

# A comprehensive strategy for confident detection of oregano adulteration by GC-Orbitrap mass spectrometry

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## ABSTRACT

Oregano is widely used as flavouring ingredient for culinary purposes because of its organoleptic properties and enjoyable taste. Oregano is a herb widely used as an ingredient in a variety of food and beverage commodities and is particularly vulnerable to fraud. Adulteration of oregano can be accidental or intentional with the latter being price and demand driven. Leaves from other plants (e.g. olive trees, thymes, majoram, sumac, myrtle and hazelnuts) are frequently used as adulterants as they are difficult to detect by visual inspection. Oregano adulteration is commonly investigated using analytical methods such as gas of liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS) that can easily identify chemical components representative for various adulterants used in ground oregano. The oregano aroma derives from a complex mixture of volatiles, mainly monoterpenes and sesquiterpenes that can be easily extracted and concentrated in one single step using the headspace solid phase microextraction (HS-SPME) technique. This technique allows for sample extraction and concentration in a single step simplifying the sample preparation step. Confident detection of the volatile compounds can be achieved through the high resolution accurate mass Thermo Scientific™ Orbitrap™ technology coupled with gas chromatography. In this study the Orbitrap technology coupled with SPME Arrow extraction was used to assess the volatile profile of oregano. Thermo Scientific™ Compound Discoverer™ 3.1 software was used for unknown compound deconvolution, identification, sample group assessment and multivariate statistical analysis. Principal component analysis (PCA) resulted in identification of the main components that are responsible for the differences observed between the samples. Thymol methyl ether, usually present in coriander seed and thyme oil, resulted to be present in one of the two samples therefore it could have been intentionally added with fraudulent purposes.

## MATERIALS AND METHODS

Two oregano samples were prepared in triplicate by weighting 150 mg from each sample and transferring it into a 10 mL crimp top headspace vial (vials P/N 10-CV, caps P/N 20-MCBC-ST3). Each jar was well mixed to homogenize the matrix before weighing. A blend (pooled sample group) was obtained by pooling together all the samples. In order to reduce the bias in the results the sample vials were analysed in a random order. A retention index mix (Sigma Aldrich, C7-C30 saturated alkanes, P/N 49451-U) was injected at the beginning of the sequence and used to derive the RI of chemical components putatively identified by NIST17 library following spectral deconvolution. In all experiments, a Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS equipped with two Thermo Scientific™ Instant Connect split/splitless SSL Injectors (SPME Arrow liner 1.7 mm ID, P/N 453A0415) was coupled with 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 26099-2910). Additional HS-SPME Arrow and Orbitrap GC parameters are detailed in Table 1. The triple coating phase of the DVB/CWR/PMDS fiber (P/N 36SA11T3) allowed for effective extraction of a wide range of volatiles such as alcohols, aldehydes, ketones and esters. Data was acquired Thermo Scientific™ TraceFinder™ software. Compound Discoverer software was used for spectral deconvolution, compound identification and multivariate statistical analysis.

Table 1. HS-SPME Arrow and Exactive GC operating conditions for volatome profile assessment of pesto samples

TriPlus RSH –HS-SPME Arrow Parameters		Trace 1310 GC Parameters	
Fiber:	SPME Arrow DVB/CWR/PMDS	Inlet Module and Mode:	SSL, split
Coating Phase Thickness (μm)	110	Split Ratio:	30:1
Coating Phase Length (mm)	20	Carrier Gas, Carrier Mode, Flow (mL/min):	He, constant flow, 1.8
Incubation Temperature (°C):	60	Oven Temperature Program:	
Incubation Time (min):	15	Temperature 1 (°C):	40
Incubation Speed (rpm)	500	Hold Time (min):	2
Extraction Temperature (°C)	60	Temperature 2 (°C):	150
Extraction Time (min)	15	Rate (° C/min):	10
Stirring Speed (rpm)	1500	Temperature 3 (°C):	260
Fiber Depth in Vial (mm)	25	Rate (° C/min):	5
Fiber Depth in Injector (mm)	70	Temperature 4 (°C):	300
Desorption Time (min)	2	Rate (° C/min):	25
Analysis Time (min)	40	Hold Time (min):	3
Inlet for Fiber Conditioning		Total GC Run Time (min):	40
Fiber Pre-Conditioning Time (min)	0		
Fiber Post-Conditioning Time (min)	15		
Carrier Gas, Carrier Mode, Flow (mL/min):	He, constant flow, 6		
Fiber Depth in Injector (mm)	70		

