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Application

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Application Note 532

Comprehensive characterisation of essential oil by GC×GC–TOF MS

Summary

This Application Note shows that BenchTOF time-of-flight mass spectrometers, in conjunction with GC×GC, provide a high-performance solution for comprehensively screening the total composition of essential oils.

Introduction

Essential oils, due to their desirable aromatic qualities, are used as raw materials in the production of a wide range of cosmetic products, including fragrances and personal care products.

The chemical composition of many essential oils exhibits a degree of natural variation, but even minor changes can adversely affect the quality of the final product. Rigorous quality control procedures are therefore needed to ensure that essential oils do not vary significantly from the initial reference batch, or contain any unwanted adulterants.

Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) is the ideal choice for screening complex mixtures, such as essential oils. The enhanced separation capacity offered by the coupling of two columns of different selectivity, combined with the highly sensitive mass spectral identification, provides a high-performance solution for rapid screening of essential oils – as evaluated in this study.

Despite the superior separation afforded by GC×GC, the identification of individual compounds in complex samples remains challenging when compounds have weak molecular ions and/or similar spectra using conventional (70 eV) ionisation – such as sesquiterpenes. This Application Note demonstrates the use of Tandem Ionisation to address these issues.

Background to BenchTOF instruments

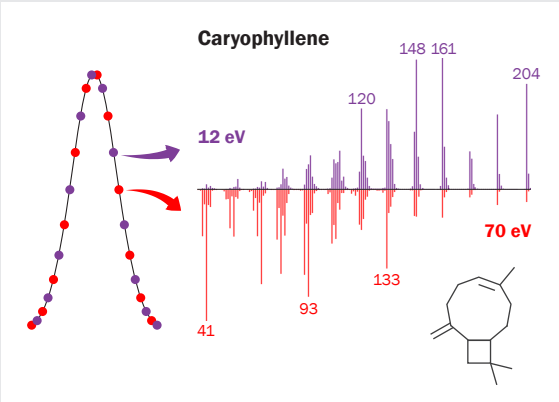
BenchTOF™ mass spectrometers are bench-top time-of-flight instruments designed specifically for gas chromatography. They are ideally suited to the analysis of trace-level analytes in essential oils, the following three features being of particular importance:

- **Sensitivity:** Highly efficient direct-extraction technology allows BenchTOF instruments to acquire full-range spectra with SIM-like sensitivity, allowing them to reliably detect trace-level targets and unknowns in a single run, which would be difficult or impossible on a quadrupole system.
- **Spectral quality:** The 'reference-quality' spectra produced by BenchTOF are a close match for those in commercial libraries such as NIST or Wiley. This enables quick and confident matching of analytes.
- **Speed:** The ability to record full-range mass spectral information to extremely high densities (10,000 transient spectral accumulations per second) enables BenchTOF to handle the narrowest peaks encountered in well-optimised GC×GC couplings.

Tandem Ionisation®

Exclusive to Markes, Select-eV® allows you to collect soft EI spectra down to 10 eV, with no inherent loss in sensitivity and full automation in TOF-DS software with no need for manual intervention.

Tandem Ionisation now adds unparalleled productivity for soft ionisation. Soft and hard ionisation spectra are obtained across every peak, in both GC and GC×GC analyses, for comprehensive sample characterisation in a single run.



One chromatographic peak, two complementary spectra. Tandem Ionisation using Select-eV enables 70 eV and soft EI spectra to be collected across every peak in a single run.

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Experimental

Sample preparation:
For the purposes of this 'blind' study, a sample of essential oil was diluted 200:1 in ethyl acetate prior to analysis.

GC:

Injector:	Split/splitless
Liner:	Single taper, deactivated, 4 mm (i.d.)
Carrier gas:	Helium, constant-flow at 0.8 mL/min
Mode:	Split 200:1
Temperature:	250 °C
Septum purge:	On, 3 mL/min

2D column set (normal):

1st dimension:	DB5, 30 m × 0.32 mm × 0.25 µm
2nd dimension:	BPX50, 1.25 m × 0.10 mm × 0.10 µm
Modulation delay loop:	1.5 m × 0.10 mm fused silica

Temperature program (normal):

