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Comparative analysis of clary sage (*S. sclarea* L.) oil volatiles by GC–FTIR and GC–MS

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Abstract

The comparative analysis of volatiles in essential oil by gas chromatography–Fourier transform infrared spectrometry (GC–FTIR) and gas chromatography–mass spectrometry (GC–MS) are investigated using a DB-wax capillary column. This technique is applied to allelochemicals present in volatiles. The identification analysis of volatile components in four kinds of clary sage (*Salvia sclarea* L.) oil is described. The GC–FTIR information obtained is complementary to the information obtained from GC–MS. With the IR subtractive spectrum technique, the GC overlap peaks can be resolved without further separate step on the other column of different polarity. Combined with GC–FTIR, GC–MS techniques, and linear retention indices (RI) of the volatile compounds, the reliability of qualitative analysis is greatly enhanced.

Keywords: Gas chromatography–mass spectrometry; Gas chromatography–Fourier transform infrared spectrometry; Retention index; Clary sage (*S. sclarea* L.) oil

1. Introduction

Clary sage (*S. sclarea* L.) (Carruba, Torre, Piccaglia, & Marotti, 2002) is a xerophytic biennial plant belonging to the family Lamiaceae. It is typical of the European Mediterranean basin and of Africa up to the Atlantic Ocean. It is widely cultivated for extractive purposes in France, Bulgaria, former USSR and USA, West of China (Lawrence, 1992). The whole plant, mostly the inflorescences, possesses a very strong aromatic scent and the essential oil, characterized by a fresh floral and herbaceous odor, has an economic value for the flavor and fragrance industries, where it is used as flavoring agent, in food and liqueur preparations, in perfumery formulations and for cosmetic purposes (Annex to the Official Journal of the European Communities, 1990).

In complex mixtures of volatiles, identification by GC alone via retention times requires knowledge of the compounds to be identified. In many extracts of samples, the identity of the major components is known but some extracts contain unknown minor compounds at low levels. The identification of these minor components can be of considerable interest. To succeed in these complex identifications structural elucidation can be achieved through the use of the hyphenated techniques such as GC–MS, the most commonly applied technique (Cai, Liu, & Su, 2001; Cai, Liu B, Lin, & Su, 2002).

Identification analysis of essential oil is widely used by GC–MS (Lee, Umano, & Shibamoto, 2005). Whereas GC–MS was a well-established technique with substantially better detection limits and the available large databases of mass spectra for identification of

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components via spectral searching. Mass spectrometry is the method of choice owing to its high sensitivity and selectivity. However, the spectral information obtained with mass spectrometry alone is insufficient for unequivocal identification. The main limitations of mass spectrometry are: firstly, the inability to distinguish closely related isomers because they have very similar mass spectra; secondly, compounds to be investigated can not quested in spectra library; thirdly, sometimes computer give some compounds to be chose which they have similar match value, and a complementary technique such as GC–FTIR is needed. This technique is an attractive alternative owing to the high discrimination properties of FTIR. FTIR provides information on the intact molecular structure rather than the fragments by MS, resulting in a unique spectrum for each molecule. Compounds with similar structures such as isomers can be distinguished. These properties make FTIR an alternative and complementary method to MS for GC detection (Vera & Chane-Ming, 1999).

Until recently, it was not possible to combine the two hyphenated techniques of GC–MS and GC–FTIR at the same level of performance owing to the relatively low sensitivity of the conventional interfaces. With this gas cell system, as for GC–MS interfaces, the spectrum is measured in real time as each separated column effluent, so that each measurement can be performed without trapping the separated column effluents. Spectra can be obtained from amounts of substance in the 1–50 ng range, depending on the absorbance of the compounds. In view of these advantages, it might be very useful to combine GC–FTIR and GC–MS for confirmatory analysis of xenobiotics. We describe here examples of identification analysis of volatile components in clary sage (*S. sclarea* L.) oil in this investigation.

2. Experimental

2.1. Reagents and materials

Four kinds of clary sage (*S. sclarea* L.) oil were kindly supplied by Huabao Fragrance & Flavor Co. Ltd (Shanghai, China).

