Supplementary Materials

Pharmacognostic Evaluation and HPLC–PDA and HS–SPME/GC–MS Metabolomic Profiling of *Eleutherococcus senticosus* Fruits

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Abstract: *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim. is a medicinal plant used in Traditional Chinese Medicine (TCM) for thousands of years. However, due to the overexploitation, this species is considered to be endangered and is included in the Red List, e.g., in the Republic of Korea. Therefore, a new source of this important plant in Europe is needed. The aim of this study was to develop pharmacognostic and phytochemical parameters of the fruits. The content of polyphenols (eleutherosides B, E, E1) and phenolic acids in the different parts of the fruits, as well as tocopherols, fatty acids in the oil, and volatile constituents were studied by the means of chromatographic techniques [HPLC with Photodiode-Array Detection (PDA), headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS–SPME/GC–MS)]. To the best of our knowledge, no information is available on the content of eleutherosides and phenolic acids in the pericarp and seeds. The highest sum of eleutheroside B and E was detected in the whole fruits (1.4 mg/g), next in the pericarp (1.23 mg/g) and the seeds (0.85 mg/g). Amongst chlorogenic acid derivatives (3-CQA, 4-CQA, 5-CQA), 3-CQA was predominant in the whole fruits (1.08 mg/g), next in the pericarp (0.66 mg/g), and the seeds (0.076 mg/g). The oil was rich in linoleic acid (C18:3 (n-3), 18.24%), ursolic acid (35.72 mg/g), and α-tocopherol (8.36 mg/g). The presence of druses and yellow oil droplets in the inner zone of the mesocarp and chromoplasts in the outer zone can be used as anatomical markers. These studies provide a phytochemical proof for accumulation of polyphenols mainly in the pericarp, and these structures may be taken into consideration as their source subjected to extraction to obtain polyphenol-rich extracts.

Keywords: *Eleutherococcus senticosus*; fruits; eleutherosides; nutri-pharmacological; metabolomics; herbs

Table S1. Chromatographic parameters and calibration data for quantification of investigated eleutherosides and phenolic acids.

Compound	Retention time (min.)	Theoretical plates	Linear regression equation	Concentration range (μg/mL)	Correlation coefficient (r)	LOD (μg/mL)	LOQ (μg/mL)
Eleutheroside B	10.55 ± 0.02	5369	y=133974820x + 6183	0.53-8.00	0.9991	0.35	1.05
Eleutheroside E	20.42 ± 0.02	126464	y=498433022x - 104028	0.53-8.00	0.9934	0.97	2.94
Eleutheroside E1	26.81 ± 0.02	41330	y=392169263x − 28199	1.20-18.00	0.9998	0.42	1.26
Protocatechuic acid	10.59 ± 0.02	17565	y=266763646x + 17984	0.75-7.50	0.9996	0.33	1.02
Neochlorogenic acid	13.72 ± 0.02	16088	y=220873737x + 14961	0.61-6.10	0.9941	0.34	1.03
Chlorogenic acid	24.27 ± 0.03	69256	y=220896903x − 52187	2.60-26.00	0.9997	0.35	1.07
Cryptochlorogenic acid	24.80 ± 0.03	51230	y= 236829164x + 16 678	0.58-5.80	0.9998	0.35	1.07

LOD and LOQ were calculated as follows: LOD = 3.3 d/S and LOQ = 10 d/S where: d=the standard deviation of y-intercept of the regression line. S = the average slope of regression lines.

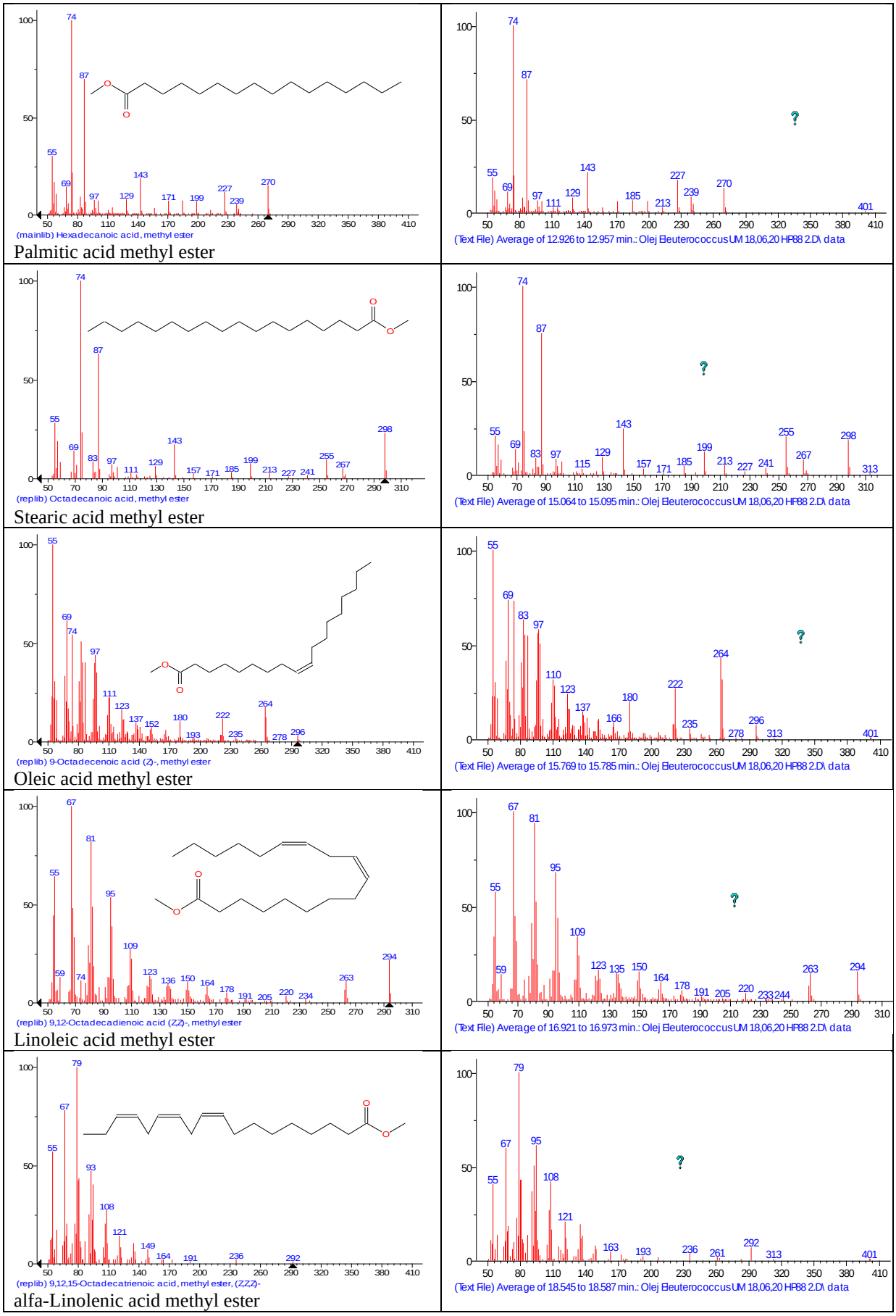
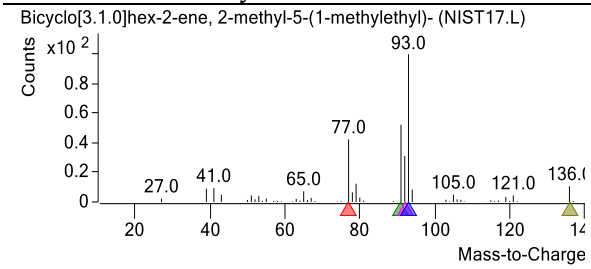
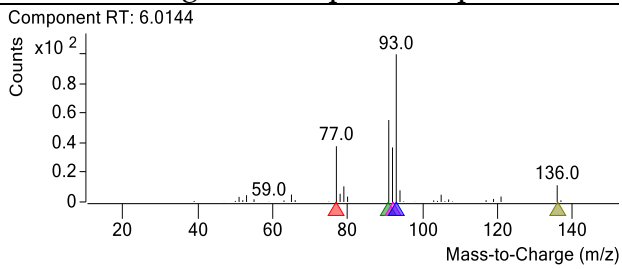