Main oven:	50 °C (2 min), 5 °C/min to 310 °C (12 min)
Secondary oven:	No offset
Hot jet:	150 °C (2 min), 5 °C/min to 400 °C (hold time matched to total run time)
Modulation period:	6 s, hot-jet pulse 350 ms
Total run time:	66 min

2D column set (inverse):

1st dimension:	Rxi®-17, 20 m × 0.18 mm × 0.18 µm
2nd dimension:	VF-5ms™, 1.9 m × 0.15 mm × 0.15 µm
Modulation delay loop:	1.0 m, as for 2nd dimension

Temperature program (inverse):

Main oven:	50 °C (2 min), 5 °C/min to 320 °C (12 min)
Secondary oven:	65 °C (2 min), 5 °C/min to 335 °C (hold time matched to total run time)
Hot jet:	150 °C (2 min), 5 °C/min to 400 °C (hold time matched to total run time)
Modulation period:	4 s, hot-jet pulse 350 ms
Total run time:	68 min

TOF MS:

Instrument:	BenchTOF-Select™ (Markes International)
Filament voltage:	1.8 V
Ion source:	280 °C
Transfer line:	300 °C
Mass range:	m/z 40–500
Data rate:	100 Hz in Tandem Ionisation mode at 70 eV and 12 eV

Software:

Image processing:	*GC Image*™ (GC Image, LLC)

Results and discussion

Initial investigations showed that considerably better spatial separation was achieved with an inverse (polar–non-polar) column set than with a conventional (non-polar–polar) setup (Figure 1).

