Application Note

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A Metabolomics Approach to Multivariate Analysis of Black Pepper Using LC/Q-TOF

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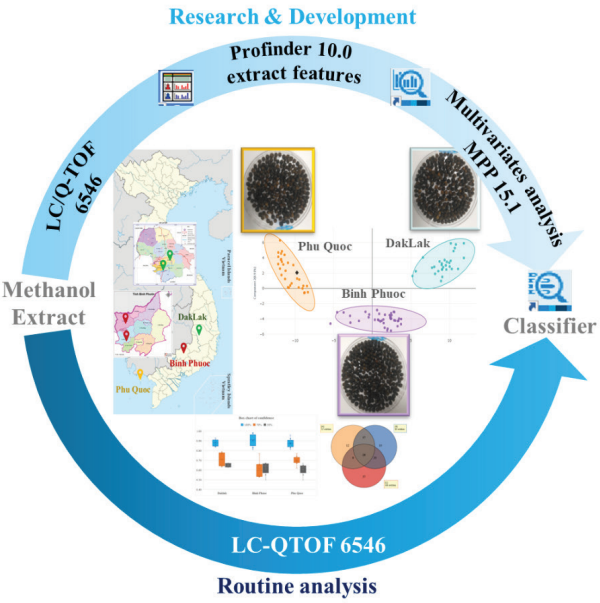
Abstract

This application note describes a combination of metabolomics profiling and chemometric approaches for discriminating black pepper samples in a narrow geographic range. This work used a comprehensive hardware and software solution that included an Agilent 1290 Infinity II LC system (UHPLC) and Agilent 6546 quadrupole time-of-flight mass spectrometer (LC/Q-TOF). Agilent MassHunter Profinder software 10.0, Mass Profiler Professional software (MPP) 15.1, and Classifier 1.0 models were used as well. The results from this workflow show high precision and accuracy and demonstrate a powerful set of tools to rapidly discover mislabeling and adulteration.



Introduction

Black pepper (*Piper nigrum* L.), known as the "King of Spices" and "Black Gold", is an important member of the native *Piperaceae* family. Because of its characteristic pungent taste, black pepper is a useful spice for dishes. However, black pepper also contains numerous bioactive compounds that may be correlated to the spices used in traditional medicine.[1] As one of the most important and popular spices in the world, black pepper is grown in tropical regions. Vietnam is the world's leading producer and exporter of pepper with many famous brands and varieties. These black peppers include Vinh Linh and Phu Quoc varieties from Phu Quoc Island, DakLak, and Binh Phuoc province.

In recent years, with the development of international markets and the demand for high-quality agricultural products, the safety and authenticity of food have become major concerns for consumers.[2] For example, black pepper with a geographical origin label, from Phu Quoc of Vietnam is considered high quality and commands a market premium. In addition to bringing economic benefits, the authentication of food helps prevent fake and inferior products. Authentication protects consumers rights and improves the credibility of producers and traders, considering the increase in global trade and free markets.

The most common techniques used for food authenticity and traceability include isotope ratio mass spectrometry, liquid and gas chromatography, elemental analysis, spectroscopic techniques, DNA-based techniques, and sensor techniques. The spectroscopic techniques for authenticity and traceability include vibrational, hyperspectral, fluorescence, and nuclear magnetic resonance. These techniques are rapid, cost-effective, and involve little or no sample preparation.[3-6] However, the main drawback to their use is lower model accuracy. The lower accuracy is partly due to lower analytical sensitivity and reduced selectivity, contributing to higher noise.

By contrast, the composition analysis method such as isotope ratio mass spectrometry (IR-MS), liquid and gas chromatography-tandem high resolution mass spectrometry (LC/ and GC/HRMS), and elemental analysis are techniques with increased selectivity and sensitivity, and often produce models with high accuracy.[7-9]

In food authenticity research, chemometric analysis plays an important role in classifying products based on their origin, variety, or other properties.[10] The most common multivariate analysis method for verifying that purpose includes two main types of algorithms. The two types are unsupervised exploratory methods and supervised methods based on regression or other types of machine learning.[11] For example, unsupervised methods including principal component analysis (PCA) and hierarchical cluster analysis (HCA) are useful tools for exploratory data analysis.[10,12] For supervised methods, regression-based techniques including linear discriminant analysis (LDA) and partial least square-discriminant analysis (PLS-DA) are among the most common algorithms.[12]

Overall, one of the key challenges for food source validation or adulteration testing is the overall complexity of the data collection and data analysis workflows. This workflow complexity creates challenges for technicians involved in routine testing. To address this challenge, this application note presents a comprehensive solution that is simple and powerful. This solution for food traceability uses a 1290 Infinity II LC system (UHPLC) and 6546 quadrupole time-of-flight mass spectrometer (LC/Q-TOF). MassHunter Profinder software 10.0, Mass Profiler Professional software (MPP) 15.1, and Classifier 1.0 models were also used. This system uses a combination of metabolomics profiling and chemometric approaches for discriminating black pepper samples in a narrow geographic range.

Experimental

Chemical and reagents

Optima LC/MS grade methanol, water, formic acid, and ammonium formate was used for mobile phase composition, which were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). For sample preparation, ultrapure water (18.2 MΩ.cm) and methanol (HPLC gradient grade) were produced by the Millipore-Q system (Merck, Darmstadt, Germany), and purchased from Sigma-Aldrich (Merck, Darmstadt, Germany), respectively.

Carbendazim D3 (methyl D3) was obtained from LGC Standards (LGC Group, United Kingdom), and is used as an internal standard for quality control of data.

