Untargeted Analysis with GC-Orbitrap: A Powerful Tool for the Authentication of Spices and Herbs

Giulia Riccardino[1], Dominic Roberts[2], Xin Zheng[3], Jason Cole[3], Lori Dolata[3] and Cristian Cojocariu[2]
[1]Thermo Fisher Scientific, IT
[2]Thermo Fisher Scientific UK
[3]Thermo Fisher Scientific, USA

ABSTRACT

Purpose: Adulteration of oregano can be accidental or intentional with the latter being driven by price and demand. Leaves from other plants are frequently used as adulterants as they are difficult to detect by visual inspection. As a consequence, the purity of oregano should be assessed to ensure the quality and consistency of the end products.

Methods: GC-Orbitrap technology coupled to solid phase micro-extraction (SPME) with Arrow technology was used to assess the volatile profile of intentionally adulterated oregano samples. Compound Discoverer 3.2 and Mass Frontier software were used for deconvolution, putative identification, multivariate statistical analysis and fragmentation prediction of unknown compounds for a more confident compound identification.

Results: The Orbitrap Exploris GC system in combination with advanced software tools allowed for easy identification of fraudulent samples representing a powerful tool for assessing food authenticity.

INTRODUCTION

Oregano is widely used as an ingredient for culinary purposes due to its organoleptic properties. Adulteration of oregano can be accidental or intentional. Olive, thyme, marjoram, sumac, myrtle, and hazelnut are frequently used as adulterants as they are difficult to detect by visual inspection. As a consequence, food manufacturers must check regularly for the quality and purity of oregano outsourced from various suppliers to ensure the quality and consistency of the end product.

Oregano is a complex matrix containing essential oils, phytosterols, and pigments; its aroma derives from a complex mixture of volatiles, mainly monoterpenes and sesquiterpenes, which can be easily extracted and concentrated in one single step using the headspace solid-phase microextraction (HS-SPME) technique. This allows for minimal sample preparation, a critical point in non-targeted analysis since every manipulation could alter the sample composition. High-resolution GC-MS approach has become very popular for the determination of aroma constituents as it offers the advantage of full-scan data acquisition combined with high sensitivity, high resolving power (up to 240,000 FWHM), and accurate mass (< 5 ppm). Moreover full-scan data acquisition allows for targeted, non-targeted, and retrospective data analysis.

MATERIALS AND METHODS

Sample Preparation

A commercially available oregano sample was purchased from a local retailer. In order to simulate an oregano fraud occurrence, thyme, marjoram, and olive leaves were purchased from local and online retailers and used to adulterate the oregano sample. "Fit for purpose" adulterated samples were obtained by mixing 600 mg of native oregano with 10% of native marjoram, thyme, and ground olive leaves, respectively. Each bulk was well mixed to homogenize the matrix prior to weight and transfer 150 mg into 10 mL crimp top headspace vials for analysis. Samples were prepared in triplicate and analysed in randomized order to reduce the bias in the results. A retention index mix (C7-C30 saturated alkanes) was injected at the beginning of the sequence and used to derive the RI of chemical components putatively identified by NIST20 library search following spectral deconvolution.

Test Method(s)

In all experiments, a Thermo Scientific™ Orbitrap Exploris™ GC 240 system equipped with two Thermo Scientific™ Instant Connect Split/Splitless (SSL) Injectors, one used for SPME Arrow fiber conditioning and the second used for GC sample introduction, both equipped with SPME Arrow liner (P/N 453A0415), was coupled to a Thermo Scientific™ TriPlus RSH™ autosampler with SPME Arrow configuration. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS capillary column, 30 m × 0.32 mm × 1.0 µm (P/N 26099-2970). The triple coating phase of the DVB/CWR/PDMS fiber (P/N 36SA11T3) allowed for effective extraction of a wide range of volatiles including alcohols, aldehydes, ketones and esters.