Fig. S1. Mass spectra for standards (based on the NIST database) and investigated fatty acids.

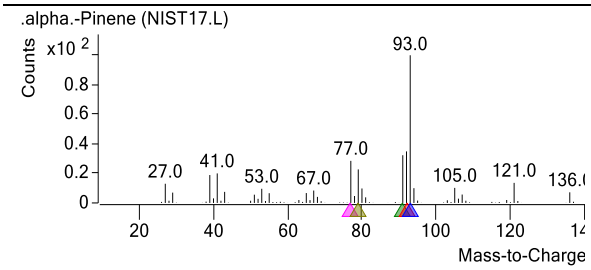
Mass spectra of HS-SPME GC-MS investigated compounds

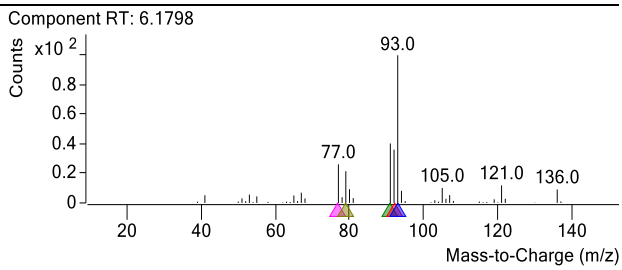
Library search results	Investigated compounds spectra



