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Black Pepper Authenticity Analysis Workflow with LC-QTOF and Novel Software Solution

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Introduction

Food authenticity as a global issue

Complex supply chains characterize the global market of herbs and spices. Black pepper is one of the most popular spices; it is used globally as a flavoring or seasoning in a wide variety of different cuisines and accordingly is produced in several countries globally. Pepper, as with most other herbs and spices, is typically grown on smaller farms, reaching the consumer after passing through a series of complex and opaque intermediaries, the ideal scenario for various frauds to be perpetrated. Geographic origin fraud is increasingly problematic in a market where tariffs and tax evasion pose significant financial risks to both businesses and governments.

Untargeted analysis using HRMS followed by statistical analysis has been demonstrated as powerful methodology to address these challenges.¹ However, such analytical workflow can be complex with multiple acquisition modes, requiring expert statistical and analytical knowledge and therefore time consuming. Herein is a novel software **MassHunter Explorer 2.0** that integrates complex analyses into a simplified workflow, making it easier to interpret various data file types and providing users with clear and confident insights.



Figure 1. Revident LC/Q-TOF with 1290 Infinity III LC. Authentic black pepper samples from 5 geographical origins (Vietnam, Cambodia, India, Indonesia and Brazil) were used for this study.

Experimental

Sample preparation

Grinded black pepper samples (20 mg) were extracted with 1.5 mL of a methanolic solution (MeOH:H₂O; 4:1 v/v). Resulting mixture were vortexed and shaken for 20 minutes at 30 C then sonicated for 30 minutes at 30 C. Samples were centrifuged and supernatant collected, then filtered before analysis on the LC/MS system. Quality Control (QC) samples were generated by aliquoting each sample into a single vial and injecting at the start of the sequence to condition the column and every 5 samples during analysis.

All data were acquired on a 1290 LC combined with Revident LC/Q-TOF (Figure 1) with MS mode and iterative AutoMSMS to increase MS/MS coverage for identification.

Table 1. LC method and Revident LC/Q-TOF source parameters.

LC Conditions		
Column	Agilent Zorbax Eclipse Plus C18, 2.1 x 150 mm, 1.8 μm	
Column temperature	45 °C	
Autosampler temp	4 °C	
Injection volume	1 μL	
Mobile phase	A: Water, 4.5 mM NH ₄ HCO ₂ , 0.5 mM NH ₄ F, 0.1% HCO ₂ H B: Methanol, 4.5 mM NH ₄ HCO ₂ , 0.5 mM NH ₄ F, 0.1% HCO ₂ H	
Flow rate	0.4 mL/min	
Gradient program	Time	%B
	0.0	2
	1.5	2
	15.0	100
	19.0	100
	19.1	2
	20.0	2
Post time	4 min	
Total run time	24 min	
Revident LC/Q-TOF MS Conditions		
Acquisition rate	MS: 3 spectra/s AutoMS/MS: 3 spectra/s	
Gas temperature, Flow	325 °C, 10 L/min	
Nebulizer pressure	35 psi	
Sheath gas temp, Flow	375 °C, 12 L/min	
Nozzle voltage	200 V	
Capillary voltage	2500 V	

Results and Discussion

Novel Software providing streamline data analysis workflow

The analysis of black pepper is complicated by the nature of the matrix, which is considered to be one of the harsher on both the UHPLC system and the MS itself. It is known to be feature rich and therefore challenging both for the instrument and the analyst to interpret results.