Purine and HP-921 (hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine) were used as reference masses for the LC/Q-TOF instrument during the analysis, which was provided by Agilent Technologies (part number G1969-85001).

Sample collection and sample set

A total of 125 samples were collected from three regions: Phu Quoc Island (Kien Giang province); Cukin, Krong Pa district of DakLak province, and Loc Ninh, Hon Quan district of Binh Phuoc province during a period starting from February to March of 2022. The number of samples for DakLak and Phu Quoc was 40 samples, while the number was 45 for Binh Phuoc.

To build and validate the classification models, the number of samples were divided into training and validation sets, respectively. The training set consisted of 100 samples (30 DakLak, 40 Binh Phuoc, and 30 Phu Quoc) and was used to build the classification model. The remaining 25 samples (10 DakLak, 10 Phu Quoc, 5 Binh Phuoc) were used to validate the accuracy of the model.

Sample preparation

All black pepper samples were lyophilized at −45 °C for 3 days, then ground with a pulverizer to obtain a fine powder (<400 µm particle size) before storing in clean plastic bags. Positive quality control (QC) samples were made by mixing the homogenate from all samples of each region. Adulterated samples were prepared by mixing the positive QC samples at known ratios, including 30:70 and 50:50.

The sample extraction procedure was based on ultrasound-assisted extraction (UAE). First, 0.5 g ±0.1 mg of sample were weighed in a 50 mL falcon tube. Second, 10 mL of a solution consisting of water and methanol (30:70, v/v) were added, and the tube was vortexed for 5 minutes. The tube was then sonicated for 30 minutes at room temperature. The samples were centrifuged at 4,000 rpm for 5 minutes and the resulting supernatant was filtered through 0.22 µm PTFE filters. Finally, each sample extract was diluted 10x with mixed solvent water:methanol (30:70, v/v) containing 500 µg/L internal standard. The samples were transferred to vials for UHPLC/Q-TOF HRMS analysis.

Instrument method

To investigate the metabolomics fingerprints of black pepper, the 1290 Infinity II LC system coupled with the 6546 LC/Q-TOF was used. The LC/Q-TOF has a high resolution of 60,000 at *m/z* 2,722 (positive mode) and *m/z* 2,834 (negative mode).

Chromatographic separation of extracted sample solutions was carried out with a total run time of 23 minutes per sample (Table 1).

Table 1. Chromatography conditions and MS parameters.

Parameter	Value
LC	
UHPLC	Agilent 1290 Infinity II High Speed Pump (G7120A) Agilent 1290 Infinity II Vialsampler (G7129B) Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
Column	Agilent ZORBAX RRHD Eclipse XDB-C18, 80 Å, 2.1 × 100 mm, 1.8 µm (p/n 981758-902)
Guard Column	Agilent ZORBAX Eclipse XDB-C18, 80 Å, 2.1 mm, 1.8 µm UHPLC guard (p/n 821725-903)
Column Temperature	35 °C
Mobile Phase	A) H_2O with 5 mM ammonium formate and 0.1% formic acid (v/v) B) MeOH with 5 mM ammonium formate and 0.1% formic acid (v/v)
Flow Rate	0.3 mL/min
Injection Volume	5 µL
Gradient Elution Profile	Time (min) %A %B 0.0 98 2 0.5 98 2 1.0 50 50 4.0 35 65 10.0 5 95 12.0 0 100 16.0 0 100 18.0 98 2 Post time: 5 min
LC/Q-TOF MS Conditions	
MS	Agilent 6546 ultrahigh-definition accurate-mass LC/Q-TOF with Dual Jet Stream electrospray ionization (ESI)
Polarity	Positive ionization
Drying Gas Temperature	325 °C
Drying Gas Flow Rate	8 L/min
Nebulizer Gas Pressure	20 psi
Sheath Gas Temperature	375 ˚C
Sheath Gas Flow Rate	12 L/min
Capillary Voltage	4,000 V
MS Range	*m/z* 50 to 1,000
Reference Ions	*m/z* 121.0509/922.0098
Mode	TOF full spectra acquisition, 3 spectra/min
Software	
Acquisition	Agilent MassHunter Data Acquisition (version 10.1)
Data Processing and Analysis	Agilent MassHunter Profinder (version 10.0.2) Agilent MassHunter Mass Profiler Professional (version 15.1) Agilent MassHunter Classifier (version 1.0)

Chemometric analysis and metabolite identification

Feature extraction was accomplished using MassHunter Profinder 10.0 software for processing the raw LC/Q-TOF data. The Recursive Molecular Feature Extraction (rMFE) algorithm was used to extract features (neutral mass and retention time) based on coeluting ions that are related by isotope group, adduct species, peak shape, and retention time.

Mass Profiler Professional (MPP) software version 15.1 was used to normalize the data. These data included filter feature (based on frequency, flag, and abundance) and statistical analysis including analysis of variance (ANOVA), fold change, volcano plot, PCA, HCA, and build classification model PLS-DA. The workflow and key parameters of data processing are shown in Figure 4.

To determine the most significant differences in compounds among three groups, MPP includes a convenient, integrated ID Browser that enables accurate mass compound matching of the list of differential metabolites to the METLIN metabolite database.

Results and discussion

Chromatography and data quality

The total ion chromatography (TIC) of black pepper samples representing each region is shown in Figure 1, indicating that there is similar TIC among black pepper samples of three regions. As shown in Figure 2, the entities are uniformly distributed over the gradient elution program. The mass of metabolites ranged from nearly 56 to 1,980 Da, and most of these compounds had masses less than 800 Da. Note that approximately 82% of entities appear in all samples (100% frequency). Based on the data, there is a high similarity in the composition of black pepper samples between the three regions.