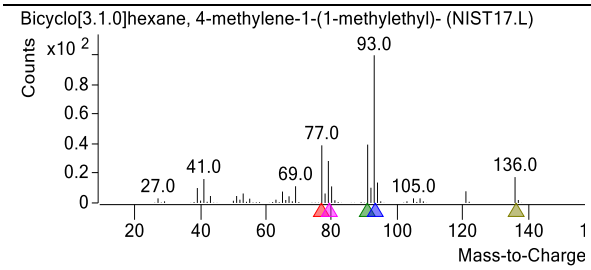


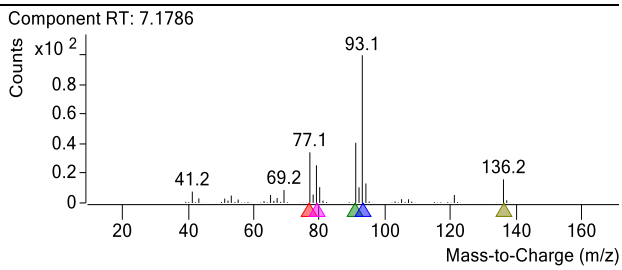
1. α-Thujene



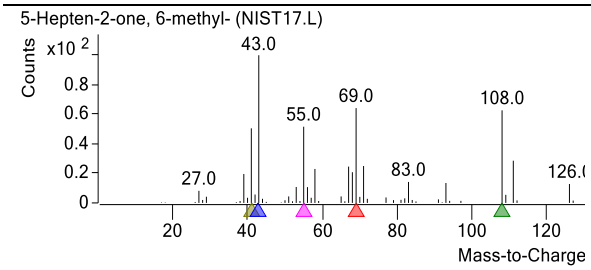


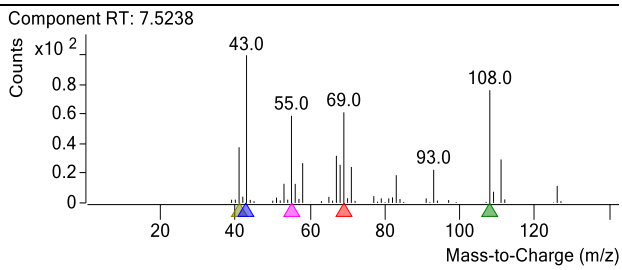
2. α-Pinene



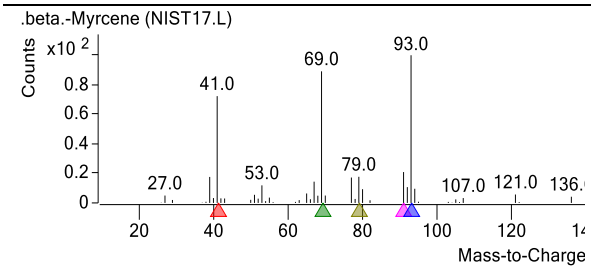


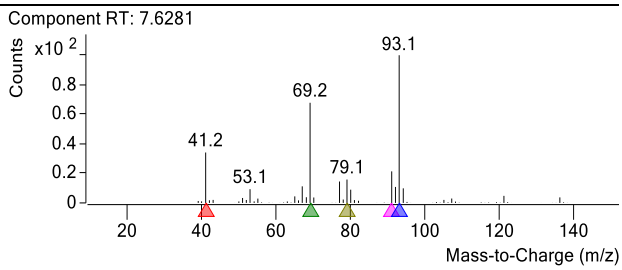
3. (Z)-Sabinene



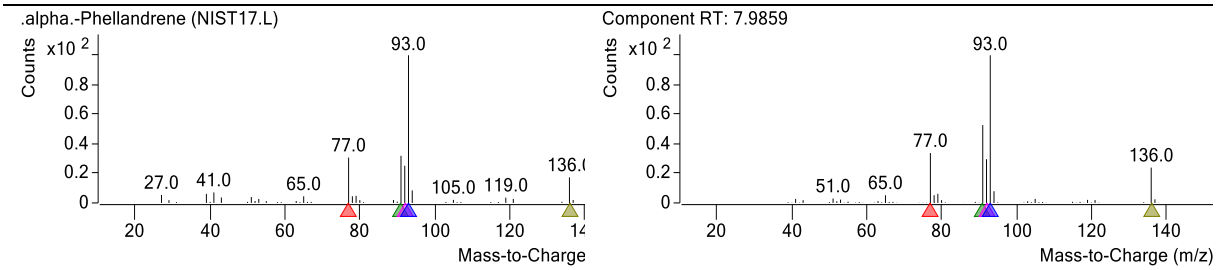


4. 6-methyl-5-hepten-2-one





5. β-Myrcene



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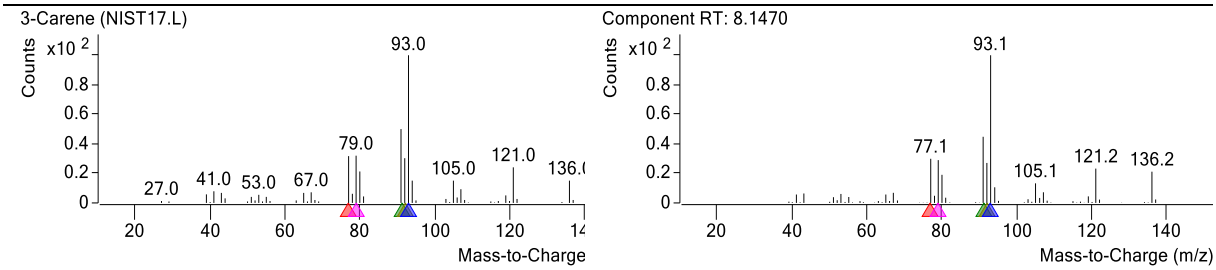
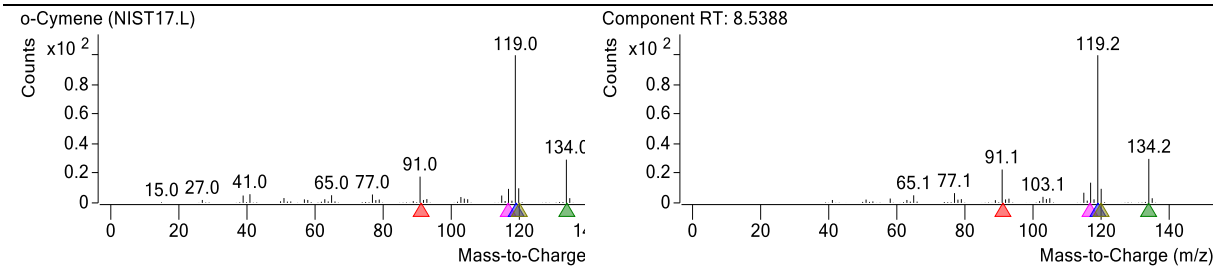
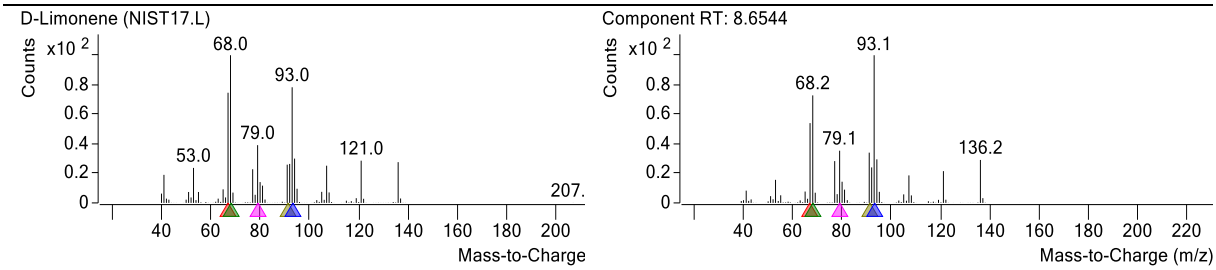