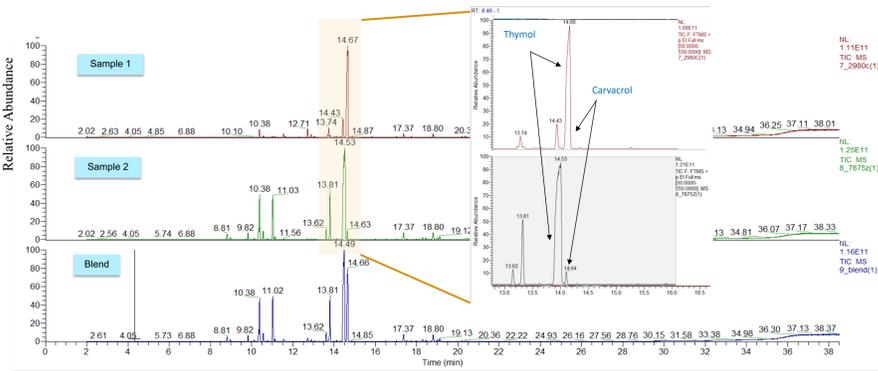
Exactive GC mass Spectrometer Parameters	
Transfer Line Temperature (°C):	280
Ion Source Temperature (°C)	280
Ionization Type:	EI/PCI
Electron Energy (eV)	70
Acquisition Mode:	full scan
Mass Range (Da)	50-550
Resolving Power (FWHM):	60,000 @ m/z 200
Lockmass	207.03235

## RESULTS

### Component identification

Samples were acquired in full-scan mode at 60,000 FWHM resolving power and subjected to Compound Discoverer for Chemometric assessment and putative identification of unique features. Differences in the chromatographic profile of the samples were visible even in the TIC as demonstrated in Figure 1.

Figure 1. Total Ion Chromatogram (TIC) obtained for oregano samples. Differences in the volatile profile can be seen visually detected



Although differences can be visually seen in the TIC comparison, it is essential that all features are extracted from the data and analysed statistically. Compound Discoverer was used to extract, deconvolute and identify the unknowns basing on mass spectral library (NIST 2017). Compounds were scored based on the total score (derived from a combination of library search index score, high resolution filtering (HRF) value and presence/absence of the molecular ions) as well as retention index difference from expected values. An example of such peak identification workflow is reported in Figure 2 for Camphene. The wide dynamic range and the <1 ppm mass accuracy ensured the detection of compounds present at high and low concentrations.

### Statistical Analysis

Multivariate statistical analysis was carried out using Compound Discoverer. The PCA is a well known statistical approach that allows easy highlight variation between sample groups and allows visualisation of strong patterns in complex datasets. By employing variance analysis in Compound Discoverer, significant differences observed between the volatile profiles of oregano samples could be related to the different varieties of oregano. Carvacrol is predominant in sample 1 while thymol is present at high concentrations in sample 2 as shown in Figure 1. The predominance of one component over the other also changes with the sample group analysed. To further assess this, differential analyses was carried out using the Volcano plots, useful to quickly identify changes in large data sets composed of replicate data. V-plots show the main components that are responsible for sample diversity between 2 sample groups (binary comparison). As an example, the Volcano plot obtained by comparing sample 1 and sample 2 is reported in Figure 3. Putative identification for selected compounds (light blue dots) is reported in the result table. This way, a unique compound, thymol methyl ether, was detected only sample group 2 (Figure 4). This component mainly originates from coriander seeds and thyme oil, suggesting that it could have been added for fraudulent purposes.