The mass accuracy and response of the Carbendazim-D3 internal standards are plotted in Figures 3A and 3B. These results serve as a quality control check for every injection and also the entire dataset. These data show that throughout the entire experiment, mass error on the 6546 UHPLC/Q-TOF was low (<2 ppm), and had a stable signal with a relative standard deviation (RSD) of 8.4% (<10%). The retention time drifted only <0.03 minutes throughout the whole worklist. This data reproducibility gives confidence in the instrument performance and confirms that the raw data can be used for further processing by MassHunter Profinder 10.0.

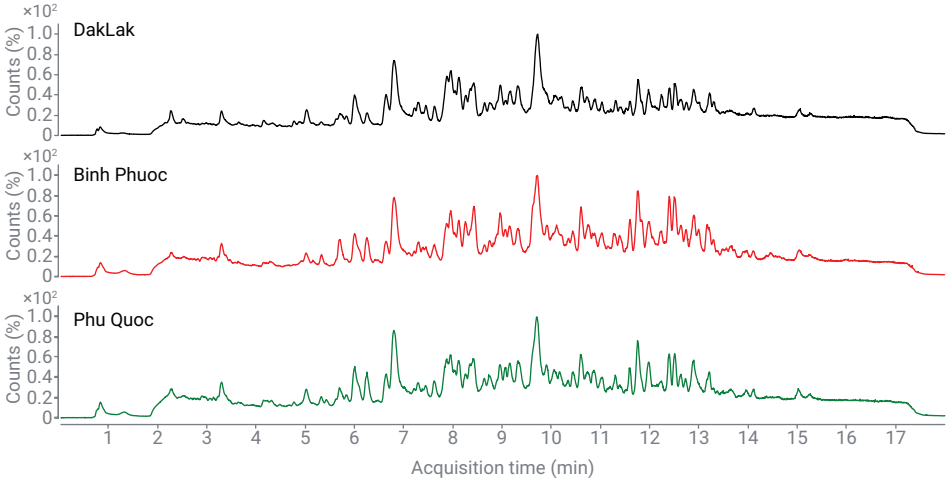


Figure 1. Chromatography of black pepper samples.

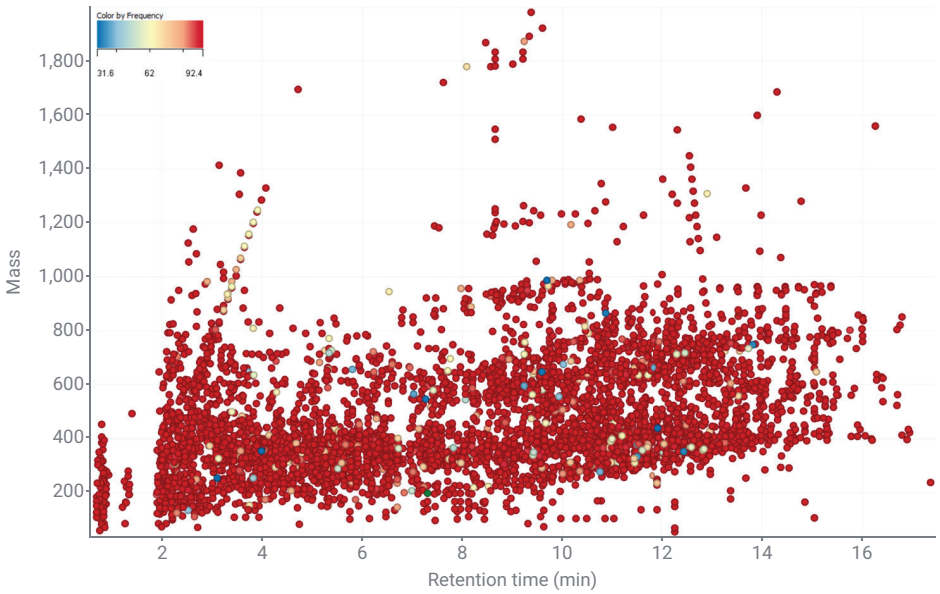


Figure 2. Distribution of RT, mass, and frequency in the sample set of the entities.

Data processing and chemometric analysis

Data processing: The 100 model samples (30 DakLak, 40 Binh Phuoc, and 30 Phu Quoc) were loaded into Profinder 10.0 and grouped by their geographical origin. Due to minimal retention time drift in this dataset, no retention time correction was needed for this analysis. The batch recursive feature extraction (small molecules/peptides) wizard was selected to detect features in an untargeted manner. A few changes were made to the default method. The protonated and ammonium adduct ion species were selected with the common organic molecules (including halogens) isotope model and a charge state limit of 1 to 2. A height filter of 1,000 was used whenever requested by the wizard. Finally, the molecular feature extractor (MFE) algorithm and the target score for feature quality were increased to 80. The results were reviewed and modified via manual extract, then exported as a .PFA file. This .PFA file was imported into MPP software before performing multivariate analysis. A simple data processing procedure was applied in this study to extract features for building a classification model, which is detailed in Figure 4.