The homologous series of *n*-alkanes (C_8–C_{28}) used for calculated retention index (RI) were purchased from Alltech (Deerfield, IL, USA). Reference compounds were obtained from Aldrich (Milwaukee, WI, USA).

2.2. Procedure

2.2.1. GC–MS

Chromatographic separations were performed using an Agilent (Palo Alto, CA, USA) 6890N instrument with split-splitless injector and fused-silica capillary column (60 m × 0.25 mm i.d.), with 0.50 μm film thickness (DB-wax, J&W Scientific, Folsom, CA, USA). The condition were as follows: injector temperature, 250 °C; carrier gas, helium (99.999% purity); column flow rate, 2.5 ml min^{-1}; splitting ratio, 100:1; injection volume, 0.5 μL; oven temperature program, held at 50 °C for 2 min, increased at 3 °C min^{-1} to 230 °C, held isothermal at 230 °C for 20 min.

Total ion chromatograms (TIC) and mass spectra were recorded using an Aligent 5973 mass selective detector (MSD) with an Agilent Chemstation Rev 6.0 in the electron impact ionization mode at 70 eV, using a scan acquisition from 35 to 500 amu in 0.3 s with the 0.2 s interval time of the scan. The transfer line is maintained at 280 °C, the source temperature at 230 °C, the quadrupole temperature at 150 °C and the voltage of the electric multiplier tube (EMT) was 1247 V after tuning.

The mass spectral identifications of the volatile compounds were carried out by comparing to the NIST98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library as well as to the Wiley 7.0 N (John Wiley & Sons, NY, USA) mass spectral library. Identification analysis (mass spectral data) was verified by comparing the retention indices and mass spectra of identified compounds with those of authentic reference substances.

2.2.2. GC–FTIR

GC separations were performed using exactly the same instrument and the same conditions as for GC–MS.

The Gram–Schmidt reconstructed chromatograms (GSC), the functional group chromatograms (FGC) and the FTIR spectra were recorded using a Nicolet (Thermo Nicolet Corporation, VA, USA) Nexus 470 FTIR spectrometer equipped with a direct deposition interface containing its own nitrogen-cooled mercury cadmium telluride-A (MCT-A) detector and with Ominic ESP software (Thermo Nicolet Corporation, VA, USA) for data acquisition and instrument control. The transfer line was maintained at 250 °C and real-time spectra were obtained by addition of eight scans, with a resolution of 8 cm^{-1}.

The carrier was helium at a flow rate of 2.5 ml min^{-1}. The analytical column outlet stretched into the light pipe inlet. Helium carrier gas was added as make-up gas at a flow rate of 2.5 ml min^{-1} at the connection between the capillary column and the light pipe. The gold-coated light pipe (12 cm × 1 mm i.d.) was heated at a constant temperature of 270 °C. The injection volume was 1.0 μL and the splitting ratio was 10: 1. The chromatographic separation was carried out on the capillary column, and the effluent was first directed to the FTIR and then to a flame ionization detector (FID).

FTIR identification were done for volatile compounds of clary sage (*S. sclarea* L.) oil; the criteria for

the identification were that the first ten hits of list position should be obtained against the Sadtler library, which contains 3240 spectra, and that the correlation value should exceed 0.5. It was required that the peak not only be seen by FID, but also necessarily by a Gram–Schmidt chromatogram. FTIR searches were also done using the FTIR search.com (2001) service.

Normalized quantitative data for volatile compounds of clary sage (*S. sclarea* L.) oil were obtained by the internal standard method using heptadacane as internal standard, without considering calibration factors (i.e., $F = 1.00$ for all compounds).

These two-coupled techniques were used here in parallel fashion.

3. Results and discussion

3.1. Analysis of volatile components of clary sage (*S. sclarea L.*) oil

Because the fused-silica column connected FTIR light pipe and FID is short and inert, with small dead volume, the separated column effluent can rapidly reach FID. Compared between GSC of GC–FTIR and GC–FID chromatogram, demonstrated in Fig. 1, they are almost same. So, some marks using the peaks of compounds identified by GC–FTIR and RI of GC–FID can easily be compared.