Data Analysis

Data were acquired using Thermo Scientific™ Xcalibur™ software. This single platform integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. Thermo Scientific™ Compound Discoverer™ software, version 3.2, was used for spectral deconvolution, compound identification, and multivariate statistical analysis. Thermo Scientific™ Mass Frontier™ spectral interpretation software, version 8.0 was used to elucidate the chemical structure of putatively identified compounds via NIST mass spectra library matching.

RESULTS

Chromatography

Chromatographic profiles of the native herbs (oregano, marjoram, thyme, and olive leaves) and the fraudulent samples showed differences when comparing the full-scan total ion chromatograms (TIC). These differences were related both to the prevalence of common aroma components but also to the presence of unique components of native oregano and native adulterants.

As an example, the comparison between native oregano, native thymol, and adulterated sample (oregano/10% thymol) is reported in Figure 1. Some components, putatively identified based on the spectral library (NIST 2020) SI score as α-pinene (RT=8.79 min) and camphene (RT=9.04 min), originate from thyme exclusively. Thymol (RT=14.23 min) and carvacrol (RT=14.41 min) are present in both native thyme and oregano but with different abundances; thymol is predominant in thyme while carvacrol is the main constituent of oregano aroma. Although differences can be observed in the TIC comparison, all features are extracted from the data and analyzed statistically.

Figure 1. FS TIC obtained for native oregano (A), thyme (B), and adulterated oregano sample (C).
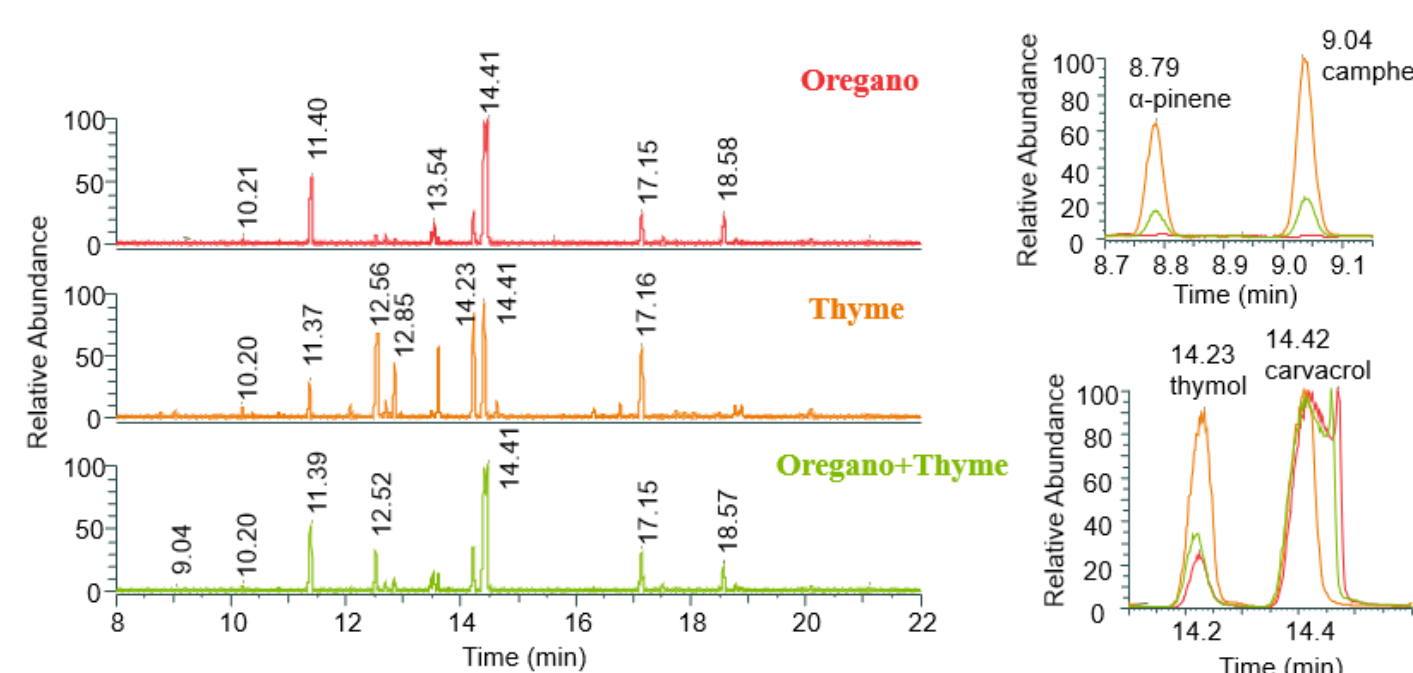
Typical components of native thyme (e.g., α-pinene, RT=8.79 min and camphene, RT=9.04) as well as differences in the amounts of the common aroma components (e.g., thymol, RT=14.23 min and carvacrol, RT=14.41 min) or oregano unique components (e.g., aromadendrene, RT=18.58 min) could be found in the simulated fraudulent sample.