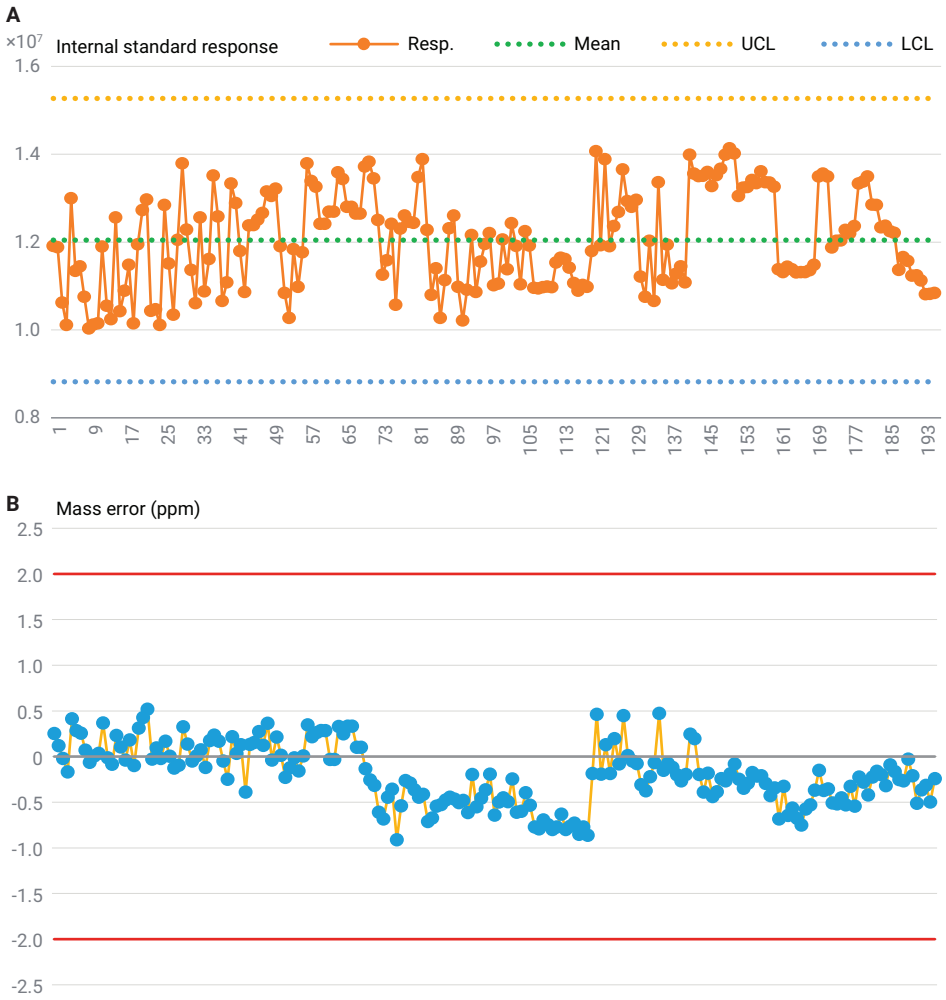


Figure 3. International standard (IS) response and mass error over 200 injections.

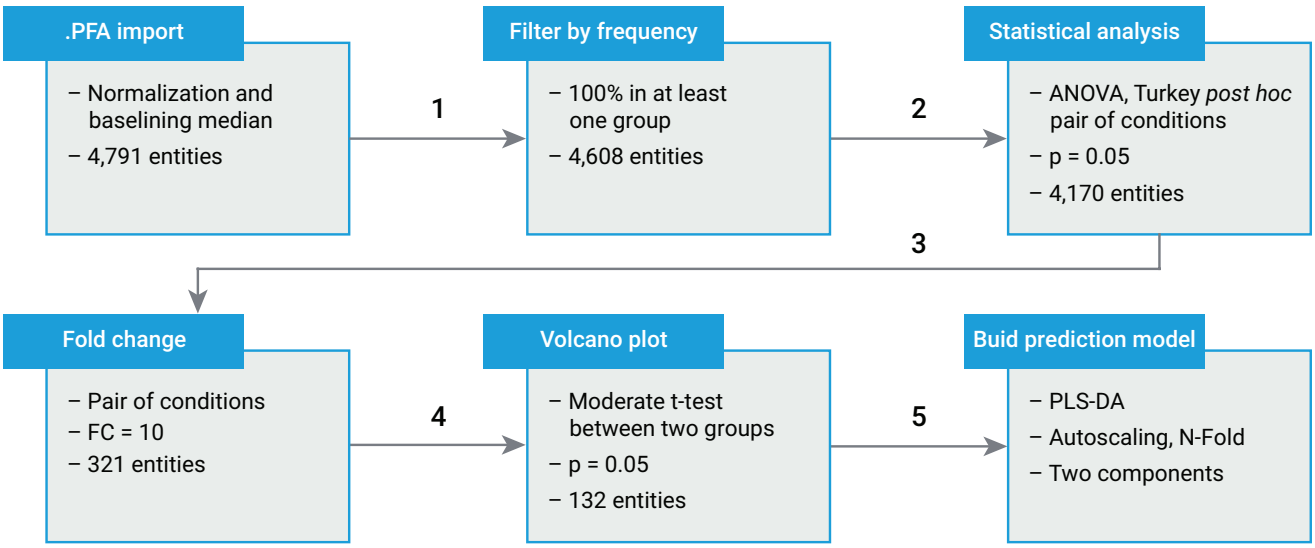


Figure 4. MPP analysis workflow and key parameters.

