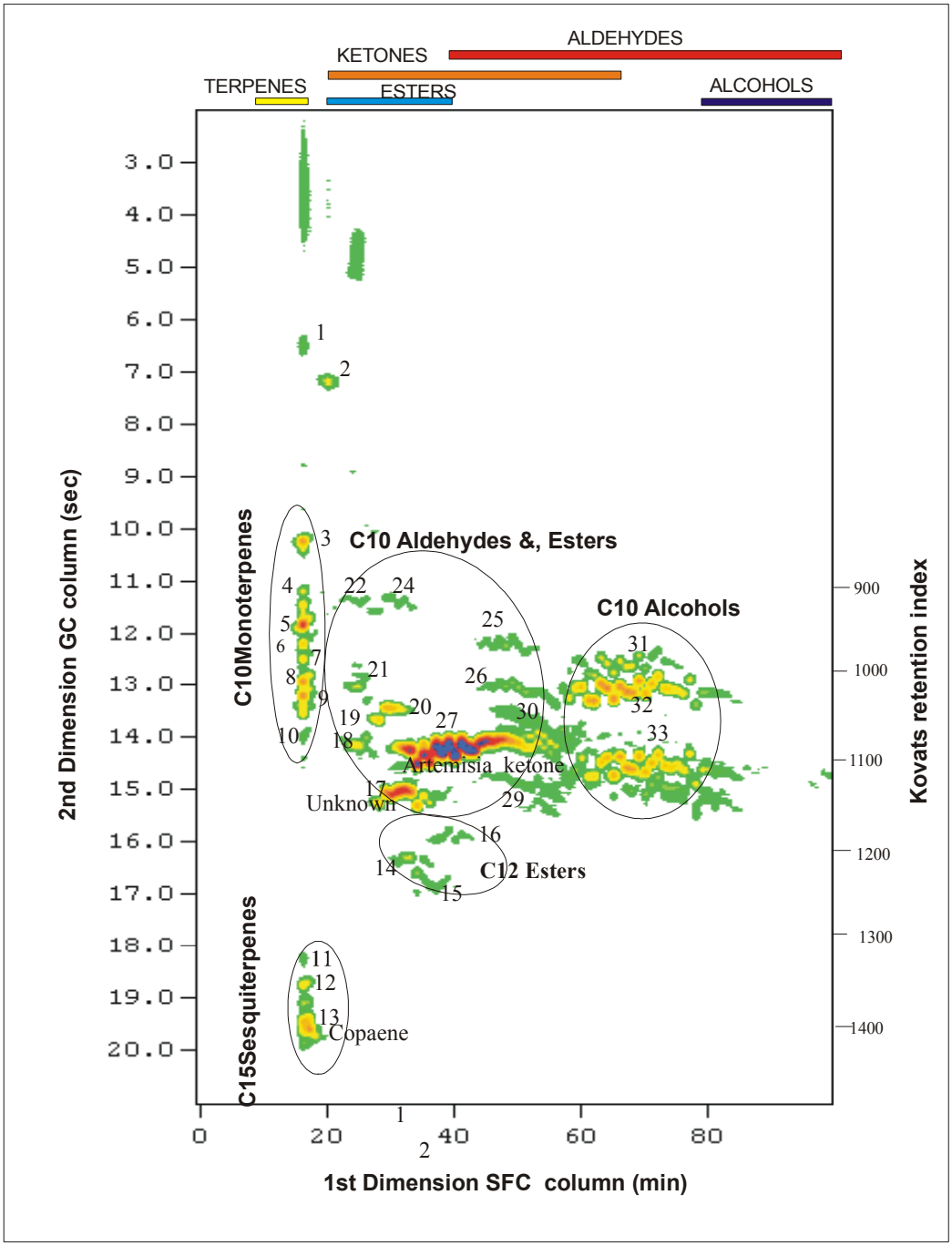

Figure 6.7. SFCxGC chromatogram *of Artemisia afra* essential oil. SFC_{PLOT} pressure ramp 110-200 atm at 1 atm/min, temperature = 28°C, Modulation :5 s collection time and equilibration time = 5 s, Fast GC ramped -50 to 300 °C at 450 °C/min. Scale for Kovats index based on retention time scale of figure 6.5 (*Pelargonium* oil)

All these oils were run at identical chromatographic condition and comparison of the oils is easily achieved by doing peak matching with identification of similar regions in the essential oil chromatograms. For true visualization of very small co-eluting peaks, that are not visible in the 2D-plane, 3D-plane structure gives a clear picture of the peaks especially for the low concentration components. Figure 6.8 shows a typical three-dimensional plane chromatogram of the comprehensive SFCxGC analysis of *Pelragonium capitatum X randens* essential oil.