Untargeted screening of volatile impurities

The workflow used to assess the volatile profile and to identify adulterated samples is shown in Figure 2. A complete workflow can be used to isolate unique components, identify compounds with a high degree of confidence, and detect the variations suggesting a possible fraud. FS data is acquired using EI mode at 60,000 FWHM resolution (1) and then imported in Compound Discoverer 3.2 software. The software deconvolutes, aligns, and filters the peaks to putatively identify the compounds using mass spectral library match (NIST 20 or other libraries) (2). Multivariate statistical analysis (principal component analysis (PCA) and volcano plots) is used to select the significant features, defined by their m/z and retention time, contributing to the group differences (3). FS data are then acquired using PCI mode at 60,000 FWHM resolution to confirm the molecular ion and propose a chemical formula (4). Additional PCI MS/MS experiments are performed to confirm the fragments of the molecular ion (5). This streamlined workflow allows for a comprehensive characterization of the aroma components in oregano samples. An example of putative identification for camphene is shown in Figure 3.

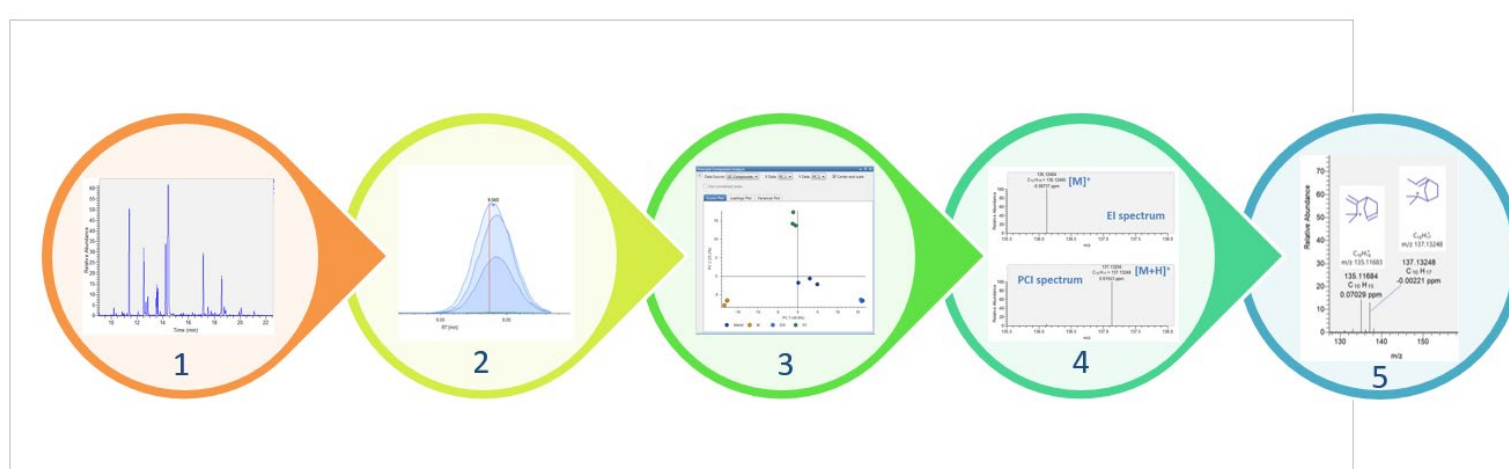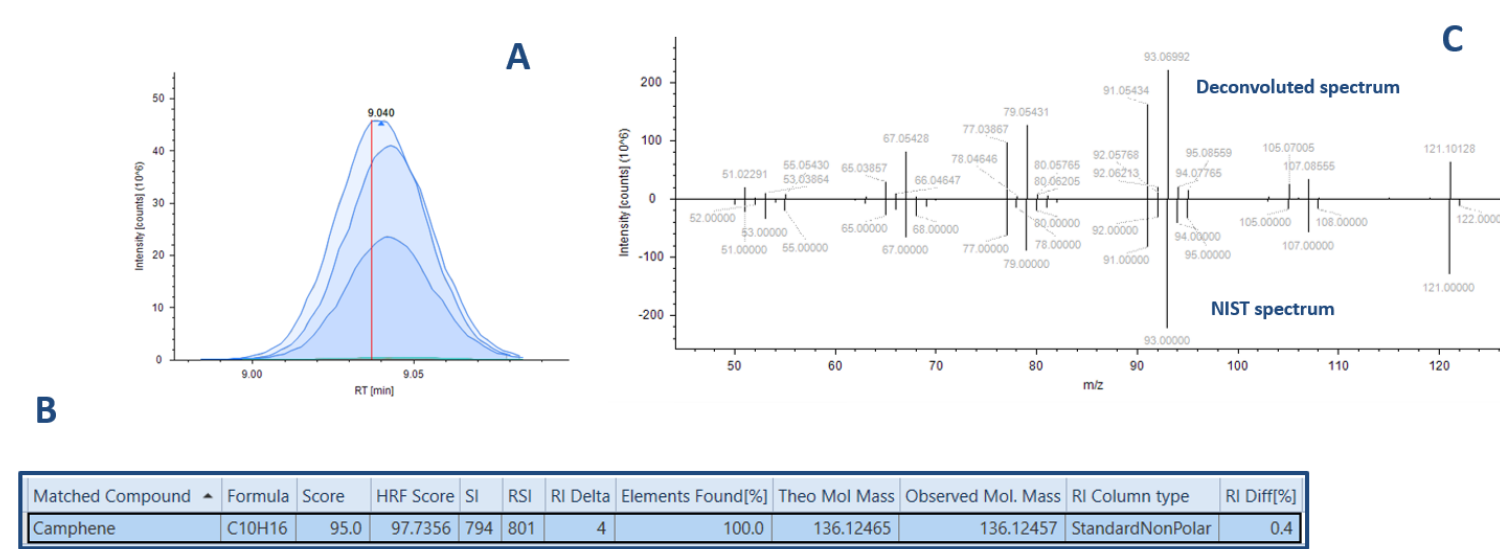**Figure 2. Workflow used to characterize aroma components in oregano samples.**

Figure 3. Compound Discoverer results browser showing an example of compound identification for the peak eluting at RT=9.04 min, putatively (RSI 801) identified as camphene.

Overlaid XIC (base peak m/z 93.06982) for camphene (A); results table with the matched compound identified based on library search and retention index (B); EI spectrum of camphene – measured vs. NIST library (C)

Multivariate Statistical Analysis

Descriptive and differential statistical analysis are carried out to investigate the differences in the analyzed samples. PCA is a well-known statistical approach that highlights variations between sample groups and allows visualization of strong patterns in complex datasets. The PCA plot in Figure 4 shows clear separation between the native oregano sample and the adulterated ones. The V-plots was used to quickly identify the changes in the data sets (Figure 5). This approach allowed the identification of suspected adulteration of native oregano, by both highlighting the changes in the amount (increase or decrease) of some compounds and the presence of compounds that are not usually found in native oregano. For oregano adulterated with 10% thyme, for example, it was possible to identify two main components such as camphene and α-pinene with a content increase by a 7-fold factor and a 3-fold factor, respectively. Allyl furoate increased by 3.5-fold in oregano adulterated with olive leaves while methyl cinnamate showed a log2-fold change of 2.5 in oregano adulterated with marjoram (Table 1).