Chromatogram obtained by integrating the absorbance in selected spectral regions, is called functional group chromatogram (FGC). The FGC is similar to the selected ion monitor (SIM) chromatogram of GC–MS. Because some volatile components contain all the three wavelength ranges (1000–1300, 1680–1800, 2700–3300 cm^{-1}), while others contain none or only partial

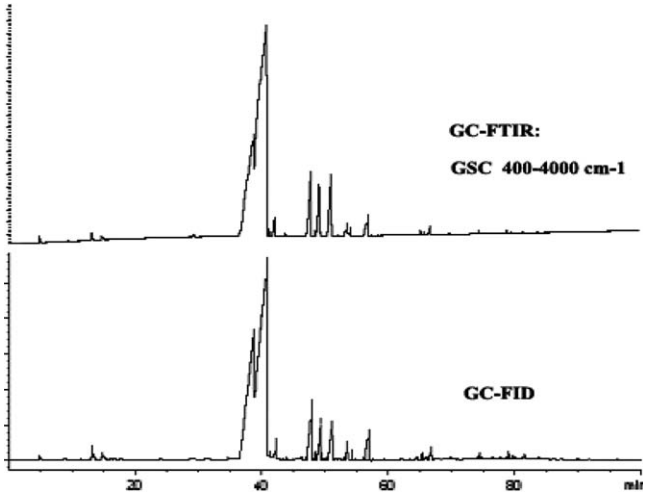

Fig. 1. The GC–FTIR's GSC (400–4000 cm^{-1}) and the GC–FID chromatogram of volatile compounds in clary sage (*S. sclarea* L.) essential oil.

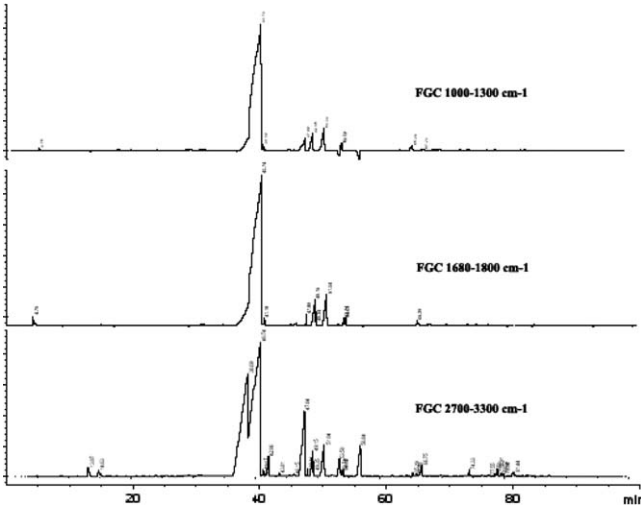

Fig. 2. The different wave numbers FGCs of volatile compounds in clary sage (*S. sclarea* L.) essential oil.

of the three wavelength ranges. The FGCs with different wavelength ranges shown in Fig. 2 nicely demonstrate that there are some similarity and difference for FGCs in the different wavelength ranges.

Forty-five volatile components of four kinds of clary sage (*S. sclarea* L.) oil were identified (Table 1). The volatile components of the oil were identified by comparing the linear retention indices of the FID peaks on column with literature values (Peterson & Reineccius, 2003), computer matching against the library spectra, and finally confirmed by comparison of mass spectra of peaks with published data (Stein, Levitsky, Fateev, & Mallard, 1998) and FTIR spectra. The homologous series of *n*-alkanes (C_8–C_{28}) were used as standards to calculate the linear retention indices. Relative amount of individual components are based on unitary peak area obtained without considering FID calibration response factors.