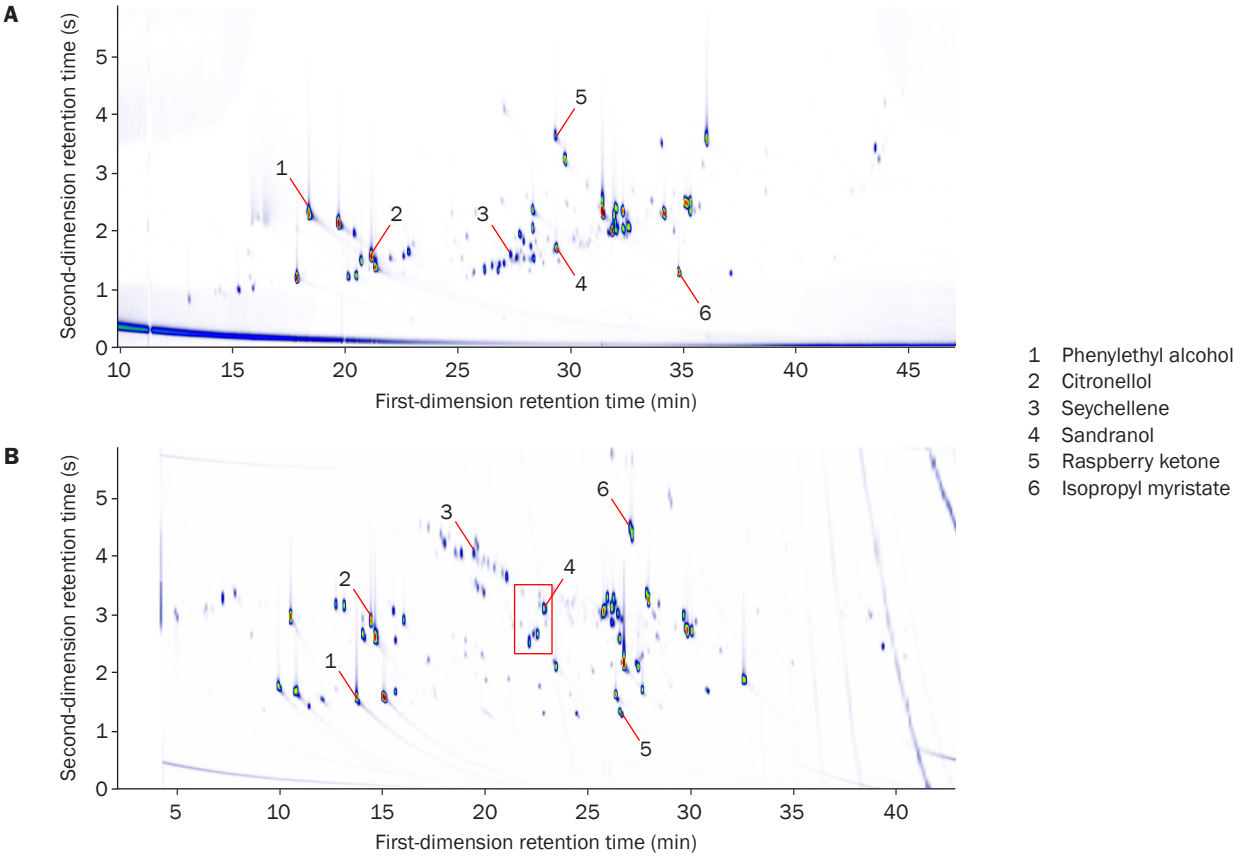

Figure 1: *GC Image*-rendered colour plots for the diluted essential oil using both (**A**) normal and (**B**) inverse column sets, with a selection of compounds highlighted to show relative elution order. The red box indicates the region expanded in Figure 2.

The chromatographic space occupation within a GC×GC contour plot can be calculated using a simple equation described by Omais *et al.*[1] It was found that the normal-phase column set used 83% of the available chromatographic space, while the inverse column set gave an improvement in orthogonality, using 92% of the total space. This enhanced peak capacity enabled more confident characterisation of the full chemical composition, which is required in the strict quality control of raw materials used in the production of fragrances.

A selection of the major components identified in the essential oil (by screening against NIST 14) are listed in Table A1 (see Appendix). The fact that high match factors are achieved for compounds across a range of chemical classes shows that GC×GC with BenchTOF is a powerful tool for confident chemical fingerprinting of complex samples that have not been subjected to extensive sample preparation.

The excellent sensitivity and spectral quality afforded by BenchTOF mass spectrometers ensures both high-loading and trace analytes can be confidently identified (Figure 2). Figure 3 illustrates the ability of BenchTOF to produce 'reference-quality' spectra for these compounds, by comparison with the NIST 14 library. In particular, note the high-quality matching of the m/z 189/190 and m/z 204/206 signals in nerolidol, which other TOF instruments would struggle to record.

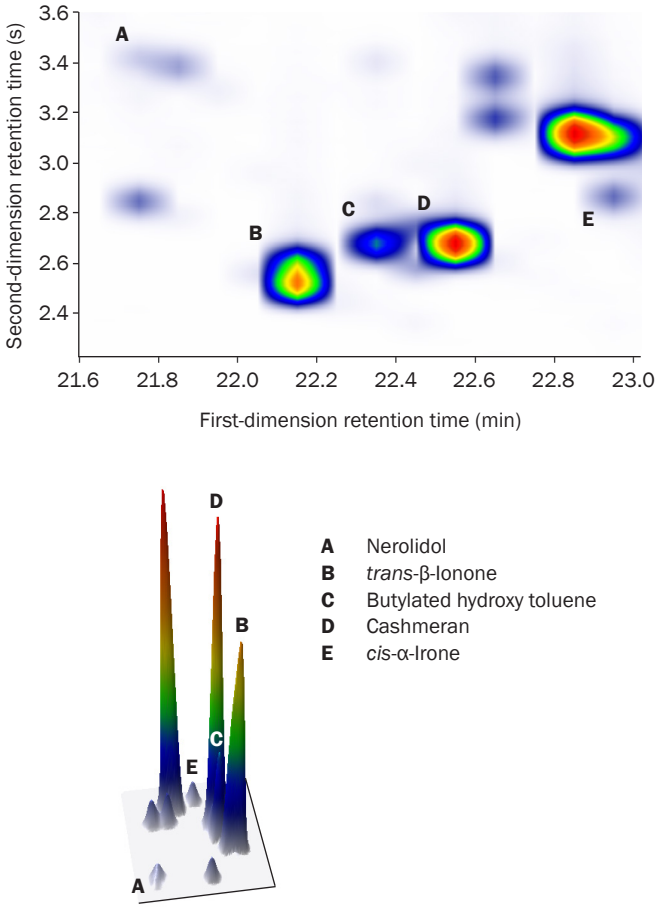

Figure 2: *GC Image*-rendered colour plot (top) with 3D rendering (bottom), illustrating the excellent separation and symmetrical peak shape for both high- and low-loading components.

