

GC-MS – Screening – Flavor and Fragrances

Screening of Terpenes with Solvent-Mediated Chemical Ionization

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Abstract

Gas chromatography-mass spectrometry (GC-MS) is a crucial analytical tool to identify terpenes. Despite being supported by large databases with retention index information, the identification of terpenes can be challenging due to the absence of molecular ion peaks in electron ionization mass spectra. Mass spectra produced by chemical ionization could instead provide ionized species of the analyte molecule for molecular weight determination. This article demonstrates the usage of solvent-mediated chemical ionization (SMCI) as a complementary technique to electron ionization. The mass spectra produced by SMCI were examined and utilized for quick screening of 21 terpenes consisting of monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. This proof-of-concept strategy can be further extended to wider coverage of terpene molecules.



Terpenes are aromatic compounds responsible for the characteristic smell of lavender (left) and orange peel (right).

Keywords:
GCMS-QP2020 NX, Solvent-Mediated Chemical Ionization, Terpenes

Highlights

- Perform chemical ionization safely with SMCI setup since it requires only common laboratory solvents as reagent gas
- Chemical ionization provides complementary mass spectral information to electron ionization for identification of terpenes in complex mixtures
- Mass spectra produced by SMCI can be used for quick screening of terpenes

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GCMS-QP2020 NX



Solvent Mediated Chemical Ionization (SMCI)



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1. INTRODUCTION

The application of the positive chemical ionization (CI) technique in gas chromatography-mass spectrometry (GC-MS) has developed into a powerful tool for the identification of organic molecules. In contrast to the electron ionization (EI) technique which utilizes electrons to ionize analytes, the CI technique is effected by gas-phase ion-molecule interaction. CI produces complementary information to EI. EI is a hard ionization technique that results in maximum fragmentation using high-energy electrons such that the molecular ions of the analyte are fully consumed. Despite that, the reproducibility of the EI spectra has allowed the creation and use of mass spectral databases for unknown identification. As CI is a soft ionization technique, it retains the ionized species of the analyte molecule (i.e., M^{+} , $[M+H]^{+}$, $[M-H]^{+}$, $[M+adduct]^{+}$) and enables the determination of its molecular weight.

The conventional CI technique relies on the usage of methane, isobutane, or ammonia as reagent gas. This requires the use of flammable gas cylinders in the laboratory. The solvent-mediated chemical ionization (SMCI) technique, an alternative procedure, enables CI techniques to be carried out with general organic solvents such as methanol or acetonitrile without the use of flammable reagent gas. This results in greater safety and lowers the overall running costs for CI analysis. In addition, the resultant mass spectra are equivalent to those obtained with conventional reagent gas.

Terpenes are natural products that comprise more than 30,000 compounds. They play a crucial role in the defense system of plants to protect against herbivores, diseases, and aid as mediators in many other ecological interactions with the surrounding environment. When extracted as essential oils, they are usually used for aromatherapy or added to many everyday products due to their characteristic flavors and scents. The identification of terpenes by GC-MS necessitates the use of a huge mass spectral database and retention index information. However, the lack of molecular ion EI spectra can sometimes hamper identification efforts via library match. As the chemical structures of terpenes can be exceedingly similar (many are positional isomers of another), the fragment information alone may be insufficient for identification. For example, the fragment of a monoterpene could also be formed from an oxygenated monoterpene or sesquiterpene. Chemical ionization could thus be a valuable complementary tool to aid the identification effort.

In this article, we will examine the usage of the SMCI technique on a mixture of 21 terpene compounds, containing monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes (Figure 1). The resultant mass spectra from SMCI using methanol and acetonitrile as reagent gas will be examined. In addition, the possibility to utilize SMCI mass spectral information for quick screening of terpene or terpene distribution is explored.