Analysis of volatile components in four kinds of clary sage (*S. sclarea* L.) oil, 45 compounds, representing differ proportion of the essential oil were characterized (95.21%, 93.68%, 95.22% and 95.40%, respectively). The relative concentrations of the volatile components identified are presented in Table 1 according to their elution order on the DB-wax column. The main constituents of the essential oil are β-myrcene (0.19–0.58%), linalool (17.03–28.76%), linalyl acetate (29.50–49.83%), linalyl formate (0.19–0.29%), *trans*-caryophyllene (0.57–1.27%), α-terpineol and geranyl formate (3.21–5.05%), germacrene (0.46–1.31%), neryl acetate (0.95–1.61%), geranyl acetate (1.68–2.79%), neryl alcohol (0.59–1.02%), geraniol (1.36–2.51%), caryophyllene oxide (0.49–0.77%), spathulenol (0.13–0.25%).

To our knowledge, gernayl formate is reported first time (Carruba et al., 2002; Lawrence, 1992; Annex to the Official Journal of the European Communities,

Table 1
Identified volatile components of four kinds clary sage (*S. sclarea* L.) oil

No.	Compound	RI	Normalized peak area				Identification methods[b]
			A[a]	B[a]	C[a]	D[a]	
1	β-Pinene	1103	0.0	0.0	0.0	t	RI/MS/FTIR
2	β-Myrcene	1162	0.6	0.5	0.2	0.4	RI/MS/FTIR
3	Limonene	1193	0.3	0.2	0.1	0.1	RI/MS/FTIR
4	1,8-Cineole	1204	0.1	0.1	0.1	0.1	RI/MS/FTIR
5	(*Z*)-ocimene	1243	0.1	0.1	0.1	0.2	RI/MS/FTIR
6	(*E*)-ocimene	1250	t[c]	0.2	0.1	0.4	RI/MS/FTIR
7	*p*-Cymene	1265	0.1	0.0	0.0	0.1	RI/MS
8	α-Terpinolene	1278	t	0.0	t	t	RI/MS/FTIR
9	3-Hexen-1-ol	1381	t	0.0	0.0	0.1	RI/MS/FTIR
10	(*Z*)-linalool oxide	1438	0.1	0.2	t	t	RI/MS
11	Acetic acid	1448	t	t	t	t	RI/MS/FTIR
12	1-Octen-3-ol	1451	0.0	t	t	t	RI/MS/FTIR
13	Nerol oxide	1464	t	t	t	t	RI/MS
14	(*E*)-linalool oxide	1466	0.1	0.1	0.1	0.1	RI/MS
15	α-Copaene	1485	0.2	0.2	0.1	0.1	RI/MS/FTIR
16	β-Bourbonene	1510	t	t	t	t	RI/MS/FTIR
17	β-Cubebene	1533	t	0.1	t	t	RI/MS/FTIR
18	Linalool	1549	28.1	17.0	28.8	28.5	RI/MS/FTIR
19	Linalyl acetate	1557	49.8	29.5	51.6	48.2	RI/MS/FTIR
20	Linalyl formate	1579	0.2	0.2	0.3	0.3	RI/FTIR
21	β-Elemene	1582	0.1	t	t	0.1	RI/MS
22	(*E*)-β-caryophyllene	1586	0.8	0.6	1.0	1.3	RI/MS/FTIR
23	α-Terpiene	1595	0.0	0.0	t	0.1	RI/MS
24	Menthol	1636	0.1	t	t	t	RI/MS/FTIR
25	Ethyl benzoate	1653	t	0.1	t	t	RI/MS/FTIR
26	α-Humulene	1657	t	t	0.1	0.0	RI/MS
27	Citral	1670	0.2	0.1	0.1	0.1	RI/FTIR
28	α-Terpineol + gernayl formate[c]	1691	5.1	3.2	4.4	5.0	RI/FTIR
29	Germacrene	1695	0.3	0.5	0.6	1.3	RI/MS/FTIR
30	Ledene	1704	t	t	t	t	RI/MS
31	Neryl acetate	1720	1.6	1.0	1.3	1.5	RI/MS/FTIR
32	α-Farnesene	1744	t	t	t	0.1	RI/MS
33	δ-Cadinene	1748	t	t	0.0	0.0	RI/MS
34	Geranyl acetate	1751	2.8	1.7	2.3	2.8	RI/FTIR
35	Dihydro-β-agarofurane	1759	0.1	t	t	t	RI/MS
36	Nerol	1795	0.9	0.6	0.9	1.0	RI/MS/FTIR
37	Geraniol	1844	2.2	1.4	2.1	2.5	RI/MS/FTIR
38	Caryophyllene oxide	1964	0.8	0.5	0.7	0.5	RI/MS/FTIR
39	Spathulenol	2110	0.3	0.1	0.2	0.2	RI/MS/FTIR
40	α-Eudesmol	2205	t	t	t	0.3	RI/MS
41	β-Eudesmol	2213	0.3	0.2	0.1	t	RI/MS/FTIR
42	Sclareol oxide	2223	0.2	t	0.1	0.2	RI/MS/FTIR
43	Dimethyl-*o*-phthalate	2276	t	0.2	t	t	RI/MS/FTIR
44	Methyl-ethyl-*o*-phthalate	2315	t	0.3	t	t	RI/MS
45	Diethyl-*o*-phthalate	2355	t	34.9	t	t	RI/MS/FTIR
Total			95.2	93.7	95.2	95.4	