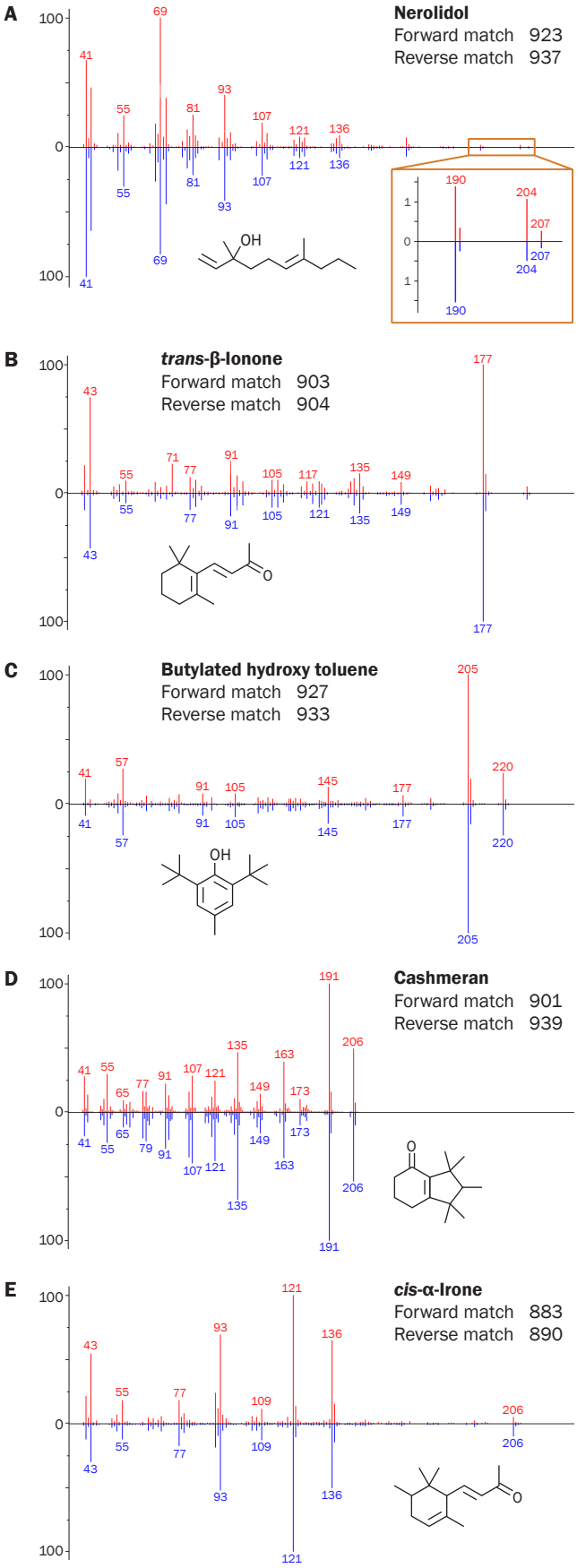

Figure 3: Comparison of the spectra for compounds **A–E** (top, red) with the NIST 14 library spectra (bottom, blue).

Increased confidence with Tandem Ionisation

The essential oil sample was analysed in Tandem Ionisation mode – meaning that both 70 eV and 12 eV data were acquired simultaneously. A selection of spectral comparisons are provided in Figure 4. Soft ionisation gave both increased intensity for the molecular ion and reduced fragmentation, resulting in simplified, more selective spectra. The intensity improvement for the important 'diagnostic' ions provides enhanced detection limits and increased confidence in the identification of ultra-trace components. Moreover, unlike other soft ionisation techniques, soft EI with Tandem Ionisation retains a degree of fragmentation, aiding structural elucidation (especially of isomers) and allowing easy library-matching.[2]

