

Analysis of Volatile and Semi-Volatile Compounds in Ground Black Pepper by Solid Probe-GC/Q-TOF with Novel High Efficiency EI Source

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Introduction

Volatile and semi volatiles in food intended for human consumption continue to be evaluated for health benefits. Of the more beneficial classes phytosterols, plant-based sterols thought to help reduce "bad" cholesterol by blocking absorption of cholesterol in the small intestine. Terpenes and alkaloids are known for anti-oxidant and anti-inflammatory properties. These compounds have varying chemical compositions and each present unique challenges in separation, identification and quantitation.

Presented here is a sample preparation-free analysis of ground *Piper Nigrum L* (black pepper) using a solid probe to thermally desorb volatiles in a GC inlet using HRAM Q-TOF equipped with a high efficiency EI source capable of ionizing analytes with lower energy for enhanced selectivity and identification.

Solids and liquid samples of complex matrix can be analyzed for GC amenable compounds via the Thermal Separation Probe (TSP), which fits into the inlet of a GC (Figures 1 and 2). Using an inlet that has rapid temperature and pressure ramps allows for volatilization of analytes, which are then directed to the GC for separation. This reduces time and cost eliminating the need to perform solvent extraction. Further, the non-volatile matrix introduction into the analytical system is greatly reduced or eliminated entirely.



Figure 1: The Thermal Separation Probe (TSP)



Figure 2: The TSP inserted into the GC Inlet

Experimental

Methods

Two to three small flakes of generic pre-ground black pepper were transferred to a 15mm disposable micro-vial, which was inserted into the TSP for analysis. The TSP was placed into the GC's Multi Mode Inlet (MMI), operated under split flow, rapidly heated from 40°C to 350°C at 300°C/min. The analyte vapour was transferred onto a DB-1HT 30m X 0.25mm X 0.10µm capillary column for separation. The helium carrier gas was set to 5mL/min during the volatilization phase reducing to 1mL/min for the GC separation. The oven, set at 35°C for 2.5 minutes, was ramped at 10°C/min to 90°C, and then ramped at 20°C to a final temperature of 350°C for 3 minutes. The transferline was set at 350°C.

Instrumentation

The studies were conducted on an Agilent 7200 series GC/Q-TOF mass spectrometer (Figure 3) equipped with a new high efficiency EI source. The source temperature was 230°C. The QTOF was operated in full spectrum mode with a mass range of 55-1050 Daltons collected at an acquisition rate of 5Hz. Mass calibration was performed externally with PFTBA infusion (Figure 2).

The high efficiency EI source can be tuned and operated at different ionization energies to give "softer" ionization, reducing fragmentation to provide increased higher mass ions in the spectrum. This capability combined with the HRAM capability enhanced the selectivity and aided identification of the target compounds. Figure 5 shows Octafluoronaphthalene spectra, obtained at standard 70eV and at 15eV.

Results and Discussion

Data Analysis

Agilent MassHunter Qualitative Analysis Software was used for data evaluation. Background subtracted spectra were searched against the NIST Library (2011 edition) to identify known constituents of black pepper.