Figure 4. PCA plot of the volatile compounds that differentiate the native oregano from the adulterated samples.
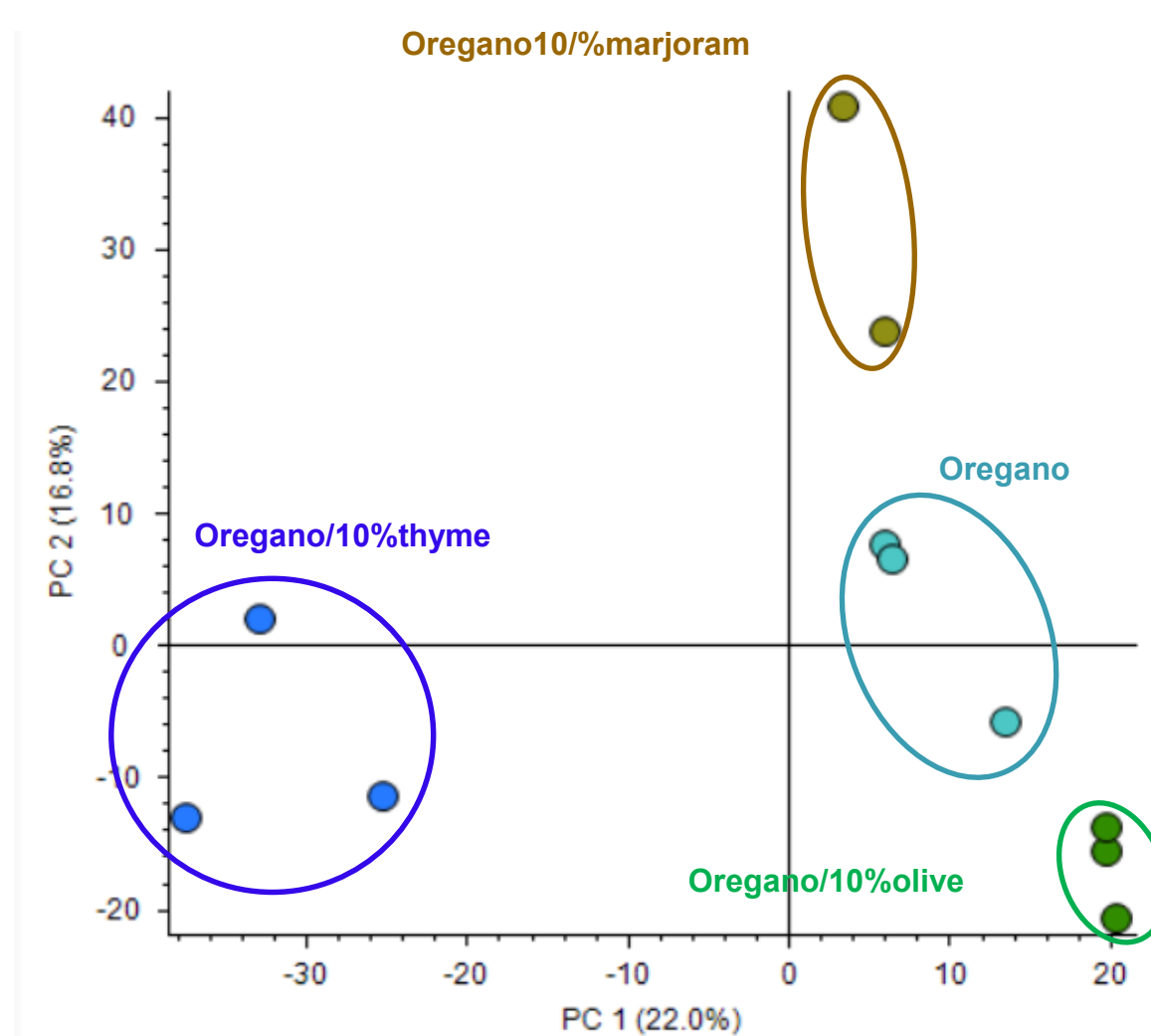