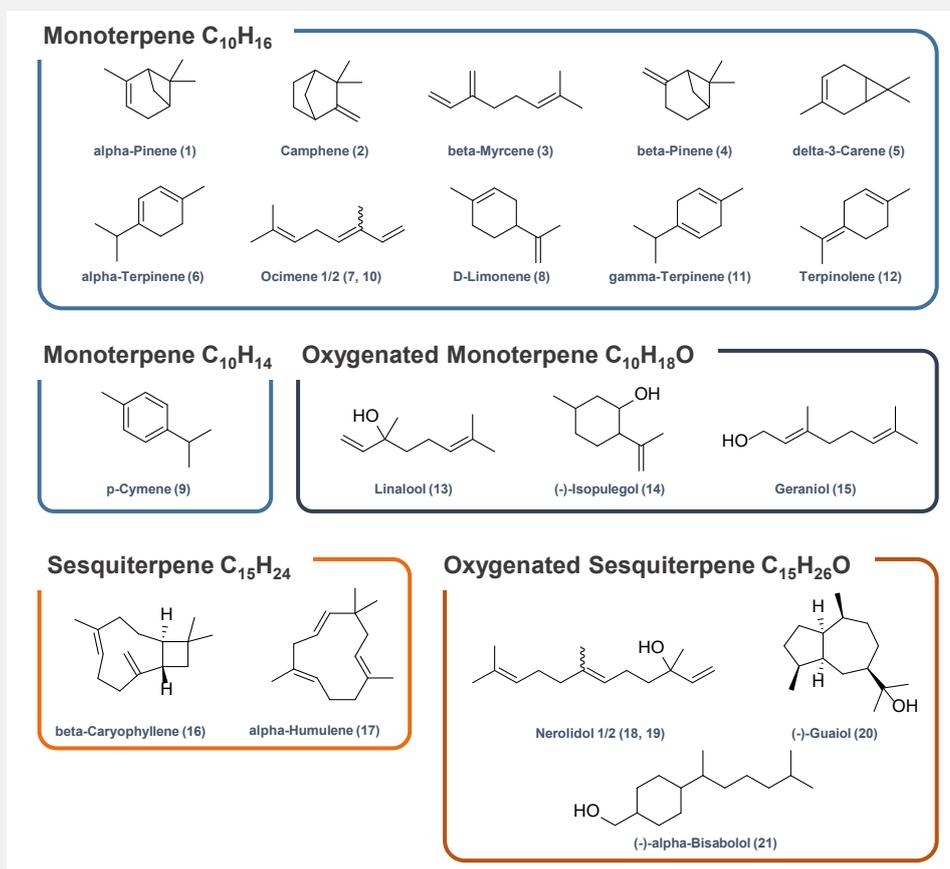


Figure 1. The monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes present in the terpene mixture.

2. EXPERIMENT

2.1 Experiment Setup

A terpene mixture (# 34095) containing 21 terpenes was purchased from Restek. HPLC grade methanol and acetonitrile were obtained from Fischer Scientific. The terpene mixture was analyzed with a Shimadzu GCMS-QP2020 NX. Positive chemical ionization was performed using the SMCI unit with methanol and acetonitrile as reagent gas.

SH-I-624Sil MS capillary column (30 m long, 0.25 mm, and 1.4 μm film thickness) was used for the analysis (P/N: 221-75962-30). Helium was used as carrier gas at a flow rate of 1.5 mL/min. The analysis was performed in full-scan mode from m/z 50 to 350. The injection temperature was 250°C and the injection volume was 1 μL with a split ratio of 5:1. The GC oven temperature was initially held at 55°C, increased to 130°C at 25°C/min and held for 6 min, and finally increased to 280°C at a rate of 30°C/min and held for 2 min. The ion source temperature was 200°C while the interface temperature was 280°C.

3. RESULTS AND DISCUSSION

3.1. Mass Spectra of Terpenes Obtained with EI and SMCI

The terpene mixture standard, which contained 12 monoterpenes, 3 oxygenated monoterpenes, 2 sesquiterpenes, and 4 oxygenated sesquiterpenes, provided a good variety of terpenes to observe the effects of SMCI on the resultant mass spectra. In conventional CI techniques using methane or isobutane reagent gas, proton affinity values can often help to predict the resultant mass spectra. With the usage of methanol and acetonitrile as reagent gas in the SMCI technique, it would be worthwhile to observe the resultant mass spectra of terpenes. Especially for acetonitrile, ensuing “self-reaction” at the ion source which produces a reagent ion of m/z 54, (1-methyleneimino)-1-ethenylum (MIE; $\text{CH}_2=\text{C}=\text{N}^+=\text{CH}_2$) enables covalent adduct chemical ionization process.