The data normalization and baselining median process of 4,791 entities was done with the purpose of transforming features to be on a similar scale and removing bias from high-abundance entities. This processing improves the performance and training stability of the model. In the next step, the filter by frequency tool was used and there were 4,608 entities that satisfied this condition with 100% presence in at least one group. ANOVA and *post hoc* ANOVA (Turkey pair of conditions) was used to find entities whose abundance had statistically significant differences among black pepper sample groups with the p-value of the hypothesis test set at 0.05. After that, the fold change and volcano plot were applied to evaluate the pair of conditions with the parameters cut-off fold change value of 10 and p-value of 0.05 for a moderate t-test, respectively. Finally, there were 132 entities used for multivariate analysis as unsupervised, including PCA, HCA, and supervised (PLS-DA). This analysis was used for building a model to distinguish black pepper from the three different growing regions.

Sample clustering overview using unsupervised PCA and HCA: The PCA algorithm was performed for the preliminary exploration of the data. The results indicated that there were only the first four components of PCA with an eigenvalue of more than 1, which can explain approximately 57% of variance in the data. Figure 5A illustrates a score plot based on the first three principal components (PC). Scores of 26.9% in PC1, 15.3% in PC2, and 9.4% in PC3 classify the black pepper samples from the three regions. According to the PCA score plot, there was a clear differentiation of Phu Quoc samples from the remaining origins along PC1. Meanwhile, PC2 acts as a tool to separate black pepper origin between Binh Phuoc and the two groups remaining.

Aside from PCA, HCA was also used to perform a preliminary data scan and to uncover the structure residing in the data. Figure 5B shows the condition tree on the group and heat map of 132 entities. This dendrogram clearly shows that the distance of the Phu Quoc sample group is far from that of the other two sample groups, contrasting the short distance between DakLak and Binh Phuoc.

Classification model

Partial least squares-discriminant analysis: In recent years, partial least squares-discriminant analysis has been used as an algorithm with high power to discriminate geographical origin. Instead of finding hyperplanes of maximum variance between the response and independent variables, this method finds a linear regression model by projecting the predicted variables and the observable variables to latent variables representing the series of linear regression components. In this study, a classification model using the PLS-DA algorithm was built using 132 entities obtained after data processing (Figure 4). The results were shown in Table 2 and Figure 6 and illustrate the extent of the contribution of the 132 entities reflected by variable importance in the projection (VIP) value to the classification model. Overall, there were 64 entities that had a VIP value higher than 1, which was found to be significant in creating a discrimination model for determining the geographical origin of black pepper. Notably, standing at nearly 2.12, the VIP value of the entity (MW 565.42, RT 10.02 minutes), which was the highest in 132 entities, indicated that this metabolite compound is the most relevant to discriminate the geographical origin of black pepper among three regions.

A satisfactory PLS-DA model was obtained with R^2Y and Q^2Y values of 0.919 and 0.833, respectively. Both R^2Y and Q^2Y values are greater than 0.5 and approximately equal to 1, which indicates that the origin discriminant model has a high correlation and good predictive power. The cross-validation discriminant accuracy of the training set was up to 100% for all groups.