It is easier to identify some of the single components separated on the essential oil samples by comparing their peak retention times with the ones of the chemical standards for individual peak identification. Therefore, comparing the *Pelargonium capitatum X radens* essential oil in figure 6.5 with the essential oil standard chromatogram (Figure 6.2), it is evident that terpineol, geraniol and linalool are some of the last eluting alcohol compounds in *Pelargonium* oil.

When displaying the chromatogram as a contour plot, within the two-dimensional plane of the two retention time axes, compounds are ordered according to their chemical or molecular functionality, structure or shape, which makes verification of the compounds relatively easy and reliable for SFCxGC, provided the composition of the sample is known from other studies (e.g. GC-MS). Two types of separation can be performed, namely, a group-type-separation and a separation of target compounds. Compounds of a particular chemical class will have comparable first dimension retention times, and are grouped together in bands along a one-dimensional plane. The identification of compounds ordered within these bands is therefore simplified by using fast GC.

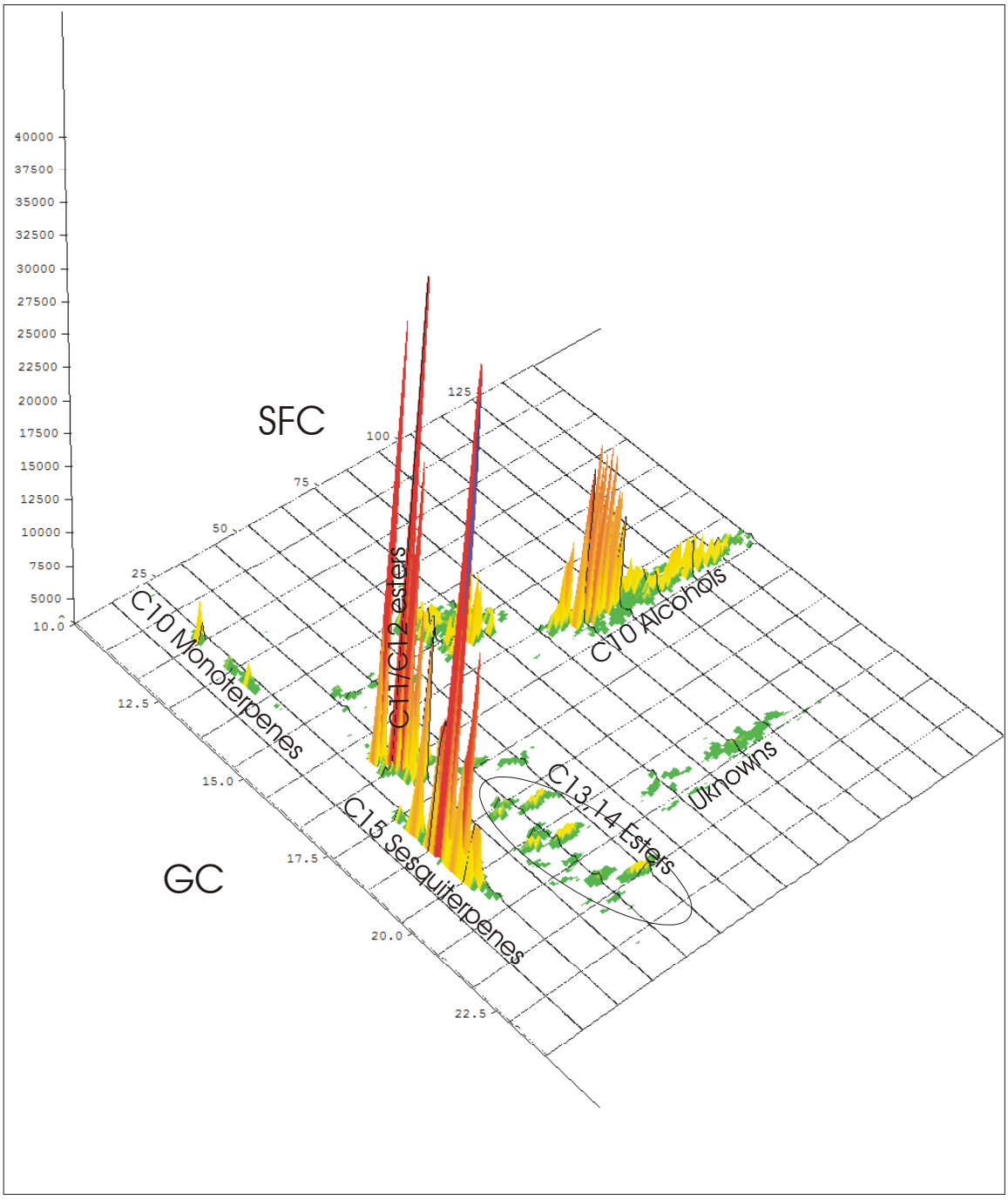

Figure 6.8 SFCxGC Three dimensional Chromatogram of *Pelargonium* essential oil.

6.3.3 Qualitative comparison of four *Cymbopogon citratus* oil samples

The quality of essential oils varies with place of origin, climate, etc. One of the objectives of this research was to evaluate the SFCxGC system for fingerprinting of essential oils in order to qualitatively differentiate oils of the same family (e.g. lemongrass) from different places. For this purpose four *cymbopogon citratus* oil samples from different locations (4,5,6,7) were analysed on the SFCxGC system using the same chromatographic conditions already outlined in section 6.3.1.1.

6.3.3.1 Results and Discussions

Figures 6.9 and 6.10 show the comparison of four lemongrass oil samples (4,5,6,7) analysed with SFCxGC. Four peaks marked A to D are highlighted in table 6.8 to show that *Cymbopogon* oil samples differ, with detection of compounds in some oils but not in others. Even in its prototype form, SFCxGC clearly can provide valuable information. Peaks integration facilities, to quantify components in the mixture, can only improve on this fingerprinting ability

Table 6.8 Qualitative comparison of four lemongrass *citratus* oil samples

Description	**A**	**B**	**C**	**D**
lemongrass 4	a	p	p	P
lemongrass 5(nduva)	p	p	p	A
lemongrass 6	a	p	p	P
lemongrass 7	p	p	a	A

Bold capital letter case (**A**, **B**, **C**, **D**)= represent chosen regions in the chromatograms for comparison of the oil sample.