Figure 2 shows the mass spectra of several representative terpenes collected using electron ionization (EI) as well as SMCI using methanol ($\text{SMCI}_{\text{MeOH}}$) and acetonitrile (SMCI_{ACN}) reagent gas. The mass spectra of the remaining terpenes which were not shown here exhibited a similar mass spectral pattern. For EI mass spectra, it was observed that the molecular ion (M^+) peaks of the terpenes were generally either present at very low intensity or missing. This increased the difficulty of identification by library match. For example, the molecular ion peak of bisabolol was missing from the EI mass spectrum, and it could be easily misidentified as a sesquiterpene if retention index information was not available.

The $\text{SMCI}_{\text{MeOH}}$ mass spectra of monoterpene $\text{C}_{10}\text{H}_{16}/\text{C}_{10}\text{H}_{14}$ showed $[\text{M}+\text{H}]^+$ ion at m/z 137 or 135 as base peak. For SMCI_{ACN} mass spectra, $[\text{M}+\text{MIE}]^+$ ion at m/z 190 or 188 was also observed in addition to $[\text{M}+\text{H}]^+$ ion. As for oxygenated monoterpene $\text{C}_{10}\text{H}_{18}\text{O}$, the $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$ ion at m/z 137 was the base peak in the mass spectra for both $\text{SMCI}_{\text{MeOH}}$ and SMCI_{ACN} . While $[\text{M}+\text{H}]^+$ ion at m/z 155 was still visible, it was present at a much lower intensity than the $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$ ion. In the SMCI_{ACN} mass spectra, the $[\text{M}+\text{MIE}]^+$ ion at m/z 208 was observed.

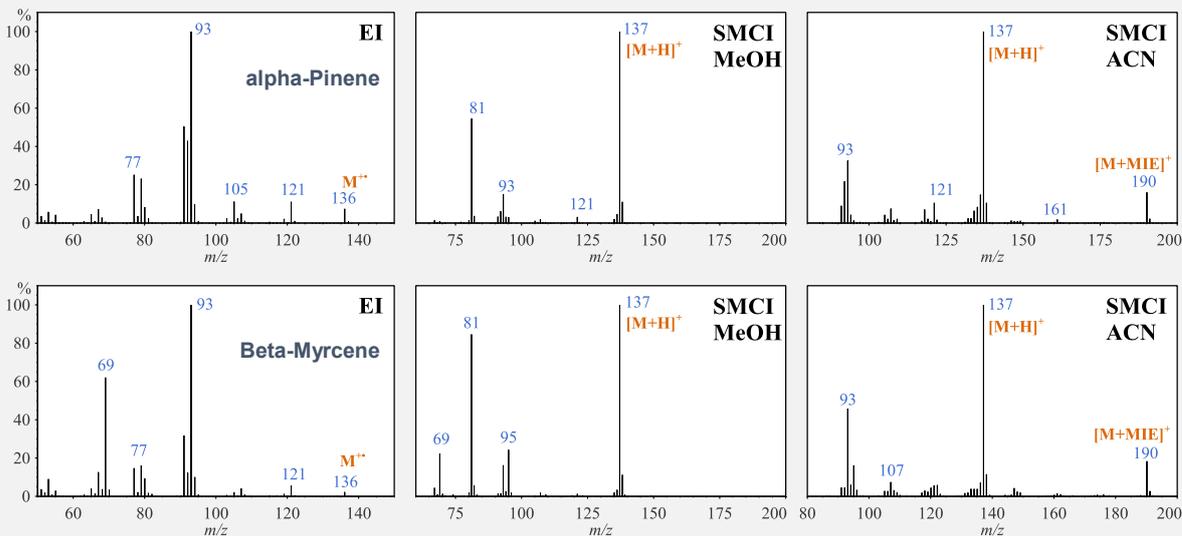
On the other hand, sesquiterpene $\text{C}_{15}\text{H}_{24}$ showed intense $[\text{M}+\text{H}]^+$ ion at m/z 205 in both $\text{SMCI}_{\text{MeOH}}$ and SMCI_{ACN} mass spectra. In the SMCI_{ACN} mass spectra, the $[\text{M}+\text{MIE}]^+$ ion at m/z 258 was present in relatively high intensity. As for oxygenated sesquiterpene $\text{C}_{15}\text{H}_{26}\text{O}$, the $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$ ion at m/z 205 was the base peak in the mass spectra for both $\text{SMCI}_{\text{MeOH}}$ and SMCI_{ACN} . The $[\text{M}+\text{MIE}]^+$ ion at m/z 276 was observed in the SMCI_{ACN} mass spectra.