### Compound Confirmation

Further confirmation in the identification of compounds was achieved by assessing the PCI spectra. The PCI is useful for confirming the molecular ion of a chemical as only molecular ions will generate adducts formation. Unlike softer EI ionisation at lower eV energies, in PCI experiments with methane as the reagent gas three adducts are typically observed: [M+H]<sup>+</sup>, [M+C<sub>2</sub>H<sub>5</sub>]<sup>+</sup>, [M+C<sub>3</sub>H<sub>7</sub>]<sup>+</sup>. Figure 4 shows EI and PCI spectra of eugenol with these adducts. The presence of these adducts confirm m/z 136.12469 as the molecular ion for camphene (RT=9.22 min).

Figure 2. Compound Discoverer result browser showing peak deconvolution results for camphene as an example (RT=9.22 min, m/z 93.07002). Overlaid XIC (extracted ion chromatogram) of m/z 93.07002 corresponding to camphene base peak ion in the analysed samples (A); results table with list of compounds detected and identified based on library search and retention index (B); EI spectrum of camphene – measure vs NIST library (C) and PCA plot (D).

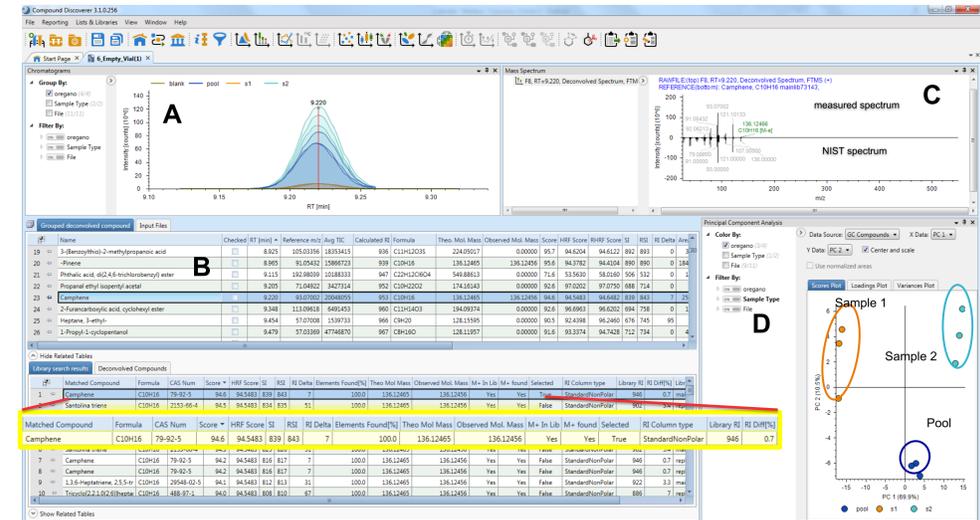


Figure 3. Volcano plot showing a comparison between oregano sample 1 and sample 2 (A). The main compounds responsible for sample diversity are selected (light blue dots, left) and their putative identification based on NIST library results, total score and RI is reported in the result table (B).