Small letter case: a= absent, p=present

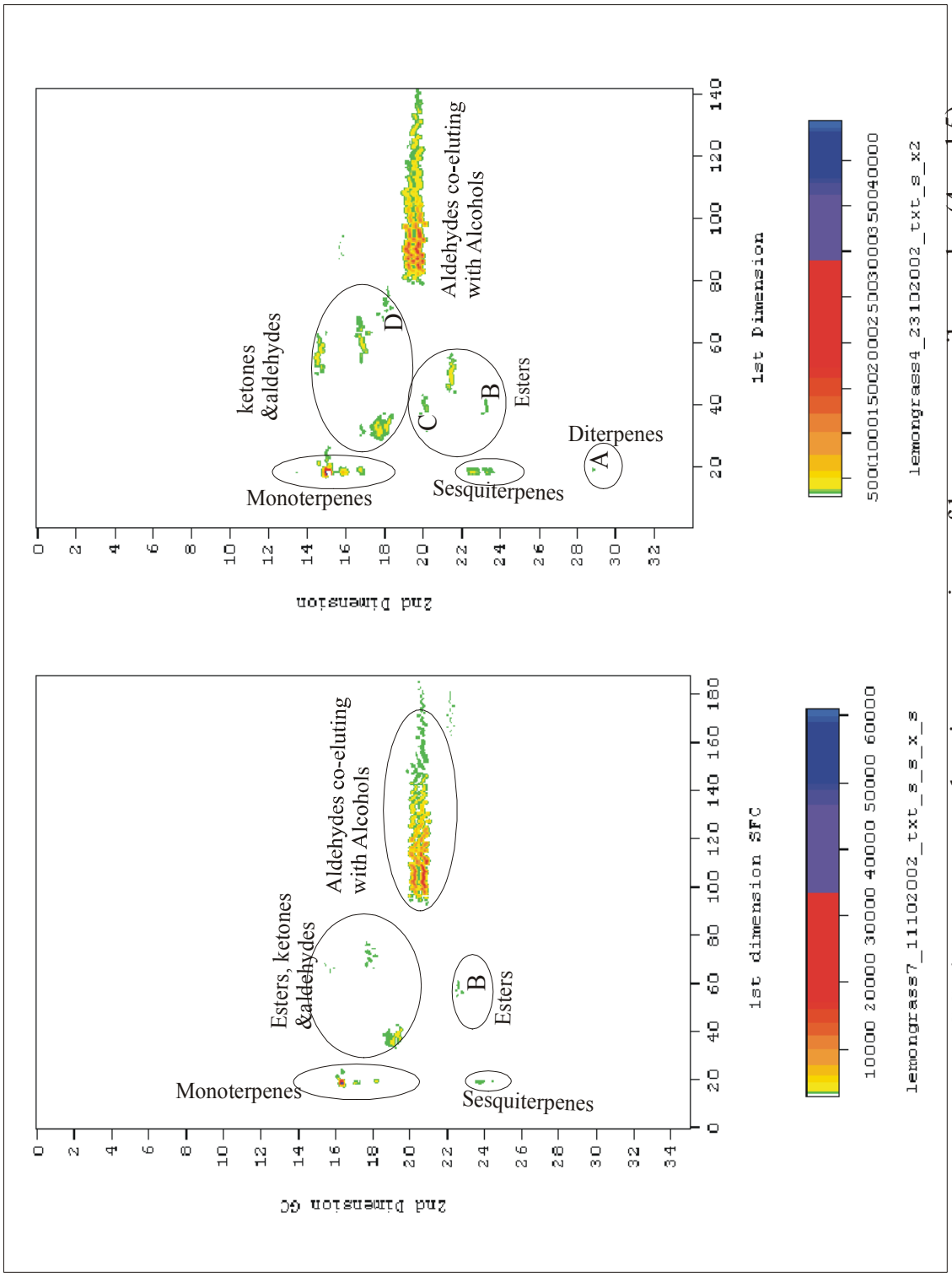

Figure 6.9 SFCxGC chromatogram showing comparison of lemongrass oil samples (4 and 5)