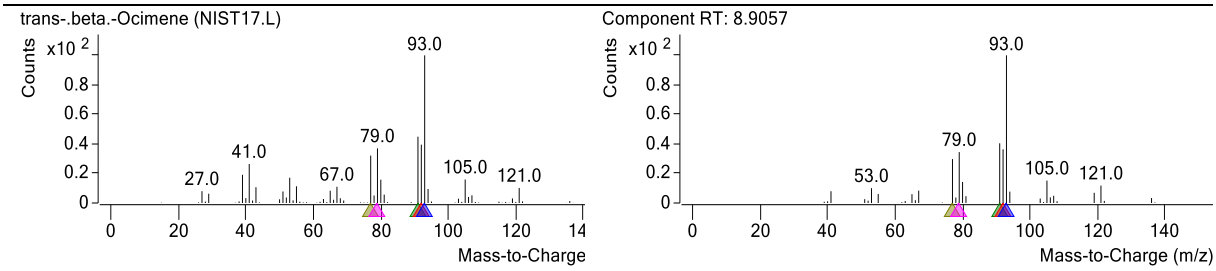
7. 3-Carene



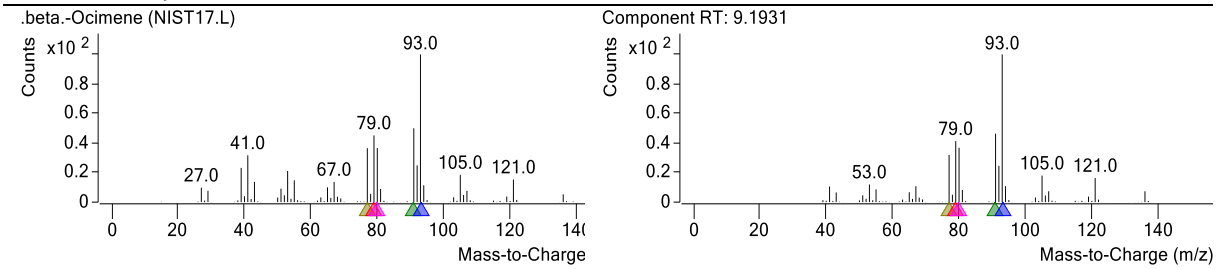
8. o-Cymene



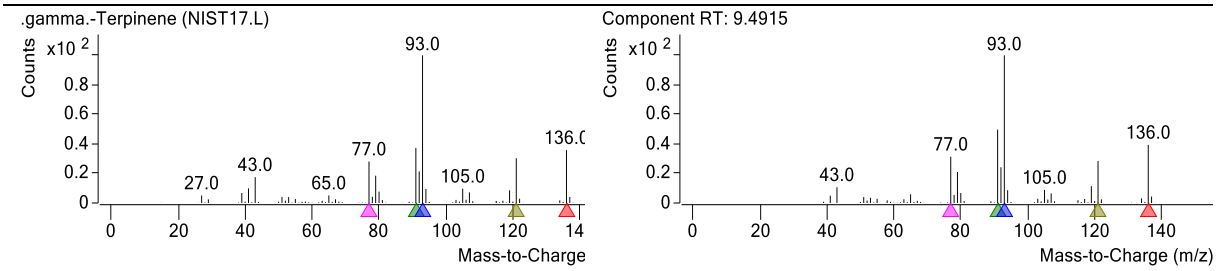
9. Limonene



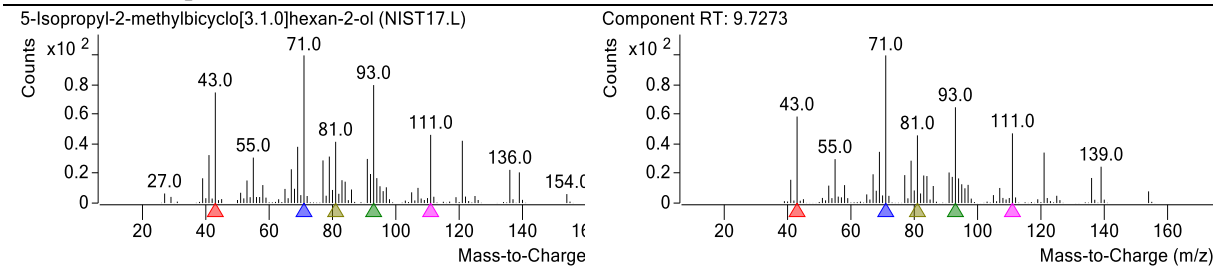
10. (Z)-β-Ocimene



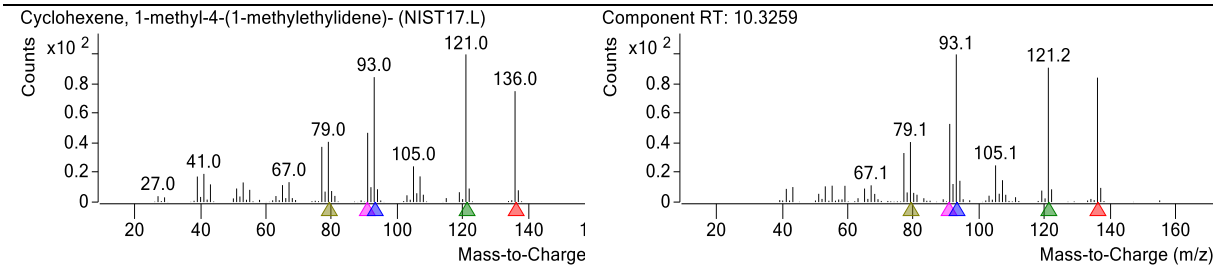
11. (E)-β-Ocimene



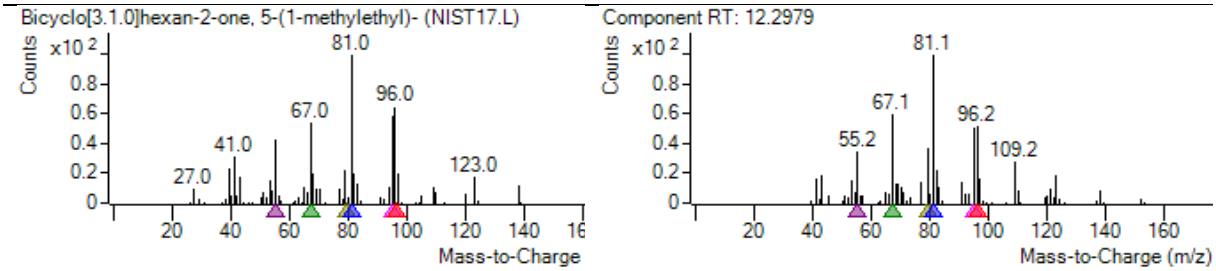