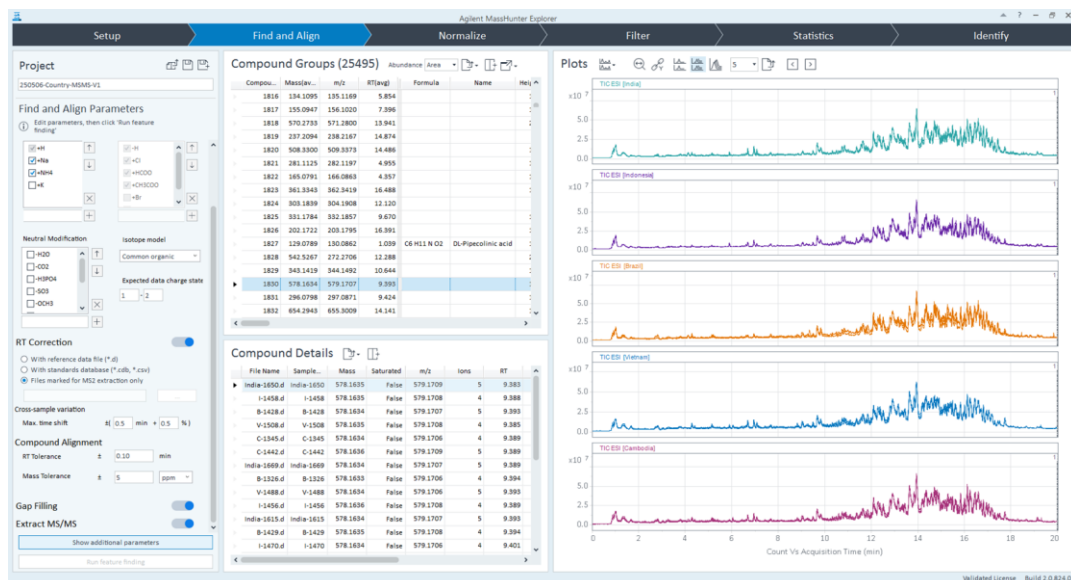


Figure 2. MH Explorer 2.0 Find and Align settings with TIC of various black pepper extracts

MassHunter Explorer 2.0 simplifies the analysis process into six key steps: setup, find and align, normalization, filtering, statistics, and identification (Figure 2). Data files are directly imported from acquisition. Subsequently, files are batched and sorted using custom groupings and easily selectable types. Feature extraction settings, such as mass extraction limits, adduct possibilities, and height filters, are chosen. Compound groups are then normalized and filtered based on abundance, variability, and frequency. Various statistical analyses, including HCA, volcano plots, PCA, ANOVA, unique features, and fold change analysis, can be performed. Finally, identification is completed using mass accuracy and isotope analysis, available retention times if present, and library fragmentation spectra.

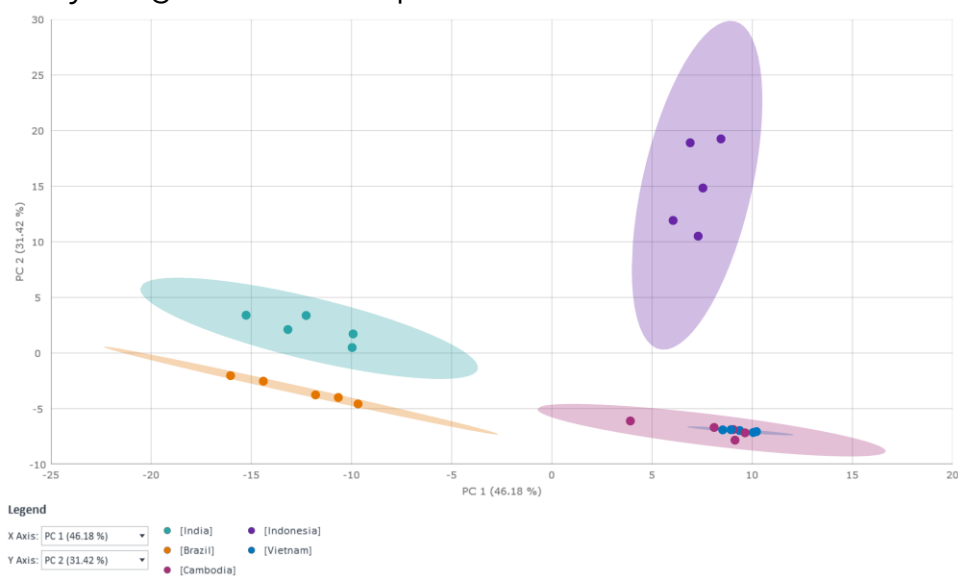


Figure 3. PCA plot of black pepper extracts after data filtration

Integrated data filtering and statistical analysis

More than 25,000 compounds were detected using a height filter of 2,000 including Na^+ and NH_4^+ adducts. Dimensionality of the data was reduced by applying a relative filter on abundance (25 to 100%), variability (less than 25% variation in the 5 group) and frequency (100% in each group).