Figure 3: Agilent 7200 GC/Q-TOF

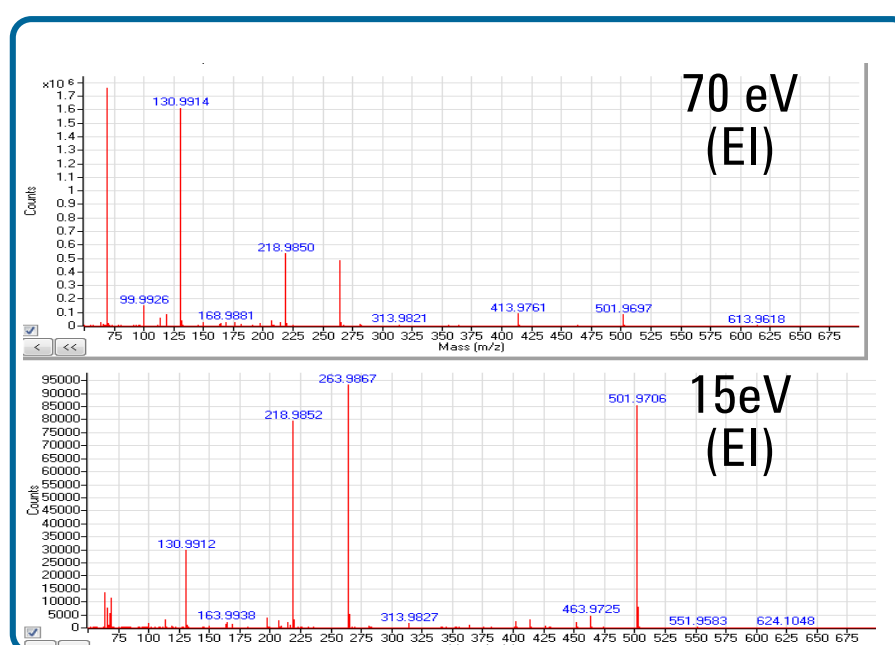


Figure 4: Mass spectrum of mass calibrant PFTBA at standard ionization mode (70eV) and at lower energy ionization (15eV). The calibrant allows for excellent mass accuracy of less than 3ppm across the entire mass range.

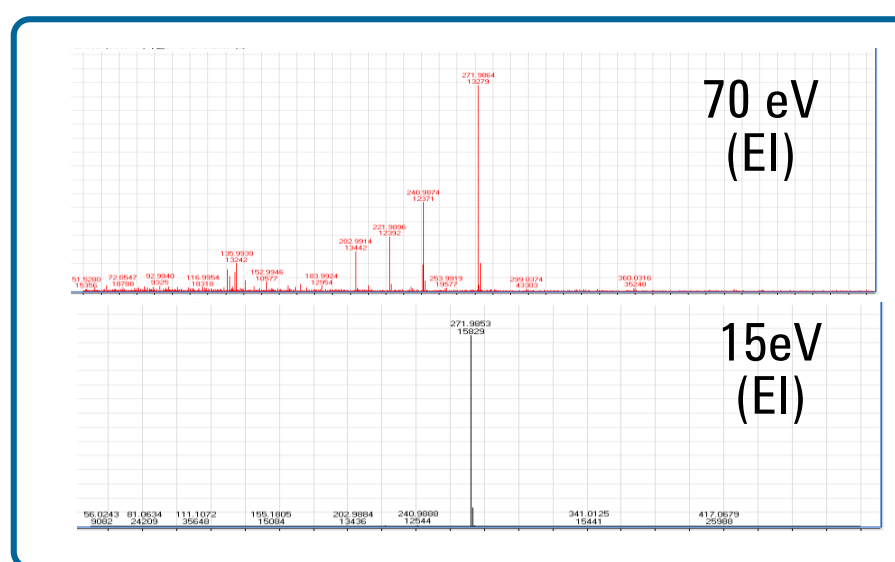


Figure 5: 1mL injection of 1pg/mL OFN standard. The molecular ion at m/z 271.9867 is dominant at 70eV and 15eV, but the smaller fragments at m/z 240.9887, 220.9899 and 202.9915 are all absent in the spectrum obtained at 15eV.

Ground Black Pepper Profile via TSP

The essential oil of black pepper (Kosher grade, extracted by steam distillation, Sigma Aldrich) was first analyzed. Mono- and sesqui-terpenes (oxygenated and non-oxygenated) were the major constituents. The total ion chromatogram (TIC) is shown in Figure 6.

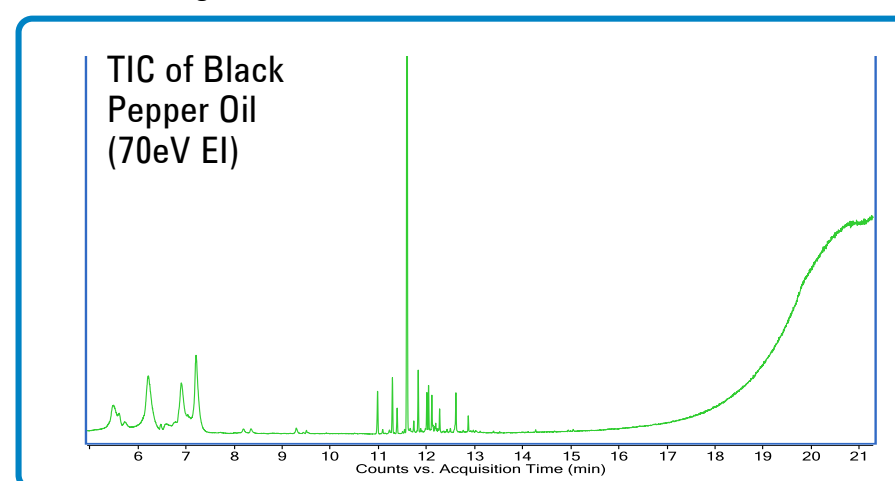


Figure 6: TIC of Black Pepper Oil (70eV EI)