12. γ-Terpinene



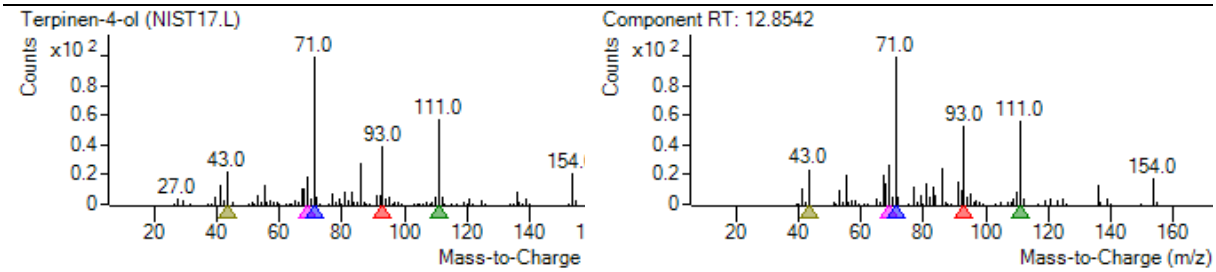
13. (Z)-Sabinene hydrate



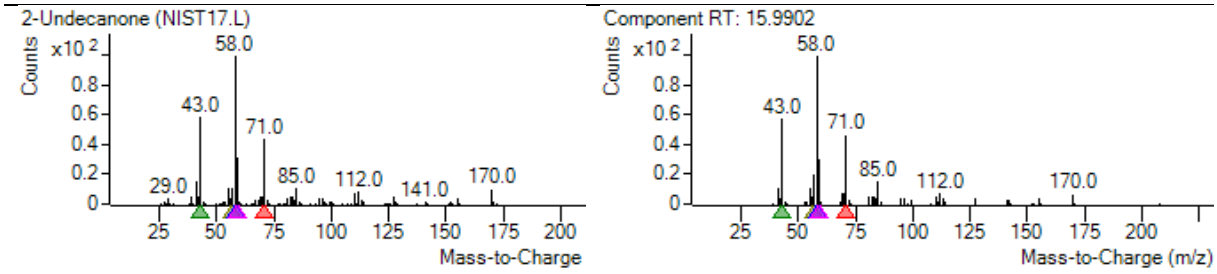
14. Terpinolene



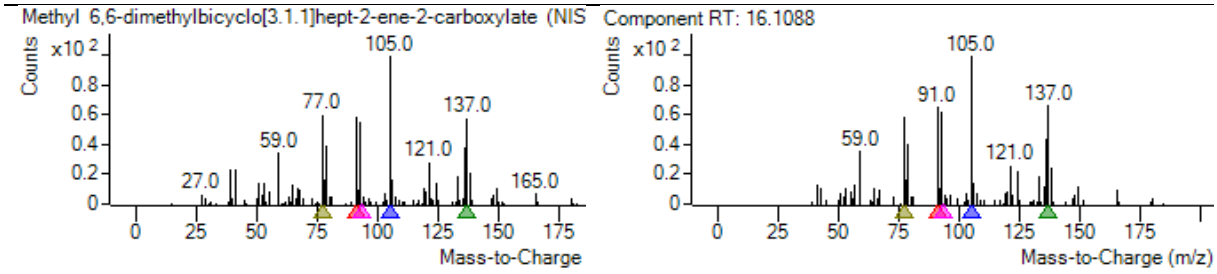
15. Sabina ketone



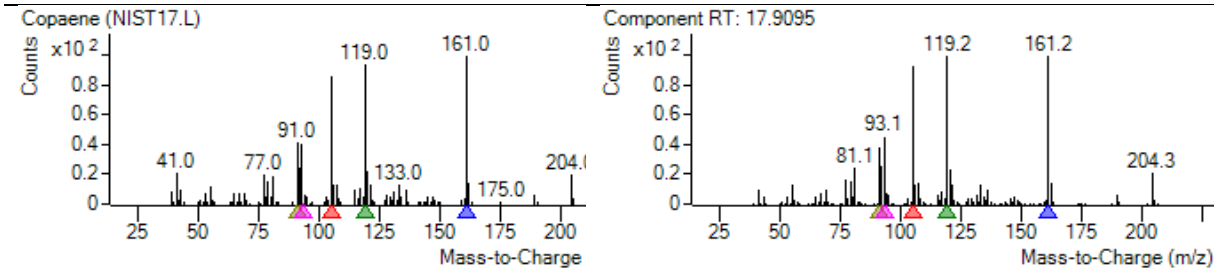
16. Terpinen-4-ol



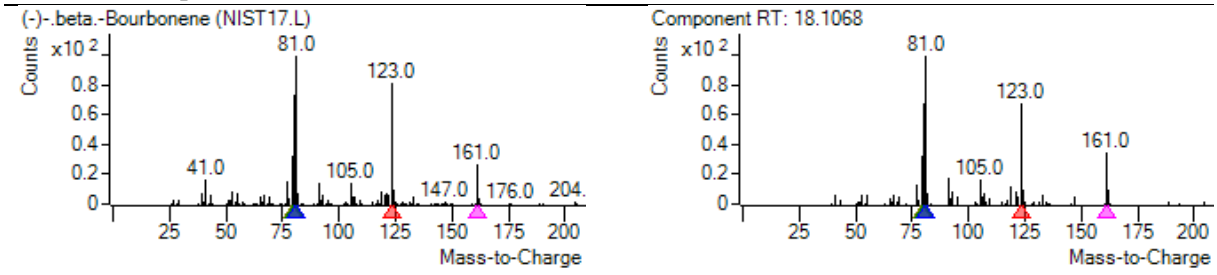
17. 2-Undecanone (IS)