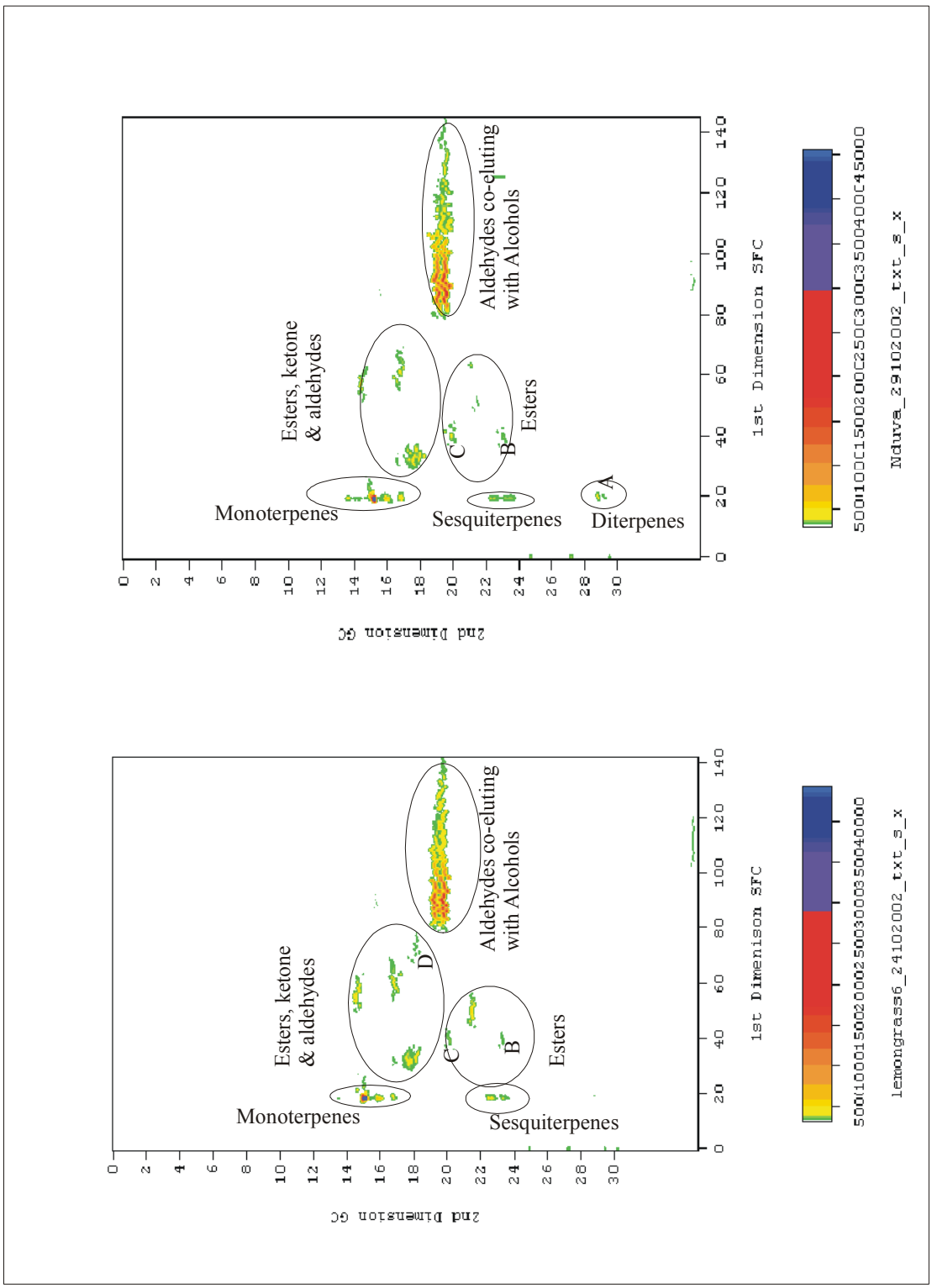

Figure 6.10 SFCxGC chromatogram showing comparison of lemongrass oil samples (6 and 7)

6.3.4 Reproducibility of SFCxGC runs

An instrument can only provide reliable information if its analytical data is reproducible. Statistical parameters such as relative standard deviation (%RSD) and standard deviation (SD) are often used to interpret the reproducibility data. For this reason one of the lemongrass oil samples *C. citratus* (no.4) was chosen to do repeated SFCxGC runs.

6.3.4.1 Result and Discussion

The chromatographic conditions outlined in section 6.3.1.1 were used for SFCxGC analysis. A single set of restrictors to control the SFC flow was used. Table 6.9 shows the reproducibility from consecutive lemongrass samples no. 4 (*citratus*) with SFCxGC (accompanying chromatograms in Appendix B, figure (B.1-B.4). The reproducibility obtained was fair in the first dimension (SFC). The relative standard deviation (RSD) of 6.16 and standard deviation of 2.35 were obtained for the SFC analysis. In Table 6.9 (A7, Appendix A) there is a shift of retention times for peaks (1-3) although they belong to the same group or fraction of terpene hydrocarbons. This retention variability seems to be caused by (1) variation in the linear velocity of the mobile phase and (2) insufficient temperature control of the SFC column at the relatively low temperature (28 ^{o}C) by the GC oven.

The second dimension (resistively heated GC) retention of individual peaks shows good reproducibility in table 6.9. With repeated SFCxGC runs the %RSD of 1.94 and SD of 0.34 s were obtained. It is important to point out that the same thermocouple was used for all runs to test the SFCxGC reproducibility. It can be concluded that the results of the present consecutive runs