The presence of $[\text{M}+\text{H}]^+$ ion in both mono- and sesquiterpene mass spectra suggested the terpenes having higher proton affinities than MeOH and ACN reagent gas, hence making the proton transfer reaction exothermic. In addition to the protonated species, the excess energy transferred further caused the fragmentation of the $[\text{M}+\text{H}]^+$ ions into smaller fragment ions. At the same time, the formation of $[\text{M}+\text{MIE}]^+$ ion in SMCI_{ACN} , which is a collision-stabilized complex, indicated that the proton affinities of the terpenes and ACN reagent gas are comparable.

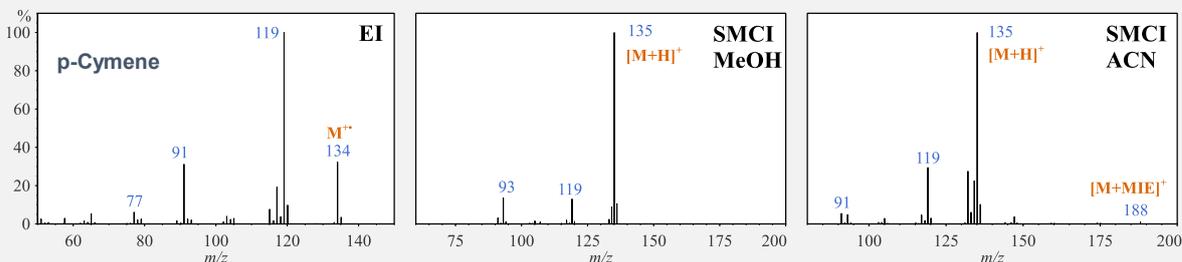
The oxygenated mono- and sesquiterpene experienced a loss of hydroxyl moiety upon chemical ionization as seen from their base peak of $[M+H-H_2O]^+$ ion. The barely visible $[M+H]^+$ ion in geraniol, (-)-guaiol, and (-)-alpha-bisabolol indicated the instability of the ROH_2^+ species and formed R^+ ion by loss of H_2O . For (-)-isopulegol, the intense $[M+H]^+$ ion suggested a possible stabilization effect conferred by the alkene moiety at the α -carbon position relative to the hydroxyl moiety. When subjected to the $SMCI_{ACN}$ process, the $[M+MIE]^+$ ion was visible, which suggested comparable proton affinities between the oxygenated terpenes and ACN reagent gas.

All in all, the use of SMCI (especially with ACN) allows additional mass spectral information to be obtained. This can help to improve the quality and confidence of identification.