t, means trace amount (not more than 0.01%).
[a] A, B, C, D represent of four kinds of clary sage (*Salvia sclarea* L.) oil, i.e., sample A, sample B, sample C and sample D.
[b] Identification methods, i.e., FTIR, Fourier transform infrared spectrum; RI, Retention index; MS, Mass spectrum.
[c] Component (29) is the overlap of α-terpineol and gernayl formate.

1990). Besides, we discovered large amount of diethyl-*o*-phthalate, methyl-ethyl-*o*-phthalate and dimethyl-*o*-phthalate in sample B (Table 1), the compound was never reported in the literature (Pesic & Bankovi, 2003) and could not be found in other sample. The presence of the three phthalates in the sample B almost certainly has resulted from contact of the extract with some plastic incorporating phthalate plasticizers. It is very unlikely that these components originated from the clary sage.

3.2. Complementarities of GC–FTIR to GC–MS

Technological advances led to the development of thecoupling of capillary gas chromatographs to FTIR

spectrometers (White, 1990). This progress allowed the on-line measurement of FTIR spectra for sub-nanogram-level analytes eluting from a capillary gas chromatographic column. Hence, individual compounds in complex mixtures could be identified by capillary GC–FTIR spectroscopy (Tomlinson, Mlotkiewicz, & Lewis, 1993). The spectral information obtained with GC–MS alone is insufficient for unequivocal identification, and GC–FTIR as a complementary technique is needed. The GC–FTIR technique is an attractive alternative owing to the high discrimination properties of FTIR. FTIR provides information on the intact molecular structure rather than the fragments by MS, resulting in a unique spectrum for each molecule. FTIR spectroscopy is a powerful identification method that can detect all molecules except homonuclear diatomics. It can distinguish structural differences that have identical mass spectra, and provides information about molecular structure even in the absence of an exact library match. Extensive vapor phase libraries, including those from Sadtler, are available in this investigation for identification of unknown compounds. These properties make FTIR an alternative and complementary method to MS for GC detection (Vera & Chane-Ming, 1999; FTIR search.com, 2001). There are some examples in this investigation as following (see Fig. 3).

A good example of why GC/FT-IR is needed to give unequivocal identification of each peak is component (19), where it is shown that a component that was identified by mass spectrometry as linalyl acetate is in fact linalyl formate (shown in Table 1). It is a little strange that the mass spectra of linalyl acetate and linalyl formate are very similar (shown in Fig. 4) in GC–MS analysis. For the component (19), with retention time 41.23 min, the MS library search proposes linalyl acetate with a very good match (the quality index of the ''identification'', i.e., the probability, is 91%). But its FTIR spectrum (shown in Fig. 3(b)) is different from the linalyl acetate standard FTIR spectrum (shown in Fig. 3(a)). The FTIR library search proposes linalyl formate with a very good match (the probability is 97%).