on essential oil sample clearly indicate the necessity of introducing important improvements in the SFC dimension. An improved means to control stable temperature conditions and proper control of the column linear velocity (i.e. pump pressure and restrictor flow) is required to better reflect the true SFCxGC

capability. However, the reproducible retention of peaks in the second dimension under ideal circumstances is impressive.

Table 6.9 Reproducibility results of SFCxGC runs *C. citratus*.

Peak No.	1st Dimension retention			2nd Dimension retention		
	Mean (min)	SD (min)	%RSD	Mean (sec)	SD (sec)	%RSD
1	20.20	1.31	6.57	14.91	0.02	0.15
2	20.64	1.30	6.28	15.59	0.25	1.59
3	20.38	1.26	6.18	16.52	0.11	0.68
5	42.43	1.87	4.40	22.93	0.13	0.57
6	44.58	3.08	6.90	20.46	0.66	3.24
7	35.90	2.72	7.57	17.99	0.16	0.78
9	66.57	3.77	5.66	17.25	0.75	4.31
8	61.49	3.49	5.71	14.89	0.63	4.21
Average		**2.35**	**6.16**		**0.34**	**1.94**

6.3.5 Effect of the thermocouple on SFCxGC separation

Although SFCxGC analysis provides enough information by spreading compounds on the two-dimensional chromatogram, the appearance of the peaks in the SFCxGC chromatogram depends on the result of the reproducibility of the fast GC retention times. To achieve reproducible retention times of the consecutive runs successfully, special care is required of the thermocouple placement onto the column. Further, direct electrical contact of the thermocouple with the column is not allowed with the present control electronics. Very slow thermal response when the glue droplet is too big (when the thermocouple is placed a fraction of a millimeter away from the column) results in irreproducible retention times. Eventually the ramp program results in an oscillating temperature and the temperature set-point is not well followed.