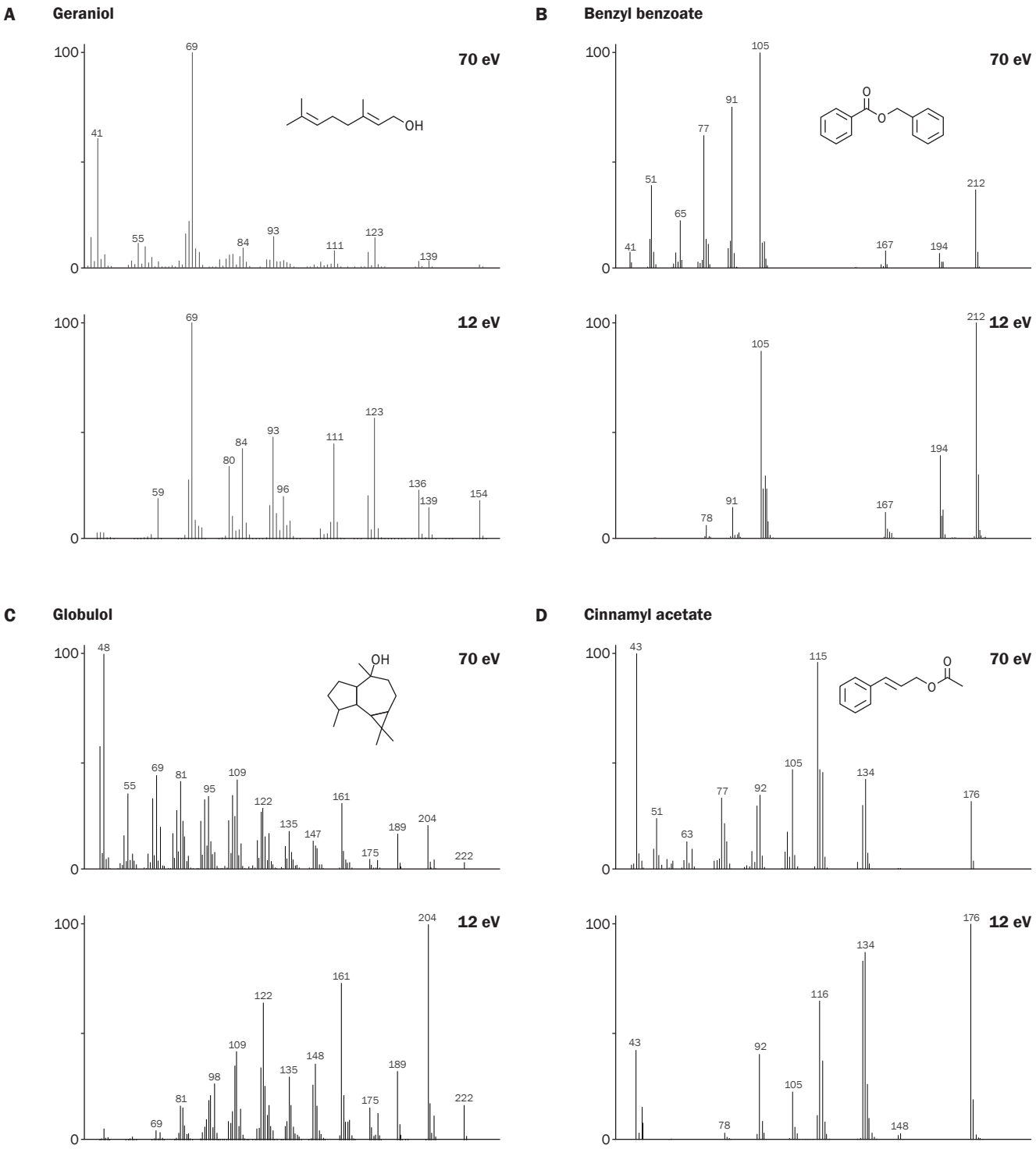

Figure 4: Spectral comparisons between 70 eV and 12 eV for a selection of compounds from the essential oil.

Conclusions

In this Application Note, we have shown that GC×GC–TOF MS can be successfully applied to the analysis of complex essential oils. Excellent compound separation was achieved, with BenchTOF providing 'reference-quality' spectra for both high- and low-concentration components. This combination ensures that both confident identification and accurate quantitation are achieved for complex mixtures – a fact that is particularly important for quality control within the fragrance industry. Furthermore, Tandem Ionisation delivers an extra level of information on sample composition, by providing enhanced confidence for the identification of challenging analytes, with no compromise on sensitivity and no additional analysis time.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

References

1. B. Omais, M. Courtiade, N. Charon, D. Thiébaut, A. Quignard and M.-C. Hennion, Investigating comprehensive two-dimensional gas chromatography conditions to optimize the separation of oxygenated compounds in a direct coal liquefaction middle distillate, *Journal of Chromatography A*, 2011, 1218: 3233–3240, http://dx.doi.org/ 10.1016/j.chroma.2010.12.049.
2. More examples of spectra acquired using Select-eV can be viewed in the Select-eV Library at www.select-ev.com.