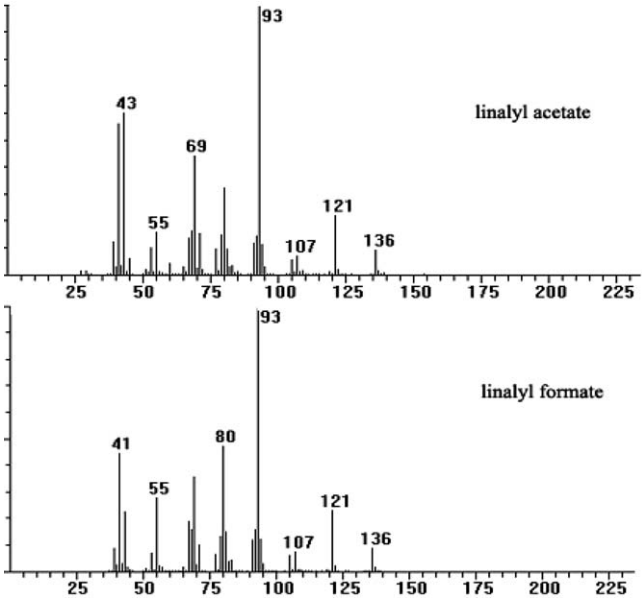

Fig. 4. The mass spectra of linalyl acetate and linalyl formate.

For the component (34), with retention time 49.19 min, the MS library search proposes nerol with a very good match (the probability is 96%), or geranyl acetate with a very good match (the probability is 91%). But its FTIR spectrum (shown in Fig. 5) shows two strong bands around 1700 and 1200 cm^{-1}, which are obviously the ester IR structural information. So the identification of component (34) is geranyl acetate, and not nerol. The FTIR library search also proposes geranyl acetate with a very good match (the probability is 98%).

3.3. Application of IR subtractive spectrum technique in GC–FTIR

Recently, an IR subtractive spectrum technique, with a subtractive similarity method (SSM) (Wang, Wei, &

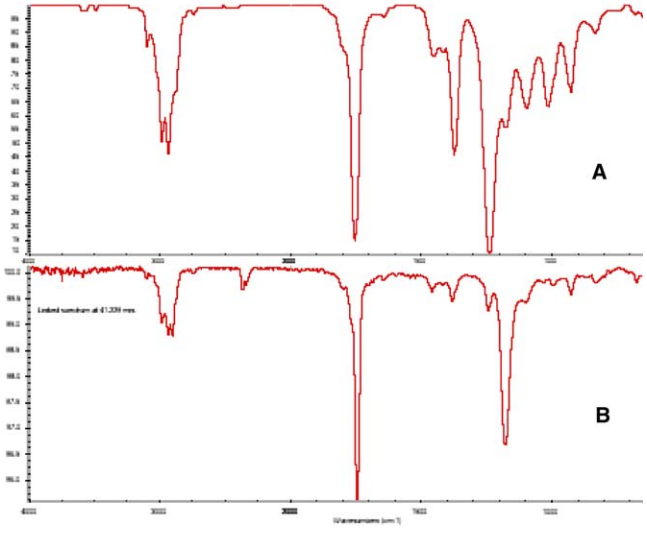

Fig. 3. The standard FTIR spectrum of linalyl acetate (A) and the FTIR spectrum of component ($R_t = 41.23$ min) (B).

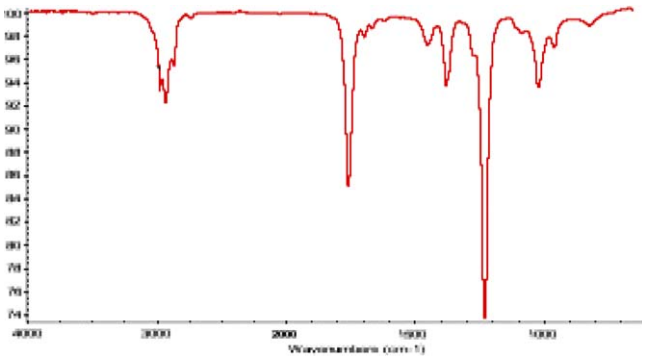

Fig. 5. The FTIR spectrum of component ($R_t = 49.17$ min).