Figure 5. V-plot scatterplot showing the statistical significance (P value) versus magnitude of change (fold change) when comparing the oregano adulterated samples (with thyme) versus the native oregano. The main chemical components that are responsible for sample diversity between two sample groups are selected with light blue dots.
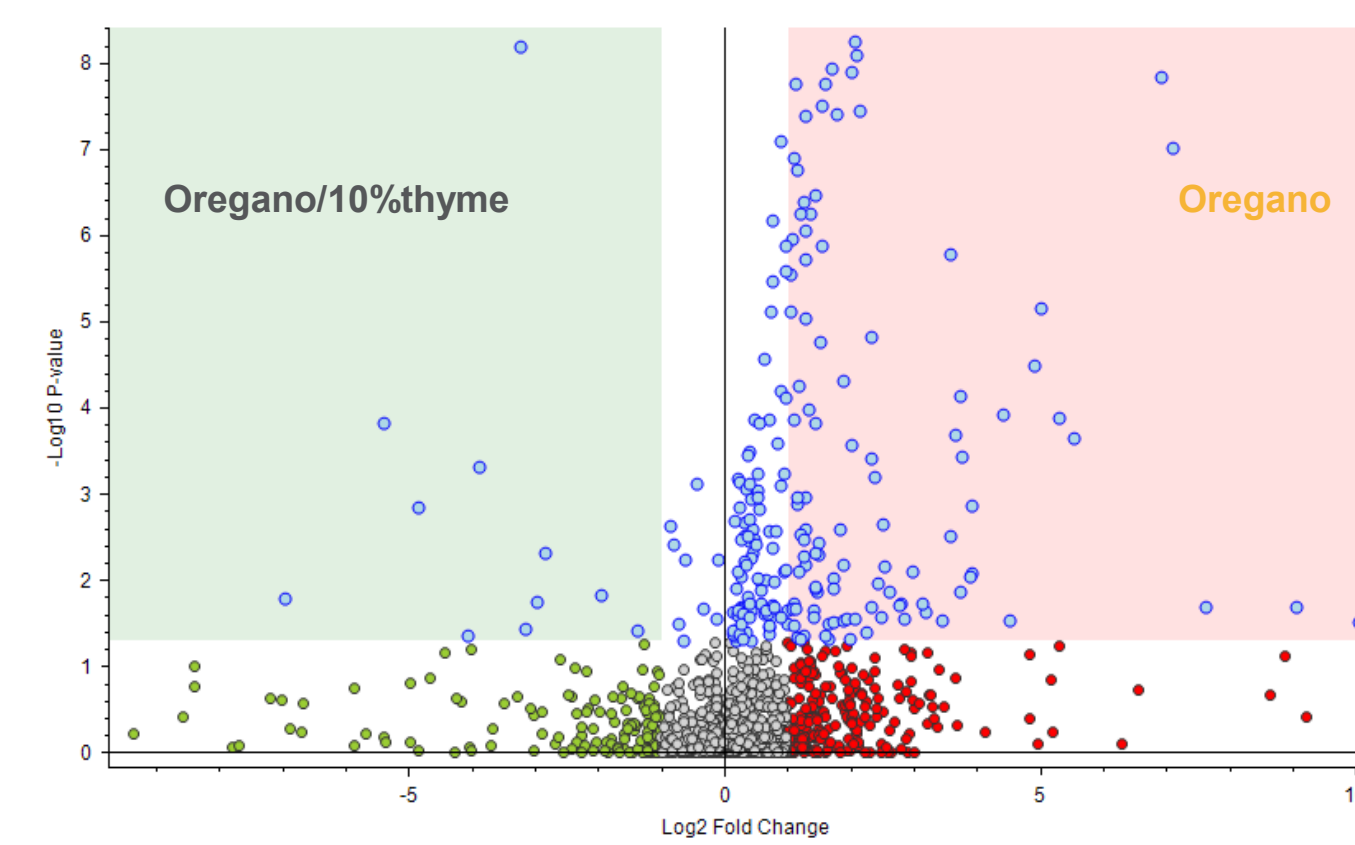