6.3.5.1 Results and discussions

Figure 6.11 shows a chromatogram of *Tagetes minuta* oil obtained with a good placement of the thermocouple on the column. This implies that the thermal contact between the column and the thermocouple is good and the resulting PID control of the temperature ramp is shown in the one in figure 6.13. Figure 6.14 shows the typical ramp obtained with a bad thermocouple placement on the column. The resulting temperature ramp is not smoothly followed as compared to the one in figure 6.13.

Figure 6.12 is a typical chromatogram obtained with a bad thermocouple placement. All individual components separated in Figure 6.11 are merged into big clusters in Figure 6.12. They are all compressed in the second dimension axis (fast GC) as one broad band. Although most of the information about individual components is lost, chemical class separation obtained in the first dimension is still maintained. Special care is needed to obtain individual compound separation and good reproducible results since bad thermocouple placement can influence the final SFCxGC chromatogram.

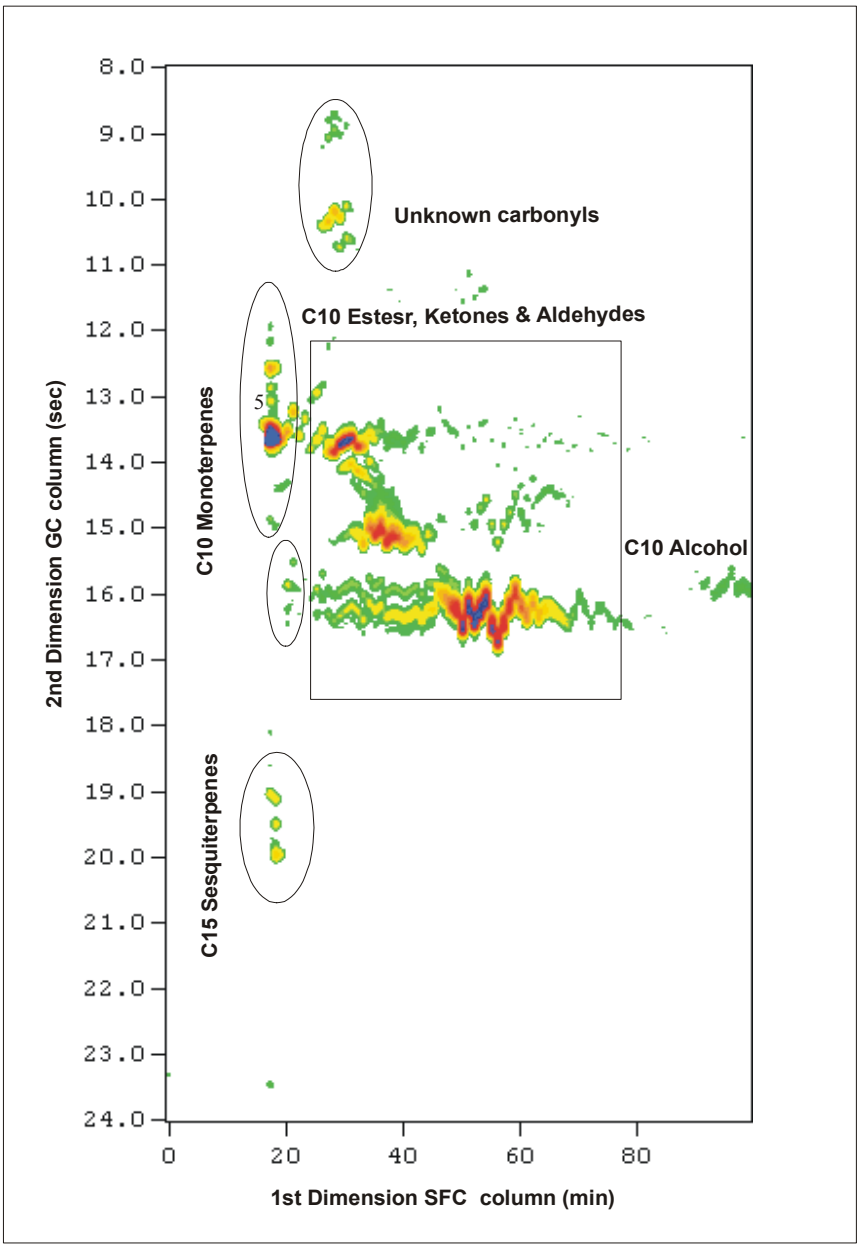

Figure 6.11 SFCxGC *Tagetes minuta* oil with a good thermocouple contact on a 1 m stainless steel , GC capillary column.