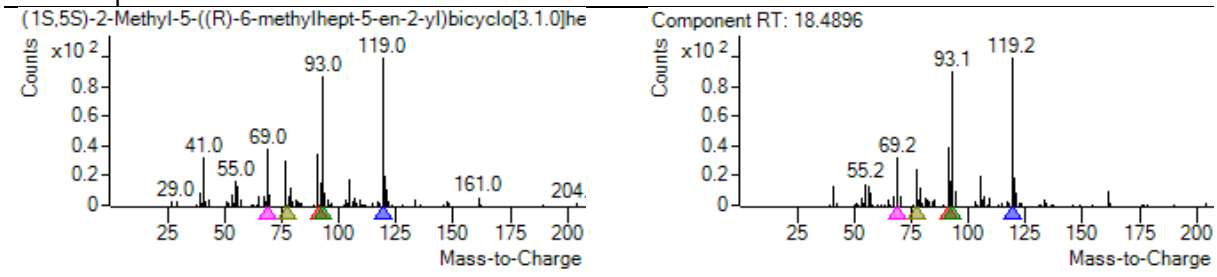
18. Methyl myrtenate



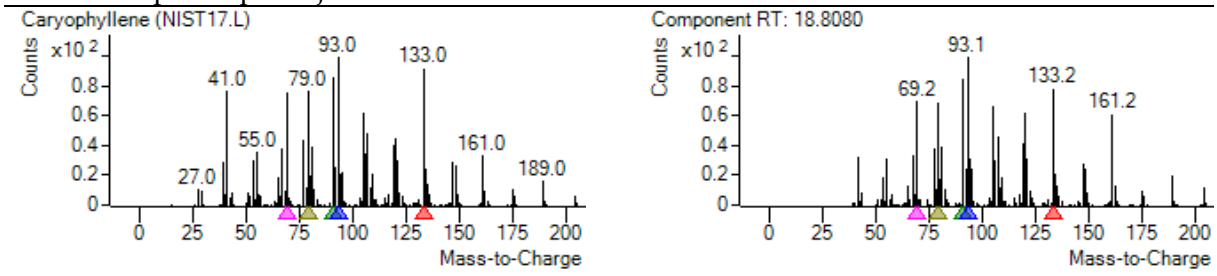
19. α-Copaene



20. β-Bourbonene



21. 7-epi-Sesquithujene



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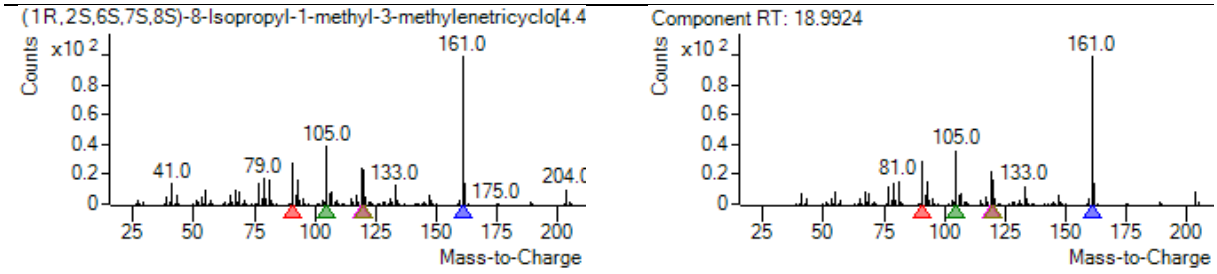
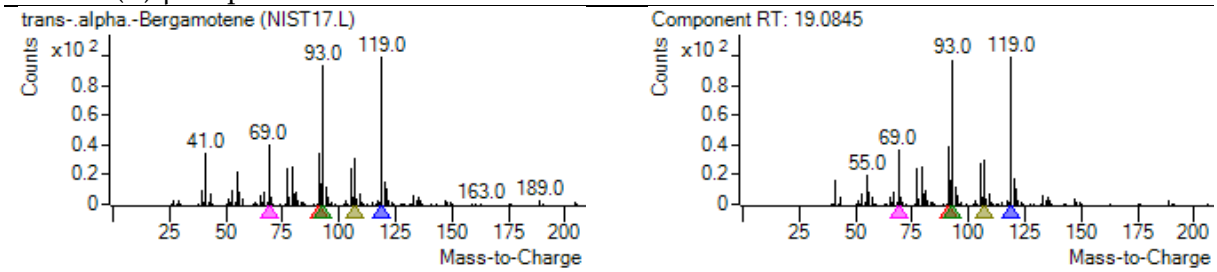
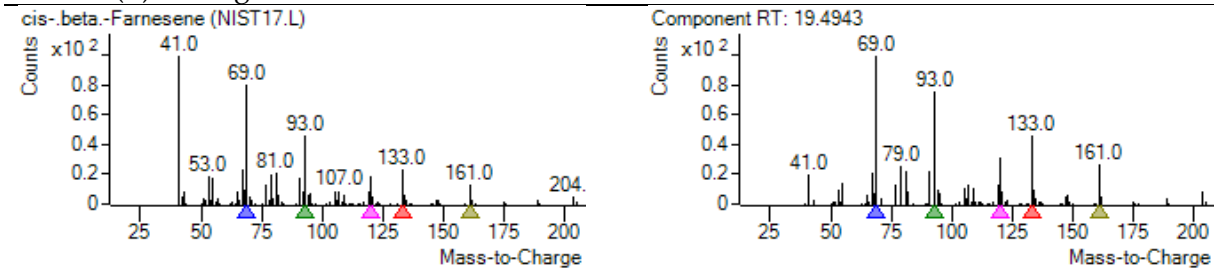