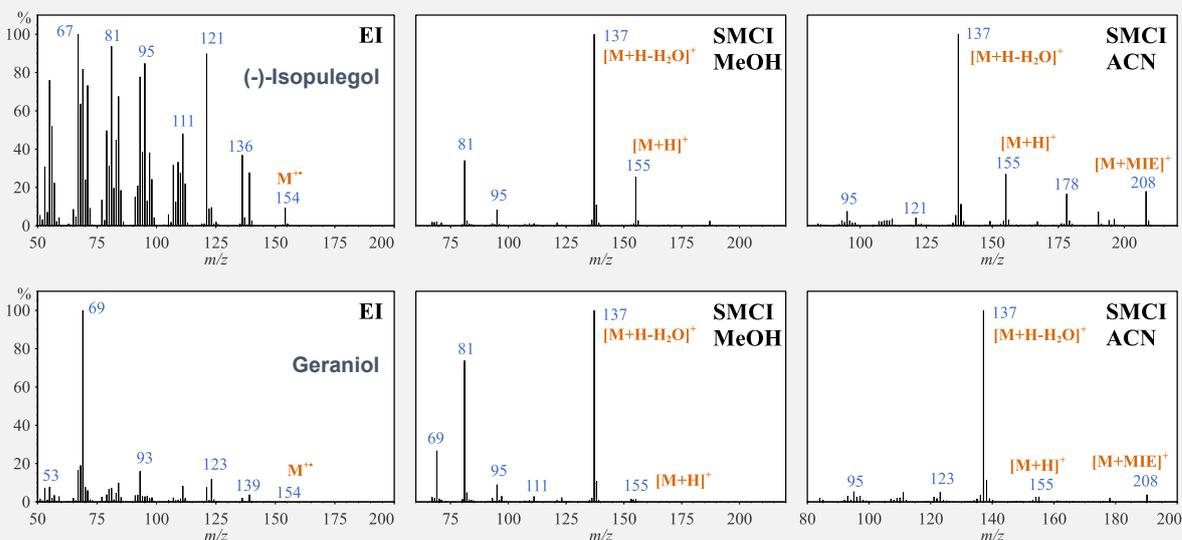
Monoterpene $C_{10}H_{16}$



Monoterpene $C_{10}H_{14}$



Oxygenated Monoterpene $C_{10}H_{18}O$



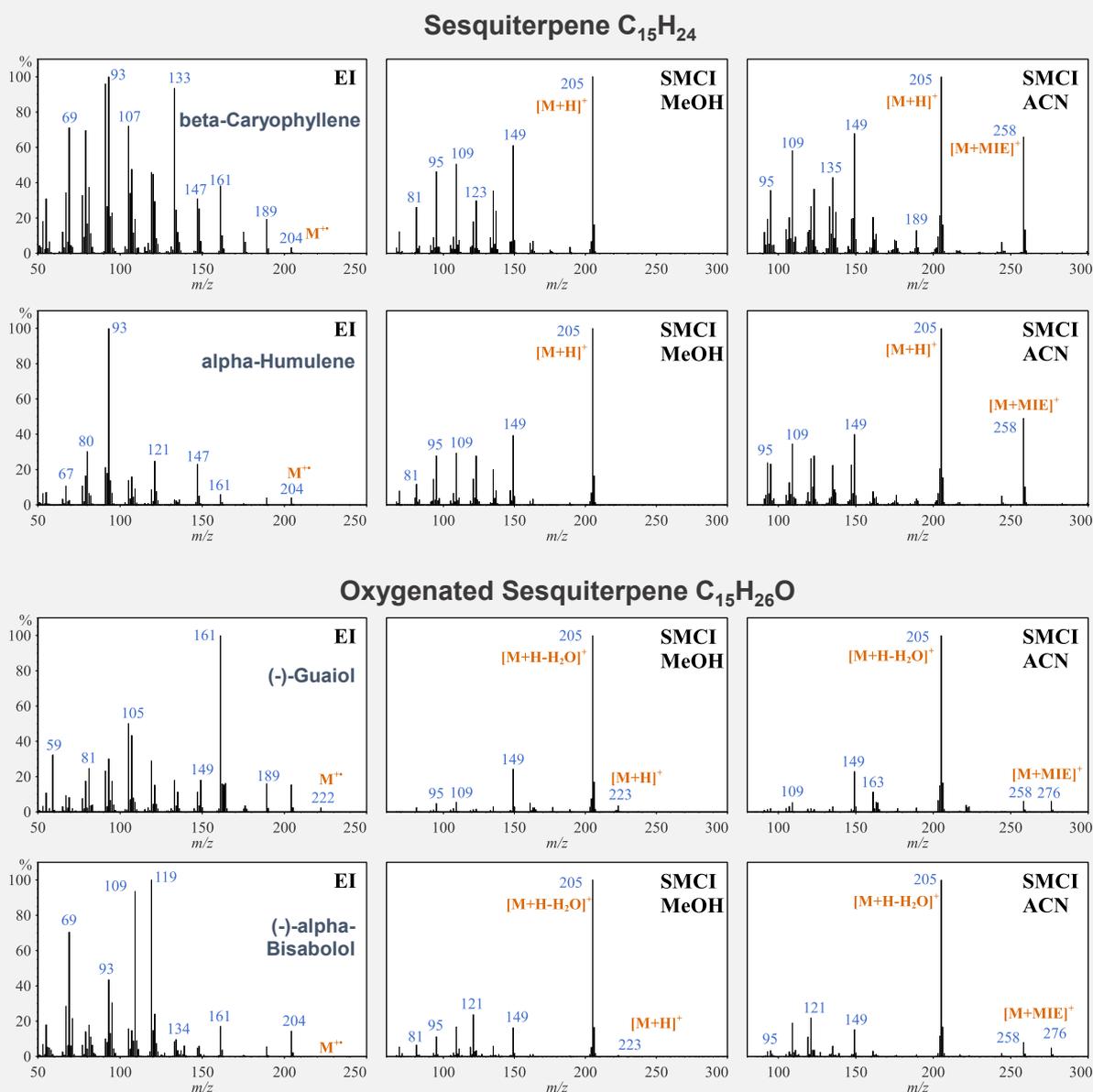


Figure 2. Representative mass spectra for monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes were collected by EI, SMCI_{MeOH}, and SMCI_{ACN}.

3.2. Screening of Terpene Distribution with SMCI_{ACN}

Table 1 lists the corresponding [M+H]⁺, [M+H-H₂O]⁺, and [M+MIE]⁺ ions of the terpenes found in the mass spectra of SMCI_{ACN}. The [M+MIE]⁺ ions of the terpenes are highly unique, even though the [M+H]⁺ and [M+H-H₂O]⁺ ions overlapped. In this regard, these ions can be applied for quick screening of the terpene distribution in an unknown sample.

	[M+H] ⁺ ion (m/z)	[M+H-H ₂ O] ⁺ ion (m/z)	[M+MIE] ⁺ ion (m/z)
C₁₀H₁₆	137	-	190
C₁₀H₁₄	135	-	188
C₁₀H₁₈O	155	137	208
C₁₅H₂₄	205	-	258
C₁₅H₂₆O	-	205	276

Table 1: Suggested monitoring ions for various terpenes in SMCI_{ACN} mode

To determine the presence of monoterpene $C_{10}H_{16}$, mass peaks of m/z 137 and 190 can be used. For monoterpene $C_{10}H_{14}$, the mass peaks of m/z 135 and 188 were used. For oxygenated monoterpene $C_{10}H_{18}O$, the presence of mass peaks m/z 137, 155, and 208 provided a decisive screening. As for sesquiterpene $C_{15}H_{24}$, mass peaks of m/z 205 and 258 can be used. To determine the presence of sesquiterpene $C_{15}H_{26}O$, mass peak m/z 276 was used in addition to m/z 205.

Given the overlapping ions between classes of terpenes, it would be crucial to examine these mass peaks simultaneously to avoid false-positive annotations. Furthermore, prior knowledge of the elution sequence of terpenes could help to improve the accuracy of screening as well. It was observed that the terpenes eluted in the sequence of monoterpene, oxygenated monoterpene, sesquiterpene and oxygenated sesquiterpene. The onset of these regions can be easily marked using retention indices and amenable to further method development processes for complex samples. The Shimadzu Automatic Adjustment of Retention Time (AART) function can be applied to correct the retention time automatically and facilitate fast method transfer.