Table 1. Table of fold change of main compounds that could suggest an adulteration of the native oregano.

Putative ID	RT [min]	Reference m/z	Total Score	Log2 Fold Change
Oregano adulterated with 10% thyme				
α-Thujene	8.63	77.03867	92.9	1.9
α-Pinene	8.79	91.05434	95.9	3.6
Camphene	9.04	93.06992	95.1	6.9
trans-β-Ocimene	10.60	91.05424	93.6	-0.3
Camphor	12.08	95.08559	95.1	1.1
Pinocamphone	12.34	95.08554	94.0	1.4
cis-Ocimenol	12.85	93.06992	95.3	1.6
cis-Dihydrocarvone	12.95	67.05428	94.4	1.6
Duroquinone	13.54	117.06986	92.3	1.2
Carvacrol	14.41	135.08051	96.2	-0.1
Germacrene	20.31	133.10118	91.1	-0.3
Levomenthol	24.97	109.10131	92.8	1.5
Oregano adulterated with 10% olive leaves				
Furfuryl disulfide	9.41	81.03341	97.1	1.2
Allyl furoate	10.69	95.04906	92.8	3.5
Thymol	14.22	135.08049	96.0	-0.3
α-Ocimene	11.40	93.06971	94.1	-0.2
Sabinene	10.84	91.05424	95.3	-0.4
4-Thujanol	10.92	93.06988	95.6	-0.2
Oregano adulterated with 10% marjoram				
α-Thujene	8.63	77.03867	92.9	1.4
Sabinene	9.42	91.05424	92.6	1.3
Thujone	11.50	110.1089	92.7	-0.2
Carvacrol	14.41	135.08051	96.2	-0.1
4-Terpinenyl acetate	14.64	94.07311	94.3	1.6
Methyl cinnamate	15.75	161.0597	93.7	2.5

Untargeted screening of volatile impurities

Additional PCI MS/MS experiments were assessed to support the proposed formula for camphene and to derive structural information. The ion m/z 137.13252 was isolated in the quadrupole and fragmented in the HCD cell using 10 V energy to generate fragments as shown in Figure 6. Mass Frontier software was used for assignment of fragment structures to measured ions in the MS/MS spectrum. The measured fragments provided detailed information with respect to the proposed chemical formula with <1 ppm mass accuracy.