Trademarks

Appendix

Compound	First-dimension retention time (min)	Second-dimension retention time (s)	Match factor	Peak volume (TIC)
α-Pinene	4.9	3.06	941	2.35×10^6
β-Pinene	6.4	3.14	940	1.82×10^6
β-Myrcene	6.7	3.10	899	4.85×10^5
3-Carene	7.2	3.30	906	6.60×10^6
Limonene	7.8	3.38	947	3.26×10^6
β-Phellandrene	8.0	3.26	914	6.33×10^5
o-Cymene	8.6	2.66	930	8.26×10^5
γ-Terpinene	9.0	3.18	796	2.50×10^5
Benzaldehyde	9.2	1.36	940	3.12×10^5
Dihydromyrcenol	9.3	3.18	810	1.11×10^5
Bis(2-hydroxypropyl) ether	9.9	1.78	883	2.02×10^7
Linalool	10.5	2.98	907	3.52×10^7
1,3-Dipropylene glycol	10.8	1.70	900	2.65×10^7
Rose oxide	10.9	2.94	910	4.52×10^5
Benzyl alcohol	11.4	1.42	913	4.23×10^6
1-Phenylethanol	11.8	1.60	895	2.19×10^5
2-(2-Hydroxy-propoxy)propan-1-ol	12.1	1.52	895	3.43×10^6
Acetophenone	12.4	1.50	896	7.47×10^4
Methyl benzoate	12.7	1.72	911	9.66×10^6
Dihydrocitronellol	12.7	3.48	864	3.90×10^5
Ethyl linalool (pk 1)	12.7	3.18	930	5.87×10^5
Ethyl linalool (pk 2)	13.1	3.16	928	1.47×10^7
Menthone	13.4	2.54	923	2.16×10^5
Phenylethyl alcohol	13.7	1.58	898	3.47×10^7
Florosa	14.0	2.68	905	1.81×10^7
Citronellol	14.4	2.90	915	2.69×10^7
Florol	14.6	2.62	893	4.92×10^7
Linalyl acetate	14.9	2.94	878	1.92×10^5
Benzyl acetate	15.0	1.60	890	4.51×10^7
Citronellyl formate	15.3	3.20	906	2.21×10^5
Phenethyl formate	15.3	1.68	866	7.75×10^4
Methyl salicylate	15.4	1.94	927	1.13×10^5
Geraniol	15.6	2.56	936	6.67×10^6
Gardenol	15.6	1.68	916	8.54×10^4
Cyprotene	15.6	4.44	853	4.27×10^6
Elemene	15.8	4.40	826	1.02×10^5
Mayol	16.0	2.92	926	1.10×10^7
Ethyl maltol	16.4	1.48	899	2.62×10^5
Copaene	16.8	4.58	921	5.30×10^5
3-Phenylpropanol	17.0	1.66	898	1.76×10^5
Hydroxycitronellol	17.2	2.22	911	1.99×10^6
β-Patchoulene	17.2	4.50	921	1.60×10^6
4-Phenylbutan-2-one	17.5	1.62	903	3.21×10^5

Table A1: Identities, retention times, match factors and peak volumes of the compounds identified in the essential oil using the inverse column set *(continued on next page)*.