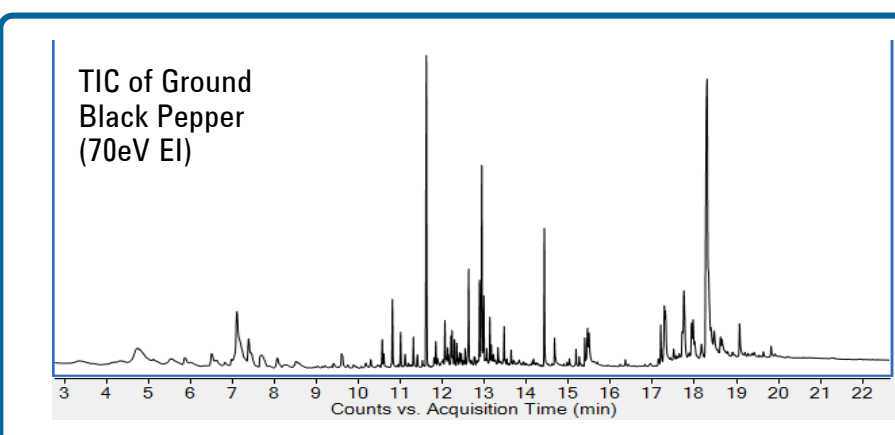
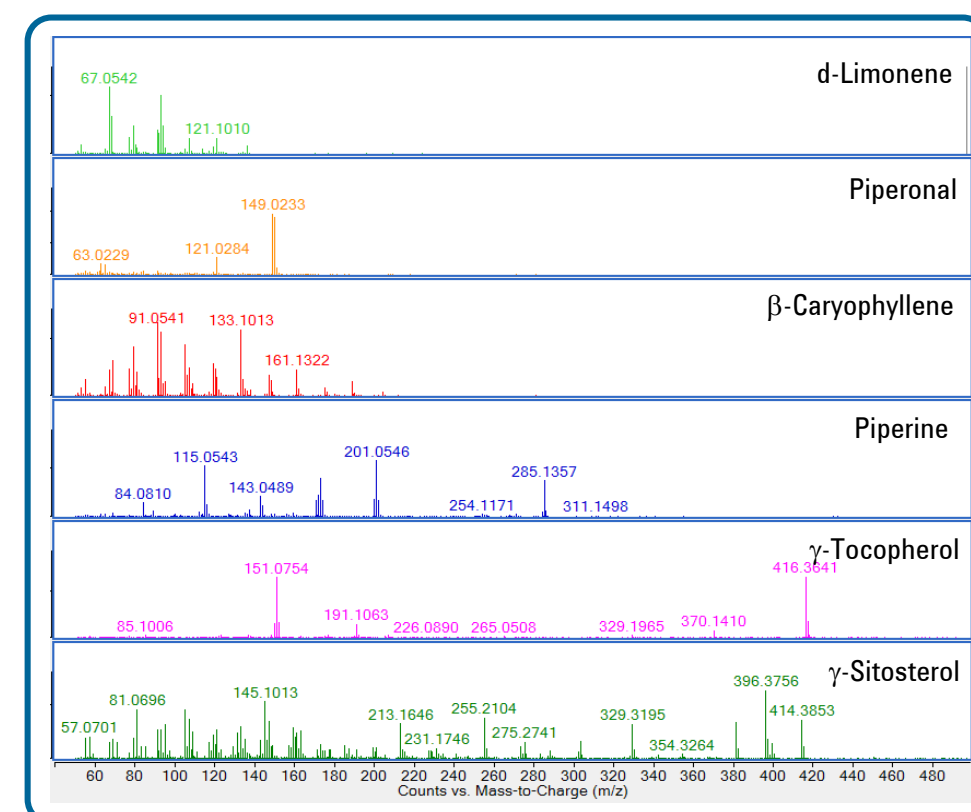


Figure 7: TIC for ground black pepper.