Figure 6. PCI MS/MS following m/z 137.13252 fragmentation in the HCD collision cell showing the product ions annotated with measured mass, elemental composition, theoretical mass, mass accuracy (ppm), and the proposed chemical structures.
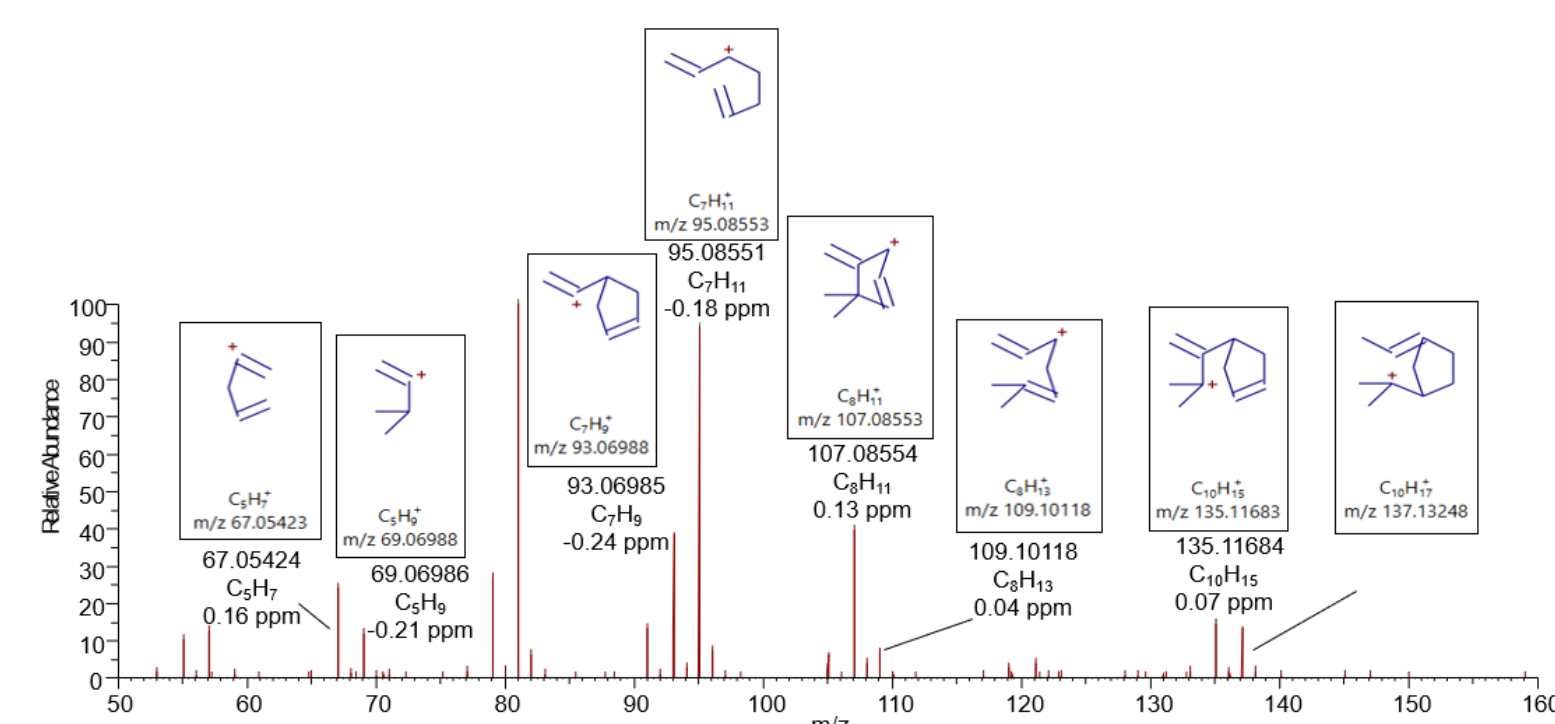
CONCLUSIONS

- The Orbitrap Exploris GC system represents a powerful tool for assessing food authenticity, providing an integrated omics approach for profiling complex samples and identifying unknown compounds intentionally added with fraudulent purposes.

- Automated headspace sampling with the SPME Arrow removes the need for sample preparation and speeds up the analysis.

- The high resolving power (240,000 at m/z 200), the consistent sub-1 ppm mass accuracy and the wide dynamic range allow for fast and confident characterization of a large number of compounds regardless of their concentration or matrix complexity.

- Compound Discoverer software allows for extraction, deconvolution, identification of unknown compounds, and multivariate statistical analysis in a streamlined data processing workflow.

- Rapid change-over from EI to softer ionization such as PCI and the ability to perform accurate mass MS/MS experiments for structural elucidation, combined with the use of Mass Frontier software for predictive fragmentation and structural elucidation, enable confident compound identification with unprecedented ease.

TRADEMARKS/LICENSING

PO66080 EN0921S