Lin, 2003), can resolve the overlap IR spectrum. A "suitable" standard IR spectrum is chosen to be subtracted from that of the overlap IR spectrum until the IR spectral area of the final spectrum with the highest "similarity" to the same of the other standard IR spectrum is produced. Through this technique, the GC overlap peaks can also be resolved without re-separate step in this GC–FTIR analysis.

For the example, the component (28) with retention time 47.90 min, the MS library search proposes a α-terpineol with a very good match (the probability is 98%). But in its FTIR spectrum, a strong band around 1680–1800 cm^{-1} is found (Fig. 6(a)), which is the typical ester IR structural information (C=O stretching bonds at 1680–1800 cm^{-1}), so the peak of this component might be an overlap peak identified by FTIR data. In order to be characterized, the IR subtractive spectrum technique is applied to produce another IR spectrum (Fig. 6(c)) by subtracted IR spectrum of Fig. 6(a) with the standard α-terpineol IR spectrum (Fig. 6(b)). The FTIR library search of Fig. 6(c) proposes a geranyl formate with a very good match (the probability is 97%). So the component (28) with retention time 47.90 min, is identified as an overlap of α-terpineol and geranyl formate.

To confirm this identification, it is found that α-terpineol and geranyl formate are well separated on apolar GC column (DB-5) and consequently they could be identified by combination of FTIR and MS data on two columns of different polarity. However, with the IR subtractive spectrum technique, the GC overlap peaks could be resolved without further separate step on the other column of different polarity.

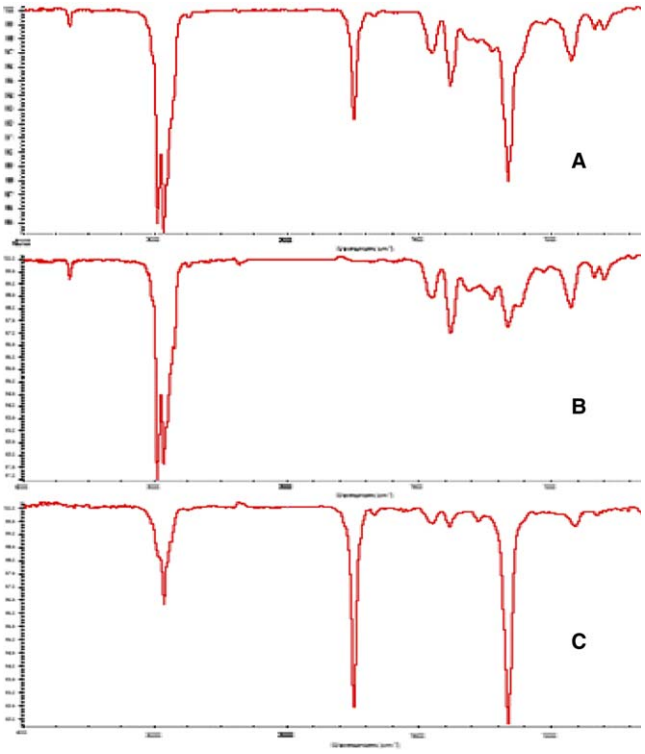

Fig. 6. FTIR spectrum of component ($R_t = 47.90$ min) (A), standard FTIR spectrum of α-terpineol (B), the obtained IR spectrum (C) by subtracted (A) with (B).

4. Conclusions

From these examples, it can be seen that GC–FTIR is very useful for identification analysis of volatile compounds. This technique produces excellent spectroscopic information and is best suited for qualitative analysis where positive identification of compounds is required. The GC–FTIR information obtained is complementary to the information obtained from GC–MS. With the IR subtractive spectrum technique, the GC overlap peaks can be resolved without re-separate step in the experiment. Combine with GC/MS, GC/FIIR technique, and linear retention indices of the volatile compounds; the reliability of qualitative analysis is greatly enhanced.

Acknowledgements

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