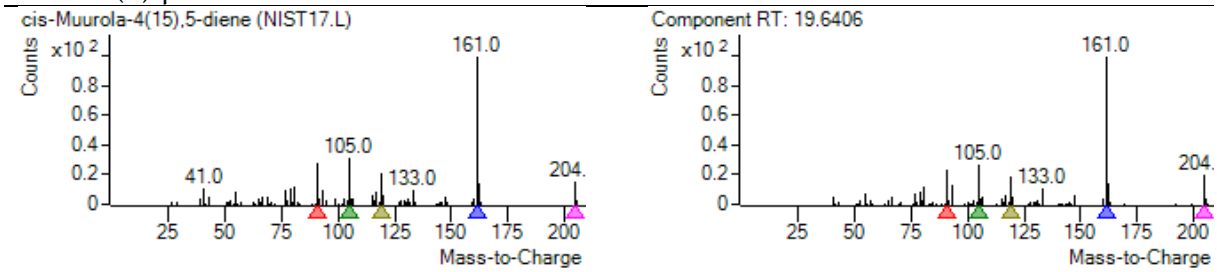
23. (Z)-β-Copaene



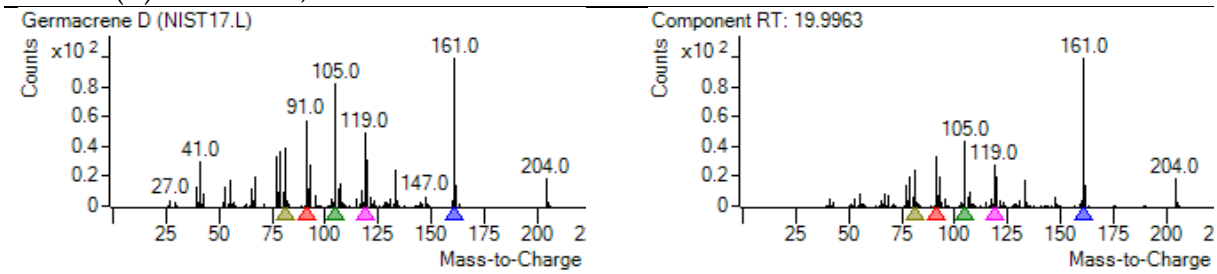
24. (E)-α-Bergamotene



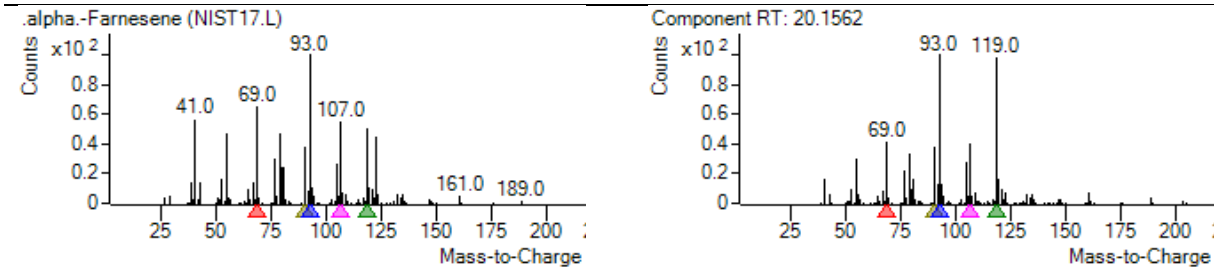
25. (E)-β-Farnesene



26. (Z)-Muurola-4,5-diene



27. Germacrene D



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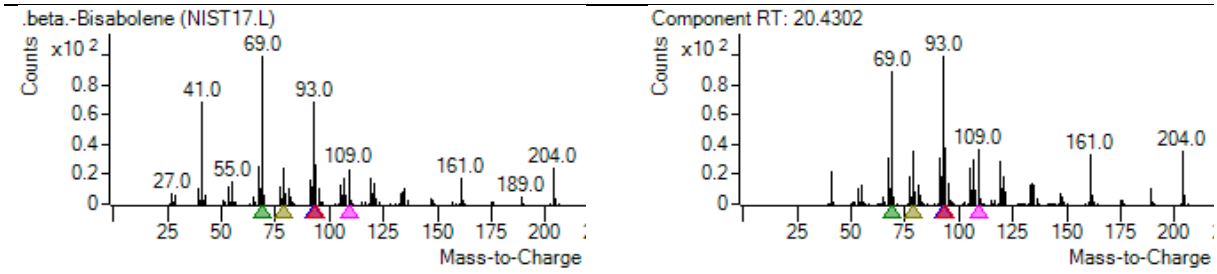
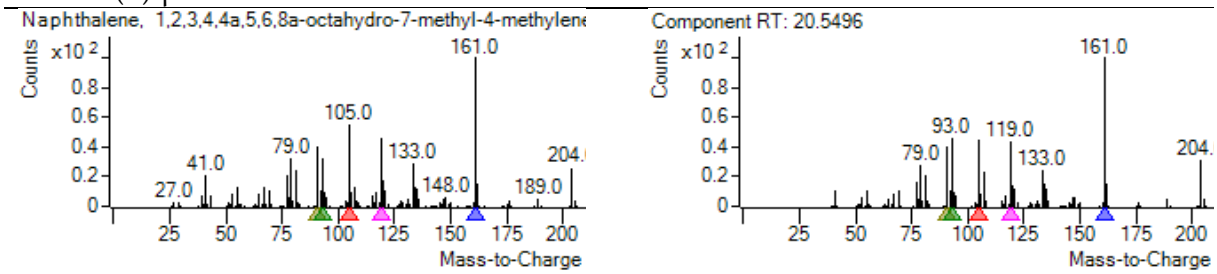
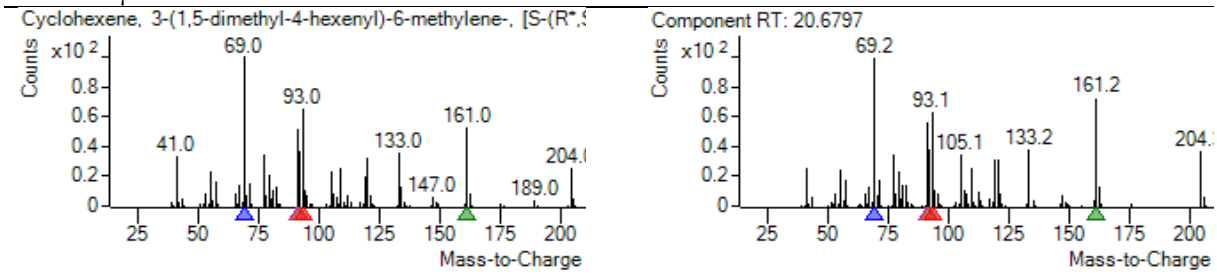


29. (E)-β-Bisabolene



30. γ-Cadinene



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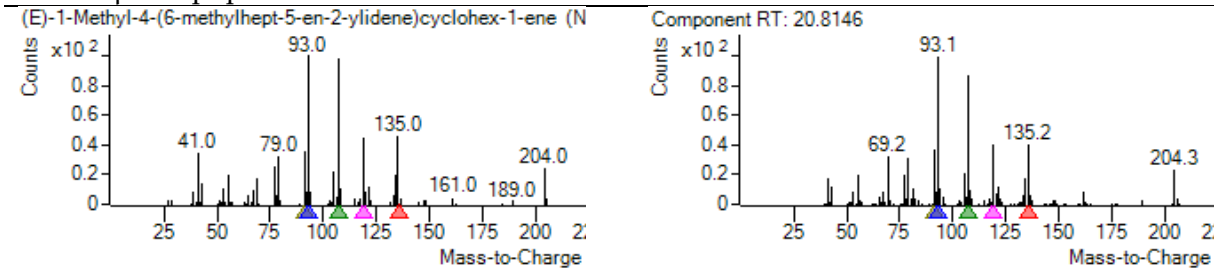
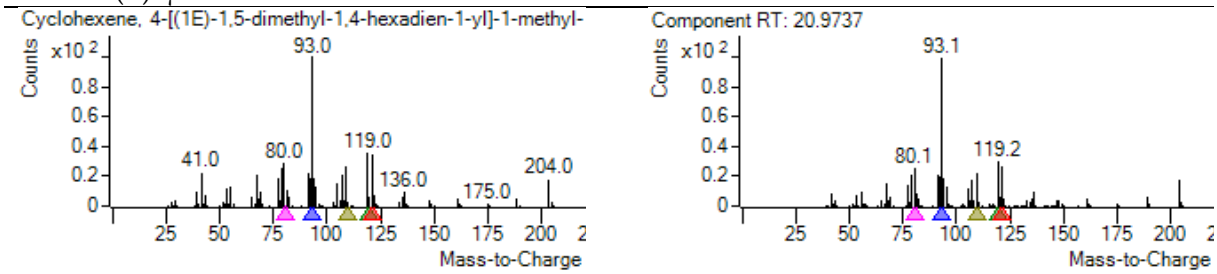