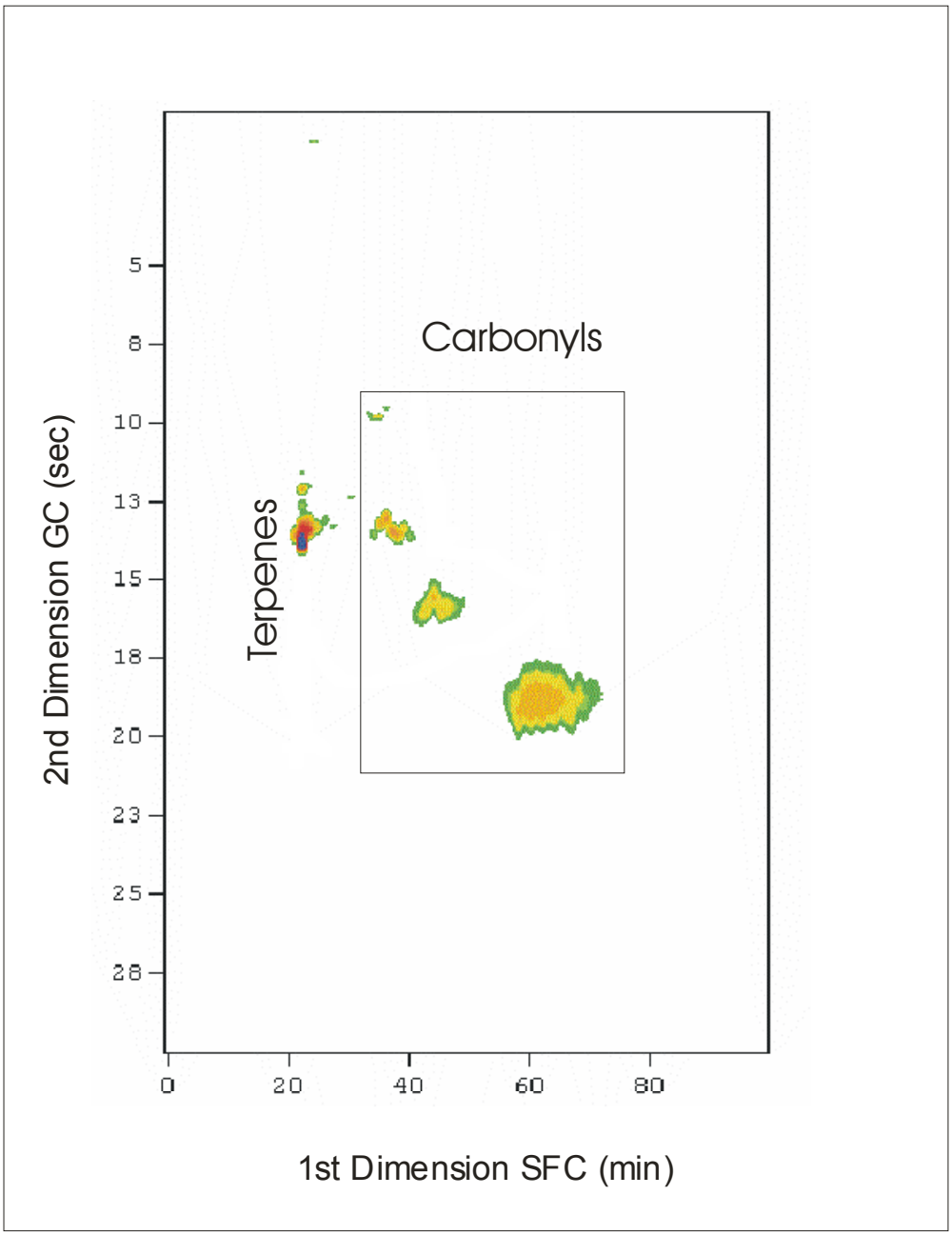

Figure 5.12 SFCxGC *Tagetes minuta* oil with a bad thermocouple contact on a 1 m stainless steel, GC capillary column.