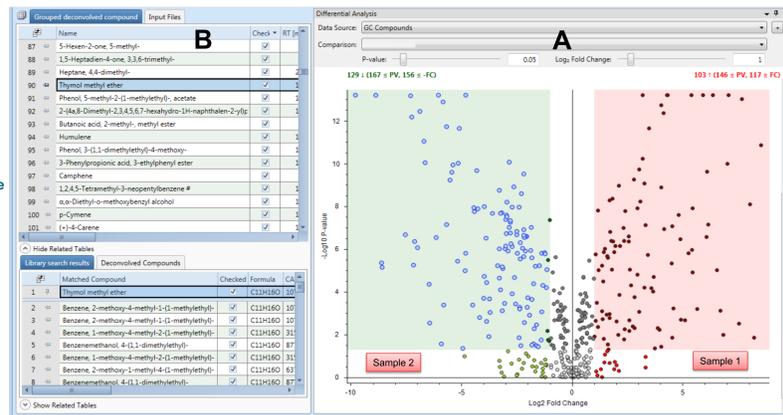
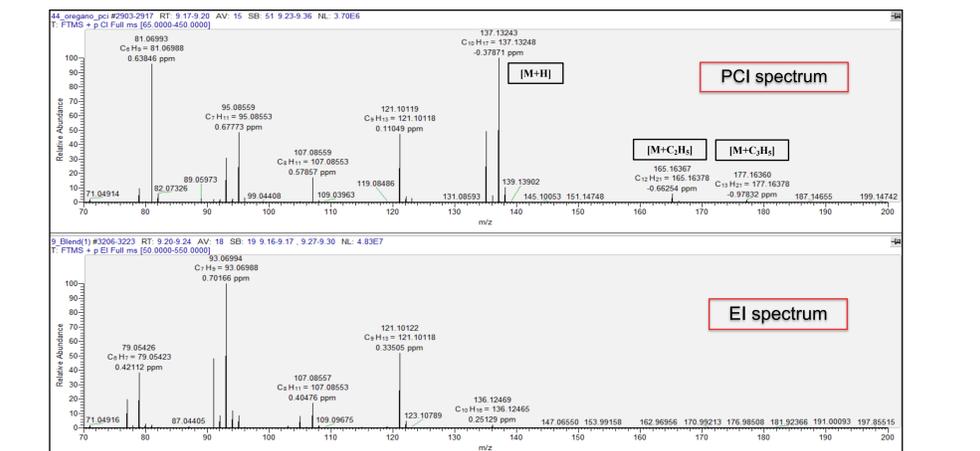
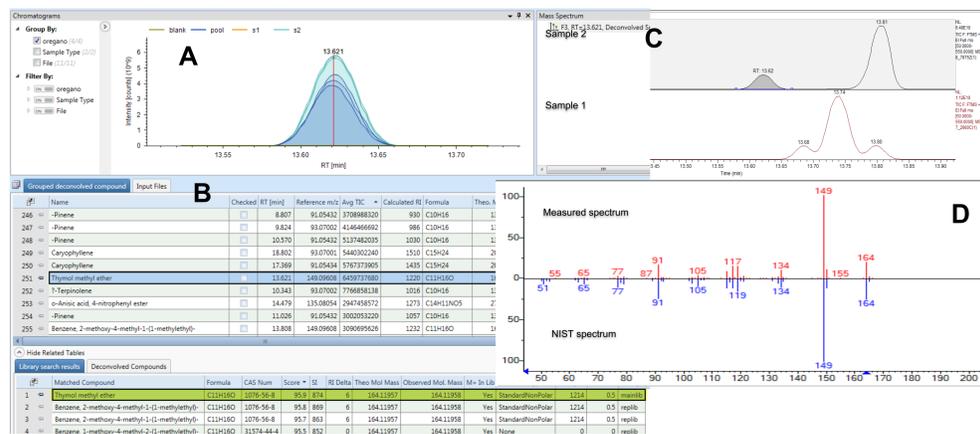


Figure 4. Compound Discoverer result browser showing peak deconvolution results for thymol methyl ether (RT=13.62 min, m/z 149.09608). Overlaid XIC of m/z 149.09608 corresponding to thymol methyl ether base peak ion in all samples analysed (A); results table with list of compounds detected and identified based on library search and retention index (B); XIC comparison for for sample 1 and sample 2 at RT=13.62 min (C) and library match for thymol methyl ether (measured spectrum, red and NIST 2017 spectrum, blue) (D).



## CONCLUSIONS

- The results presented in this study indicate that the Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with SPME Arrow technology and Compound Discoverer software is a powerful combination with significant potential for the detection of food authenticity and integrity, and especially so when using an integrated omics approach for profiling complex samples and identifying unknown peaks that can be intentionally added with fraudulent purposes.
- The power of routine high resolving power and consistent low ppm mass accuracy as well as the wide linear and intraspectrum dynamic range allows for a large number of compounds to be detected regardless of their concentration or matrix complexity.
- Rapid change-over over for EI (important for spectral library search) to softer ionisation such as PCI and the availability of the retention index information allow for confident compound identification with unprecedented ease.

## REFERENCES

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