Compound	First-dimension retention time (min)	Second-dimension retention time (s)	Match factor	Peak volume (TIC)
Phenylethyl acetate	17.5	1.70	930	4.68×10^5
Cyperene	17.8	4.40	896	1.88×10^6
Dihydrosafrole	17.9	2.16	832	6.01×10^4
α-Gurjunene	18.0	4.24	918	5.84×10^6
cis-Thujopsene	18.2	4.36	837	7.75×10^5
Caryophyllene	18.5	4.08	942	3.22×10^6
α-Guaiene	18.8	4.06	945	6.17×10^6
Geranyl acetate	19.0	2.56	908	9.35×10^5
Hydroxycitronellol	19.0	2.32	870	8.76×10^5
Seychellene	19.4	4.08	938	6.38×10^6
1-Phenylbutan-2-ol	19.5	2.48	807	1.70×10^5
Benzyl butyrate	19.5	1.96	891	2.45×10^5
Humulene	19.6	3.94	903	3.30×10^6
Ebanol (pk 1)	19.6	3.46	919	9.31×10^5
Damascenone	19.8	2.32	910	1.39×10^6
Ebanol (pk 2)	19.9	3.38	914	4.47×10^6
Eugenol	20.0	1.88	928	9.43×10^5
α-Guaiene	20.8	3.72	898	2.40×10^6
α-Bulnesene	21.0	3.66	945	7.87×10^6
Piperonal	21.0	1.40	929	7.41×10^4
Jasmone	21.1	1.90	912	9.98×10^5
Cashmeran (pk 1)	21.3	2.82	911	5.81×10^5
Cashmeran (pk 2)	21.7	2.84	862	8.97×10^5
cis-Nerolidol	21.8	3.38	914	1.21×10^6
trans-β-Ionone	22.1	2.52	903	1.02×10^7
Butylated hydroxy toluene	22.3	2.66	927	3.02×10^6
Cashmeran (pk 3)	22.5	2.68	905	1.24×10^7
trans-Nerolidol	22.6	3.34	923	1.30×10^6
Cinnamyl acetate	22.6	1.76	933	1.28×10^6
Sandranol	22.8	3.12	917	1.85×10^7
Vanillin	22.8	1.30	922	1.36×10^6
cis-α-Irone	22.9	2.86	824	1.20×10^6
Caryophyllenyl alcohol	23.3	3.44	888	1.19×10^6
Phenoxanol	23.4	2.10	898	1.49×10^7
Caryophyllene oxide	24.0	3.04	870	1.83×10^5
Phenylethyl isovalerate	24.0	2.22	853	2.09×10^5
Methyl isoeugenol	24.1	1.62	906	4.85×10^5
trans-2-Hydroxy-cinnamic acid	24.4	1.30	927	2.94×10^6
Jasmine lactone	24.5	1.54	798	1.90×10^5
Isobutylquinolene	24.5	1.96	909	2.26×10^5
Methyl piperonyl ketone	24.7	1.40	887	1.27×10^5
Globulol	24.8	3.12	825	1.99×10^5
8-*epi*-γ-Eudesmol	25.0	3.06	824	8.95×10^4
Eugenol acetate	25.1	1.46	840	1.17×10^5
Peach lactone	25.2	2.02	926	1.06×10^6
Isocyclemone E	25.7	3.06	925	3.93×10^7
Aromandendrene	25.9	3.30	816	1.30×10^7
Neophytadiene	26.1	5.80	936	2.79×10^6
sec-Butylquinoline	26.1	2.14	906	8.77×10^5
Patchouli alcohol	26.1	3.14	913	2.48×10^7
Helional	26.3	1.62	911	1.76×10^7
Raspberry ketone	26.5	1.34	936	1.35×10^7
cis-Hex-3-enyl salicylate	26.5	2.60	909	1.47×10^7
Cepionate	26.7	2.18	915	6.78×10^7
Isopropyl myristate	27.1	4.40	929	3.38×10^7
α-Santalol	27.4	2.66	854	6.96×10^5
Triethyl citrate	27.6	1.70	879	1.22×10^7
Cyperenone	27.9	2.38	900	5.54×10^5
Ambrox	27.9	3.26	897	3.90×10^7
2-Ethylhexyl salicylate	28.4	3.18	818	2.40×10^6
Vanillylacetone	28.5	1.42	893	4.96×10^5
Veramoss	29.0	1.76	896	2.80×10^5
Isophytol	28.9	5.08	929	3.13×10^6
Methyl hexadecanoate	29.4	4.16	744	9.34×10^4
Pentadecanolide	29.6	3.00	933	1.40×10^7
Habanolide	29.8	2.74	909	3.84×10^7
Ambretone	30.2	2.88	876	1.71×10^6
Nootkatone	30.3	2.30	820	9.27×10^4
Benzyl benzoate	30.8	1.68	922	4.12×10^6
Benzyl salicylate	32.5	1.88	873	2.67×10^7
Phytol	32.7	4.36	879	1.96×10^5
Phytol acetate	34.6	4.16	887	2.81×10^5
Ketodecanolide	34.9	2.18	872	5.03×10^5
Hercolyn	38.7	3.00	863	1.84×10^6
Octinoxate	39.3	2.46	899	4.00×10^6
Methyl dehydroabietate	40.4	2.28	862	5.15×10^5

Table A1 *(continued)*.