32. (E)-γ-Bisabolene



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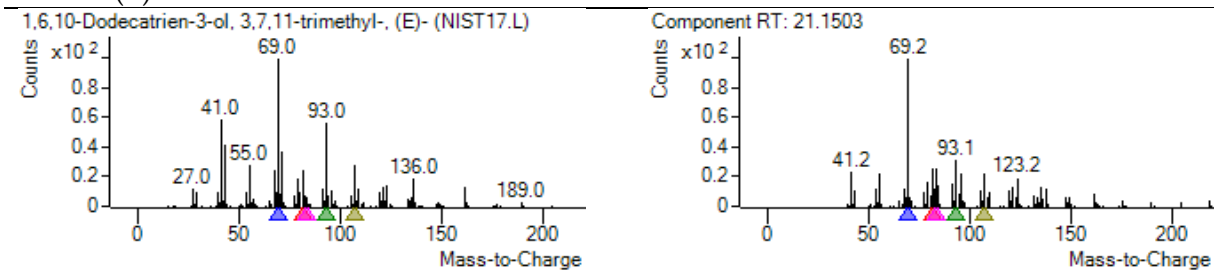
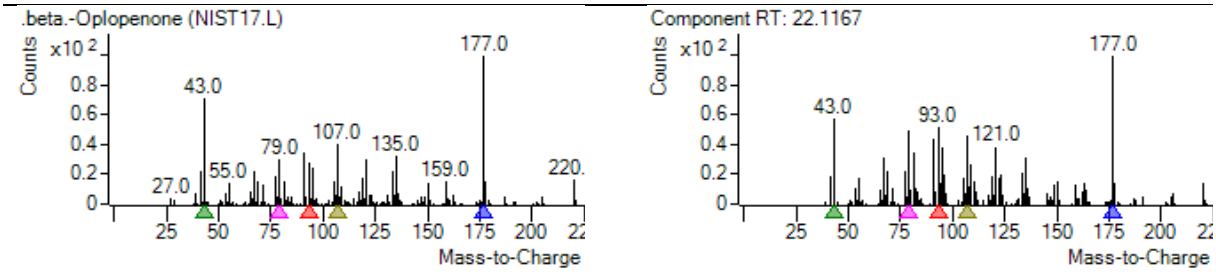
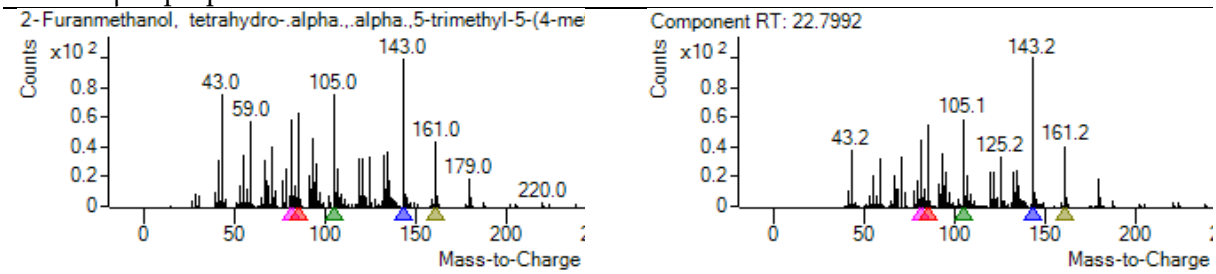


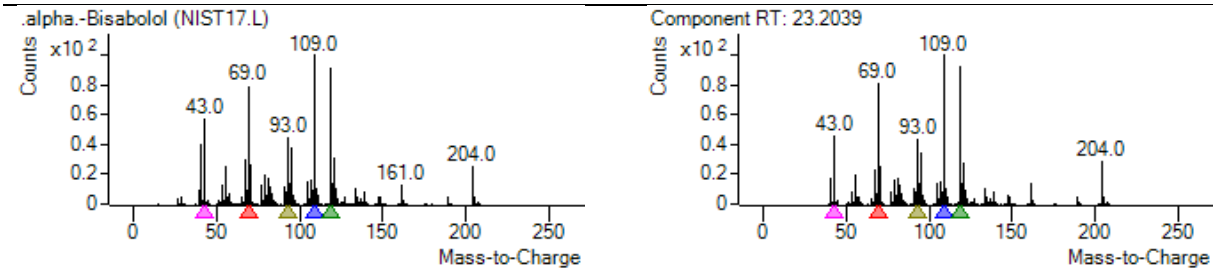
34. (E)-Nerolidol



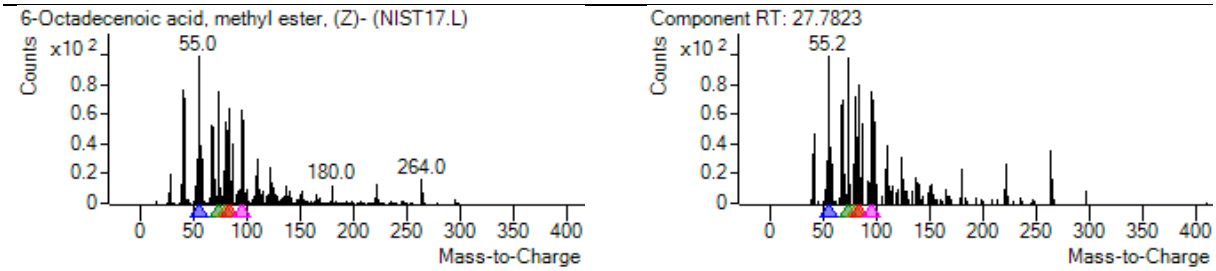
35. β-Oplopenone



36. α-Bisabolol oxide B



37. α-Bisabolol



38. 6-Octadecenoic acid, methyl ester, (Z)-

Fig. S2 Mass spectra of HS-SPME/GC-MS investigated compounds.