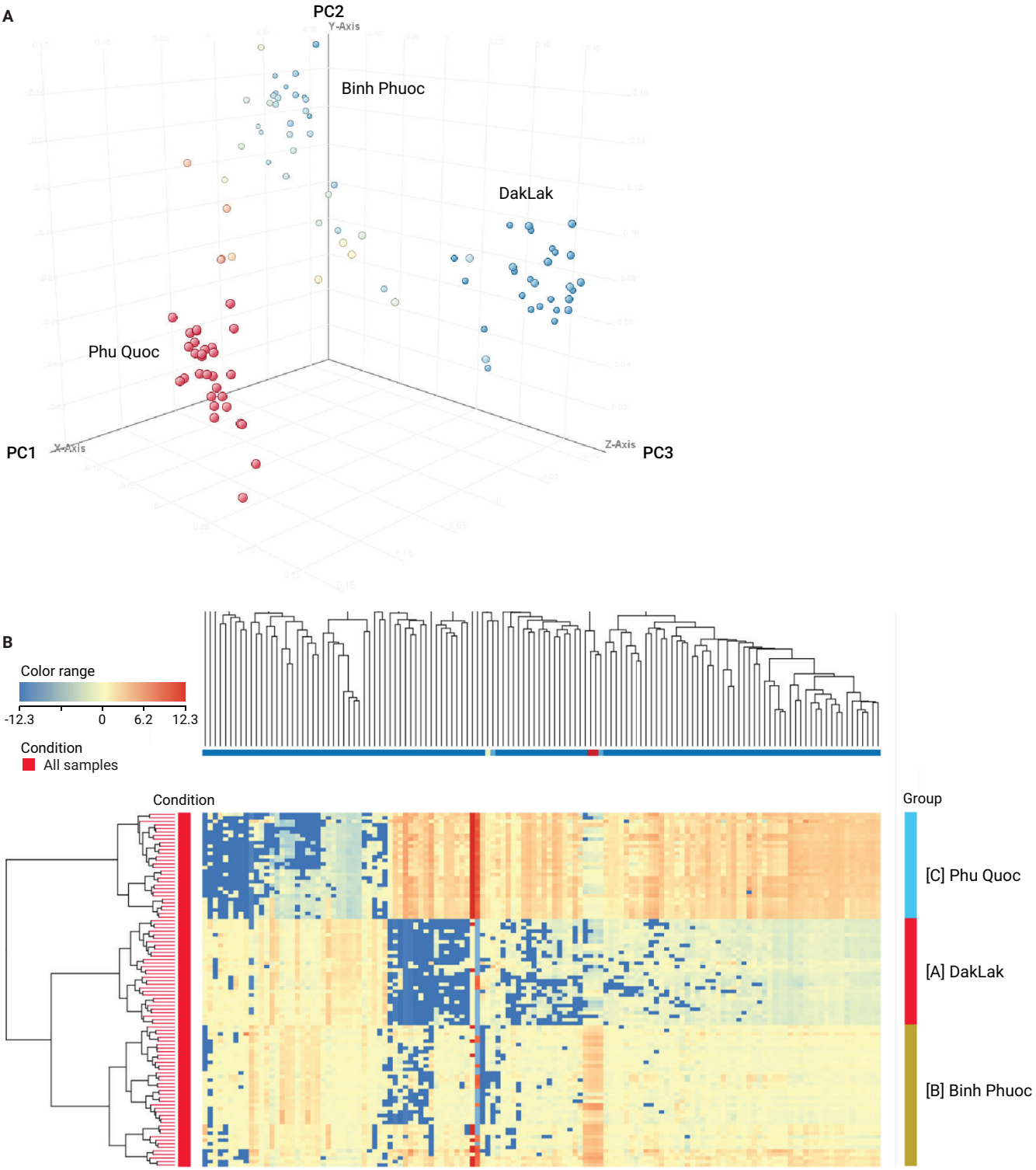


Figure 5. (A) PCA scatterplot and (B) hierarchical cluster analysis (HCA) dendrogram for black pepper samples from three regions (tree on group).

Model validation: For the accuracy test of the model, raw data from validating set, QC, and adulterated samples combined with the Profinder method, and the PLS-DA model were imported simultaneously into Classifier 1.0 software. The imported data were used to perform the discrimination process without performing any other data processing operations. The classification results are displayed in Figure 7 and Table 2.

The black diamonds in the PCA plots in Figure 7 represent the reviewing samples. The sample position relative to the Hotelling ellipses (95% confidence ellipse) is indicative of their purity, and the confidence value of the sample is also shown in the sample table. When the sample is a pure QC sample, the black diamond is in or very near the black pepper grouping to which it belongs (Figure 7A). When the sample is adulterated, the black diamond is plotted further away from the grouping (Figures 7B and 7C). As shown in Table 2, all the validated and QC samples are classified with 100% accuracy. These data show that the metabolomics approach using UHPLC/Q-TOF to identify fingerprints is a powerful tool for food origin classification.

The repeatability of the model was also evaluated using the QC sample and the confidence value was obtained from the Classifier software. As shown in Figures 8A and 9, the mean confidence values of the QC samples (purity 100%) ranged from 0.88 to 0.90 for the groups. The RSD values of six replicates fluctuated from 4.5 to 8.0% and this figure was lower than 10%, indicating that the precision of the method was also very high.

Table 2. Prediction results of PLS-DA model for traceability of black pepper samples.

Sample Origins	Training Set			Validation Set and QC Samples		
	Number	Mistake	Correct Rate	Number	Mistake	Correct Rate
DakLak	30	0	100	10+6	0	100
Binh Phuoc	40	0	100	5+6	0	100
Phu Quoc	30	0	100	10+6	0	100