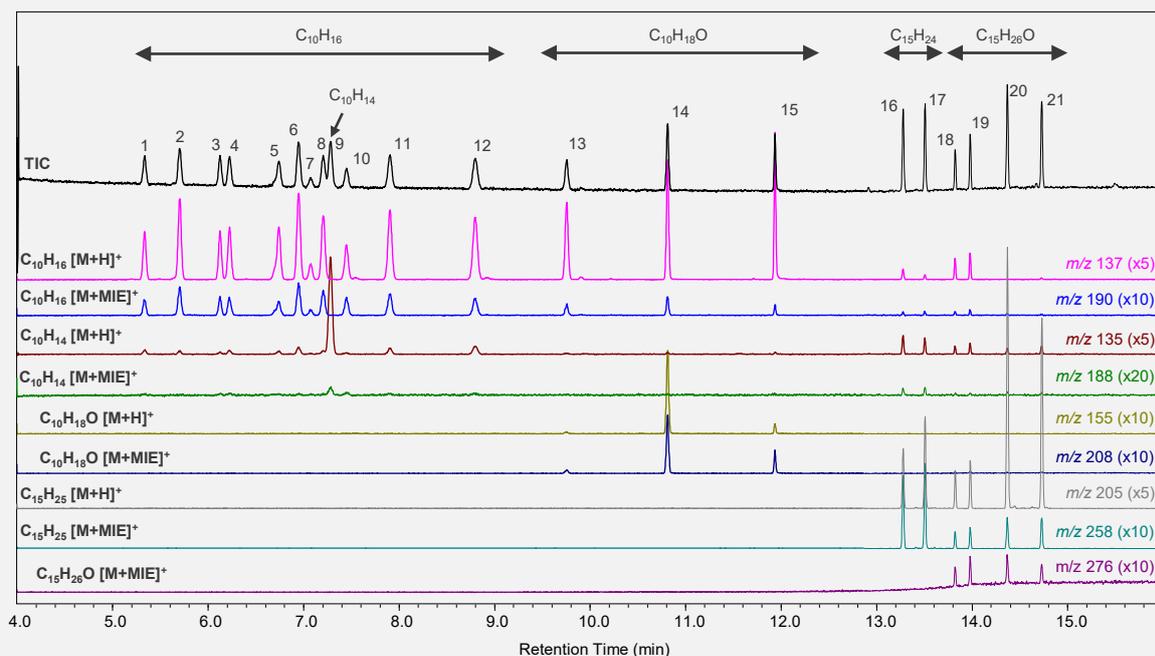


Figure 3. Total ion chromatogram of the terpene mixture. Extracted ion chromatograms for the $[M+H]^+$, $[M+H-H_2O]^+$ and $[M+MIE]^+$ ions of the terpenes. The elution order of the terpenes is according to the numbering indicated in Figure 1.

4. CONCLUSION

The SMCI technique based on methanol and acetonitrile as reagent gas can be used to retain the ionized species of terpene molecules. Mono- and sesquiterpene generated predominantly $[M+H]^+$ ions. Oxygenated mono- and sesquiterpene generated predominantly $[M+H-H_2O]^+$ ions as a result of H_2O loss from an unstable ROH_2^+ species. With the usage of acetonitrile as reagent gas, additional $[M+MIE]^+$ ions were generated. Knowing the presence of these ionized species enabled the usage of SMCI as a complementary tool for the molecular weight determination of these terpenes. These ionized species can be subsequently used as a quick screening strategy for the presence or distribution of terpenes and with further potential for extrapolation to other types of terpenes.

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SMCI

SMCI introduces headspace reagent gas from a bottle into the GC-MS ionization unit for use in positive or negative chemical ionization. Conventional chemical ionization techniques require the use of flammable reagent gas cylinders, but SMCI can be carried out with general organic solvents, such as methanol or acetonitrile, together with nitrogen or argon gas. This results in greater safety and lower running costs.

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FFNSC 4



The Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC), 4th edition library contains mass spectra for 4030 flavor and fragrances compounds. The library also contains retention indices using three types of columns including non-polar, semi-polar, and polar columns. This provides highly accurate identification results and makes it ideal for flavor and fragrance analysis in complex mixtures such as essential oils and plant extracts. The library was developed by Prof. Mondello and his team of the University of Messina, Italy using the Shimadzu GC-MS system.

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