Statistical analysis was then applied to the remaining 6000 Compound Groups to focus on analytes that were significantly different between the groups. A 1-way ANOVA (pair group) test was followed by:

a Fold Change (pair group) to obtain a list of 239 features exacerbating significant difference between the different country of origin for the black pepper extract and

PCA plot (Figure 3) showed clear difference between 4 groups with samples originating from Cambodia and Vietnam having high similarities likely due to geographical reasons, making them difficult to differentiate.

Integrated MS and MS/MS identification workflow from curated spectral libraries and databases

The identification procedure has been enhanced to evaluate fragmentation information from MS/MS data in complement of accurate mass and isotopologue matching (Figure 4).

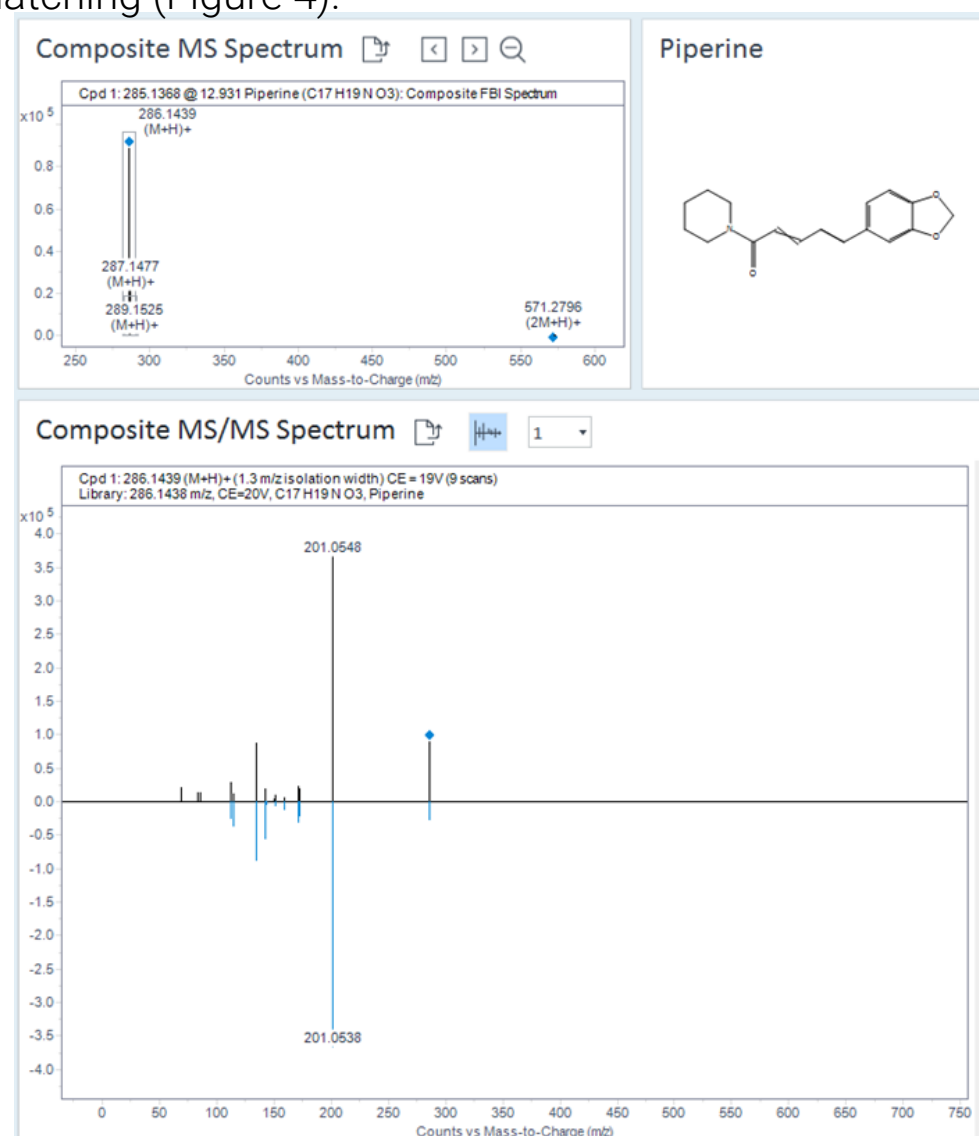


Figure 4. Identification step displaying isotopologue matching (top) and fragment spectra mirror plot (bottom).