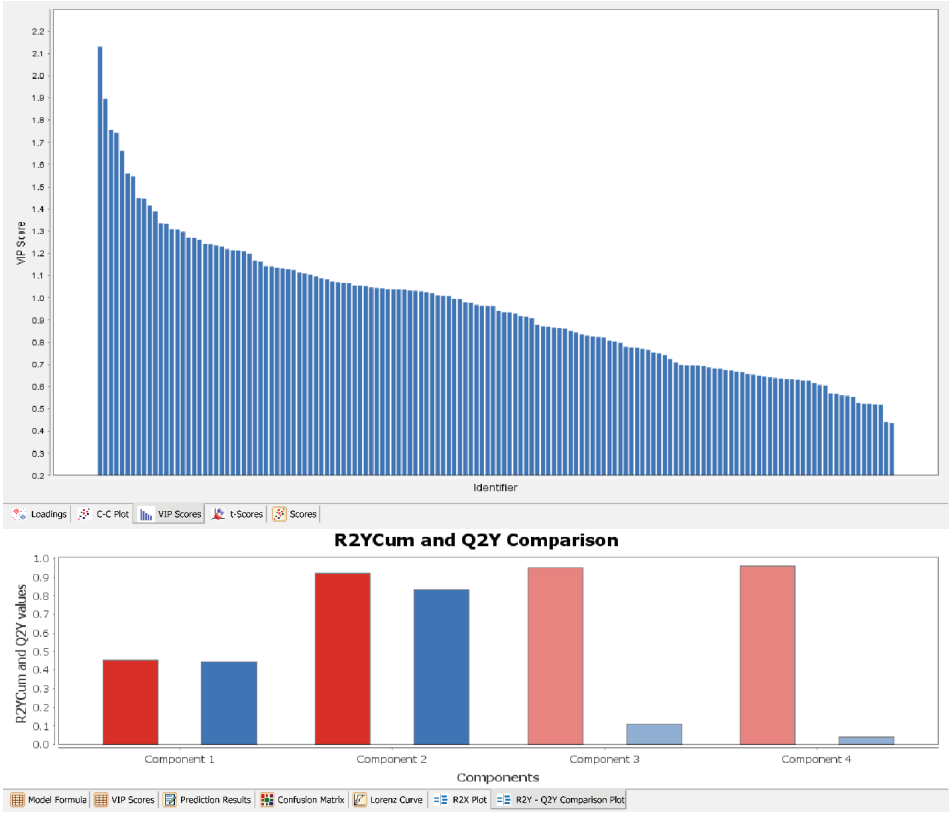


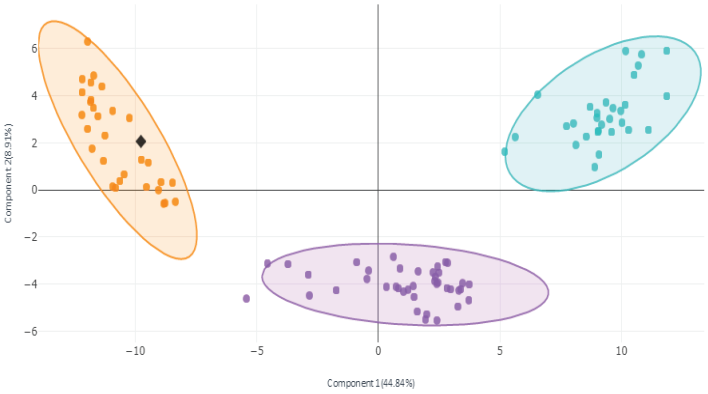
Figure 6. VIP value of entities combined with R^2Y and Q^2Y comparison among components.

Moreover, the lowest confidence threshold used to distinguish pure and adulterated samples is also defined in this application note. Figures 7B, 7C, and 8 clearly show that the confidence values of the pure samples are much higher than those of the doped samples at a ratio of 70:30 and 50:50. The reliability was lower when the adulterated ratio was larger. In this case, a cutoff value of 0.8 was used to accurately identify pure or adulterated samples.

A Sample Table

Sample	Class	Confidence
QC Binh Phuoc L1	B	0.98
QC Binh Phuoc L2	B	0.91
QC Binh Phuoc L3	B	0.81
QC Binh Phuoc L4	B	0.98
QC Binh Phuoc L5	B	0.91
QC Binh Phuoc L6	B	0.83
QC Daklak L1	A	0.84
QC Daklak L2	A	0.86
QC Daklak L3	A	0.91
QC Daklak L4	A	0.93
QC Daklak L5	A	0.84
QC Daklak L6	A	0.91
QC Phu Quoc L1	C	0.96
QC Phu Quoc L2	C	0.83
QC Phu Quoc L3	C	0.87
QC Phu Quoc L4	C	0.81
QC Phu Quoc L5	C	0.87
QC Phu Quoc L6	C	0.92

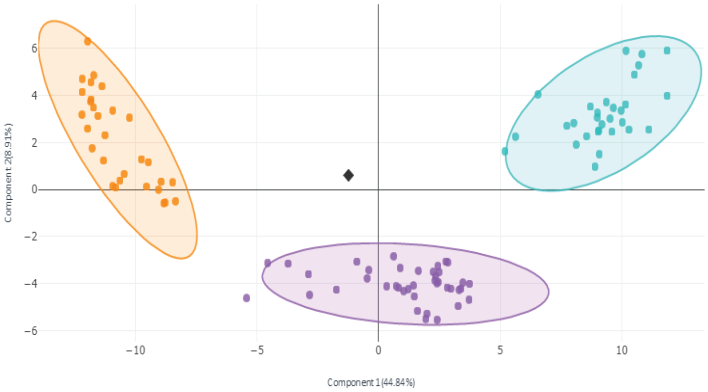
QC Phu Quoc L6



B Sample Table

Sample	Class	Confidence
Binh Phuoc-Phu Quoc 50-50 L1	C	0.65
Binh Phuoc-Phu Quoc 50-50 L2	C	0.55
Binh Phuoc-Phu Quoc 50-50 L3	C	0.50
Daklak-Phu Quoc 50-50 L1	C	0.63
Daklak-Phu Quoc 50-50 L2	A	0.63
Daklak-Phu Quoc 50-50 L3	C	0.67
Binh Phuoc-Daklak 50-50 L1	B	0.67
Binh Phuoc-Daklak 50-50 L2	A	0.67
Binh Phuoc-Daklak 50-50 L3	A	0.63

Daklak-Phu Quoc 50-50 L1



C Sample Table

Sample	Class	Confidence
Phu Quoc-Binh Phuoc 70-30 L1	C	0.67
Phu Quoc-Binh Phuoc 70-30 L2	C	0.62
Phu Quoc-Binh Phuoc 70-30 L3	C	0.77
Phu Quoc-Daklak 70-30 L1	C	0.70
Phu Quoc-Daklak 70-30 L2	C	0.74
Phu Quoc-Daklak 70-30 L3	C	0.69
Daklak-Binh Phuoc 70-30 L1	A	0.64
Daklak-Binh Phuoc 70-30 L2	A	0.77
Daklak-Binh Phuoc 70-30 L3	A	0.63
Daklak-Phu Quoc 70-30 L1	A	0.79
Daklak-Phu Quoc 70-30 L2	A	0.65
Daklak-Phu Quoc 70-30 L3	A	0.78
Binh Phuoc-Daklak 70-30 L1	A	0.65
Binh Phuoc-Daklak 70-30 L2	B	0.54
Binh Phuoc-Daklak 70-30 L3	A	0.53
Binh Phuoc-Phu Quoc 70-30 L1	C	0.53
Binh Phuoc-Phu Quoc 70-30 L2	C	0.66
Binh Phuoc-Phu Quoc 70-30 L3	B	0.77