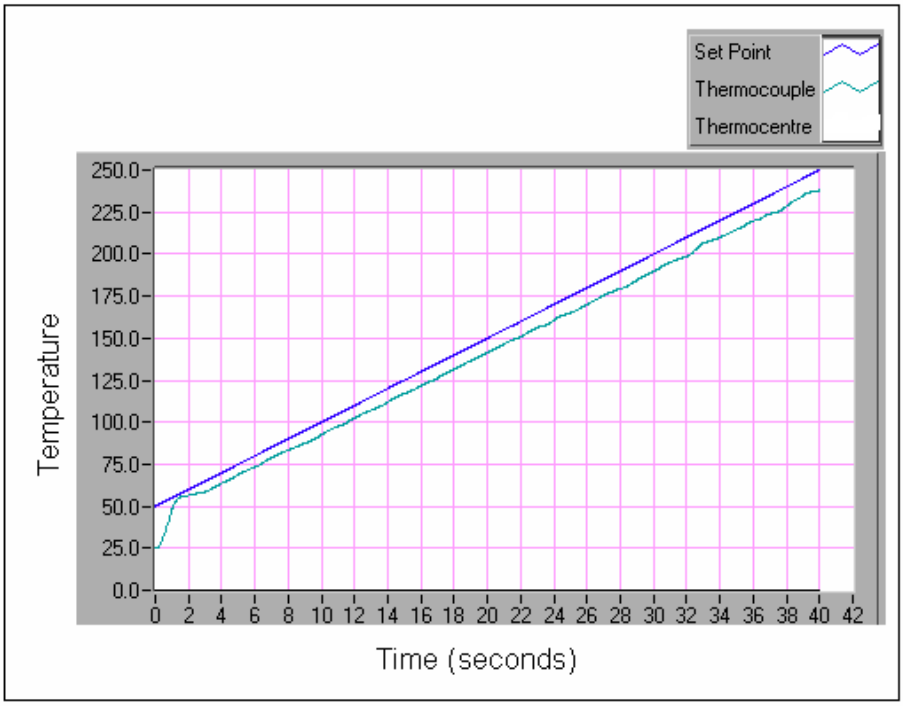

Figure 6.13. Fast GC temperature ramp: good thermocouple contact with the column.

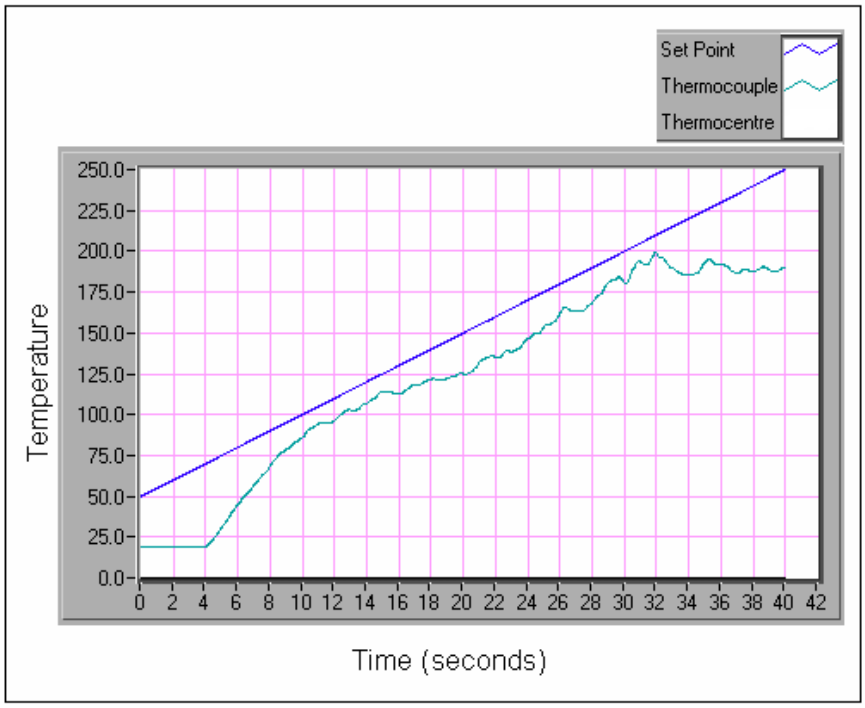

Figure 6.14 Fast GC temperature ramp: bad thermocouple contact with the column.

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