Results and Discussion



Compound	Class	Formula	Mono Mass (m/z)	Mass Error (ppm)
d-Limonene	Monoterpene	C ₁₀ H ₁₆	136.1247	-2.9
Piperonal	Fragrance	C ₈ H ₆ O ₃	150.0311	-1.3
b-Caryophyllene	Sesquiterpene	C ₁₅ H ₂₄	204.1873	-2.0
Piperine	Alkaloid	C ₁₇ H ₁₉ NO ₃	285.1359	-0.7
g-Tocopherol	Vitamin	C ₂₈ H ₄₈ O ₂	416.3649	-1.9
g-Sitosterol	Phytosterol	C ₂₉ H ₅₀ O	414.3856	-0.7

Table 1. Properties of select components of the ground black pepper identified using TSP with theoretical monoisotopic mass and measured mass error.

Soft Ionization and Isomer Differentiation

Not all of the compounds identified can be distinguished by their mass spectrum and RI alone. Many of the mono-terpene isomers have RI values less than 20 units apart and near identical mass spectrum. In these cases, softer ionization can assist in confirming specific isomers. Compounds that have the same mass spectra at 70eV may be differentiated at lower energy ionization, which is demonstrated below in Figure 9.

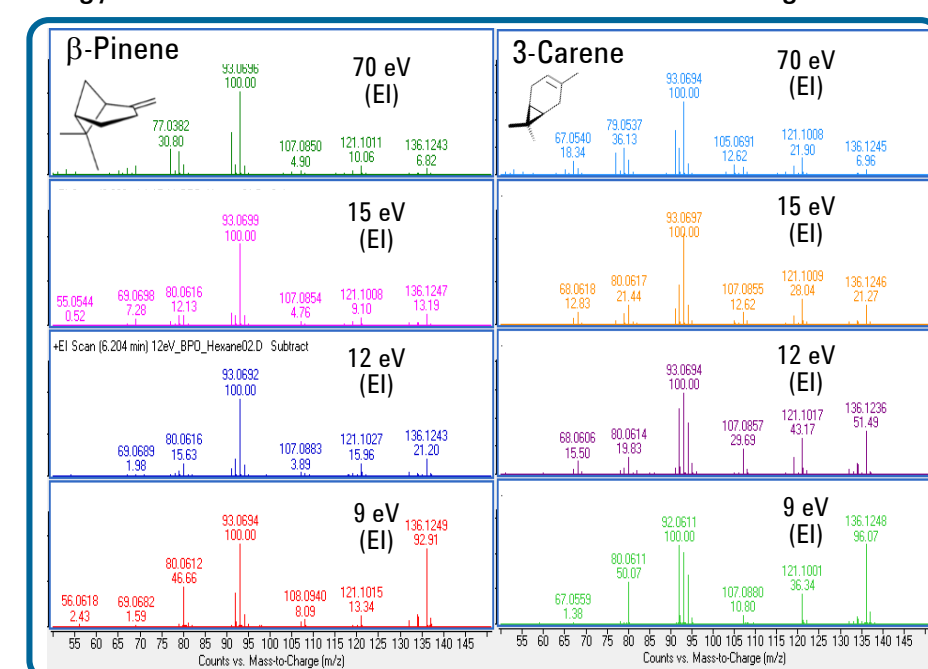


Figure 9: 3-Carene and b-Pinene at various ionization energies.

At 70eV, 3-Carene (calc. RI=992) and β -Pinene (calc. RI=971), two mono-terpenes with the molecular formula C₁₀H₁₆, (m/z 136.1247) have nearly identical spectra. At lower electron energies, different ionization patterns can be observed. Both compounds have enhanced molecular ion (MI) at lower electron energies, however 3-Carene has a relative abundance of fragment m/z 121.1012 (C₉H₁₃⁺) that is significantly higher than β -Pinene. The 3-Carene spectra also features significant changes in the m/z 93 ion cluster. Fragments m/z 92.0621 (C₇H₈⁺) and m/z 94.0777 (C₇H₁₀⁺) become dominating peaks at lower electron energies in 3-Carene, while there is no significant increase for these ions in the low electron energy mass spectra of β -Pinene.

Conclusions

- **TSP** combined with **GC/Q-TOF** is a powerful solution for sample-prep free analysis.
- **Soft Ionization** with the **Novel High Efficiency EI Source** combined with accurate mass greatly enhance the ability to detect and identify compounds