Phu Quoc-Binh Phuoc 70-30 L1

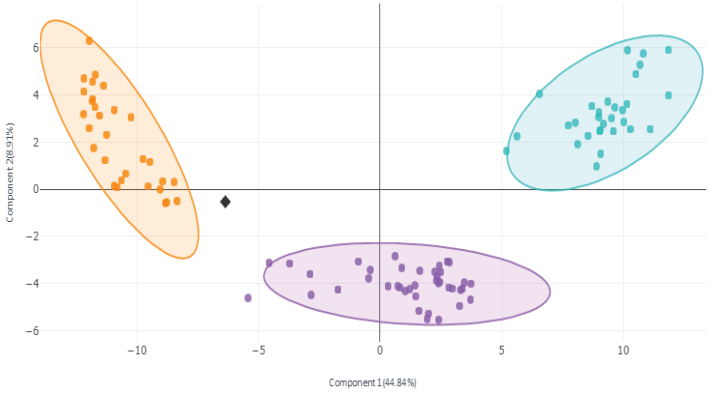


Figure 7. Classifier 1.0 results for (A) QC sample and (B) adulterated black pepper sample at 50:50 ratio. (C) Adulterated black pepper sample at 70:30 ratio. The Phu Quoc class is orange, the Binh Phuoc class is purple, and the DakLak class is teal. The black diamond represents the selected sample.

Identity of unique compounds

MPP software was used to identify the number of unique entities in the different black pepper sample groups. Venn diagrams are shown in Figure 9, indicating that out of a total of 132 entities, there were 37 entities unique to the Phu Quoc pepper pattern; the number for DakLak was 12 entities, and 15 entities for Binh Phuoc. Half of the unique compounds were tentatively identified (Table 3) using the ID Browser identification tool integrated on MPP software combined with the metabolite and lipid database and library (METLIN).

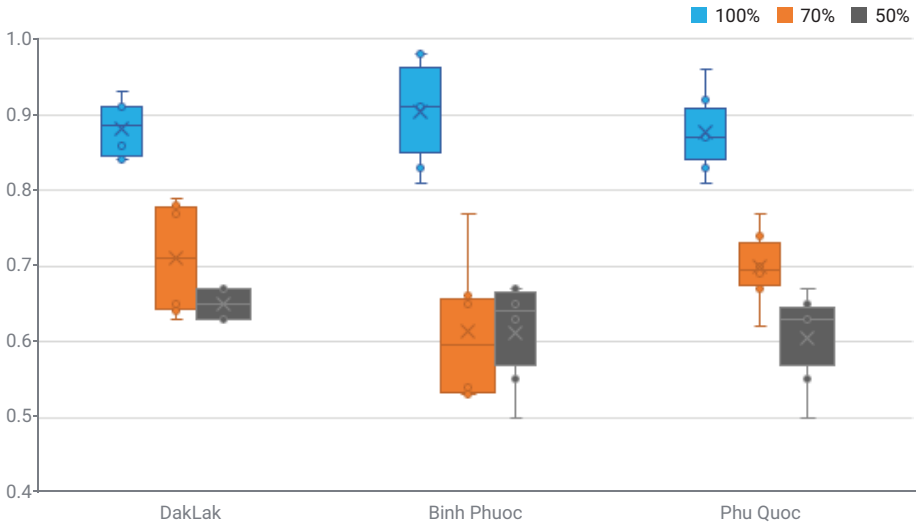


Figure 8. Summary plot of confidence results of purity and adulterated samples from Classifier 1.0.

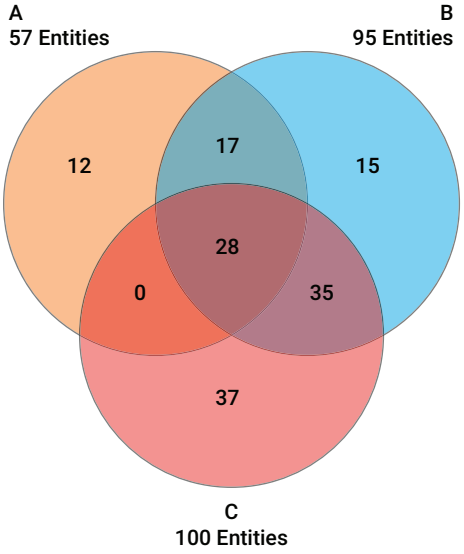


Figure 9. Venn diagrams for unique entities in black pepper sample of each region.

Table 3. Unique compounds in black pepper from each region by Venn diagrams.

No.	Tentatively Identified Compound	RT (min)	Accurate Mass (*m/z*)	Adduct	Error (ppm)	VIP Value
Phu Quoc black pepper						
1	Oleic acid-2,6-diisopropylanilide	11.03	459.4311	$[M+NH_4]^+$	0.41	0.97
2	6β-Naltrexol	8.18	344.1858	$[M+H]^+$	0.37	0.82
3	(2-Oxo-3-pyridin-3-ylchromen-7-yl) acetate	3.55	282.0761	$[M+H]^+$	0.36	0.69
4	1-Hexadecanoylpyrrolidine	11.43	310.3107	$[M+H]^+$	0.87	1.66
5	N-oleoyl phenylalanine	12.50	452.3146	$[M+Na]^+$	2.66	0.67
6	(S)-Codamine	8.19	366.1677	$[M+Na]^+$	1.51	0.63
7	Moupinamide	3.03	314.1289	$[M+H]^+$	1.9	0.96
8	1-(2,3-Dihydro-1H-pyrrolizin-5-yl)-1,4-pentanedione	2.02	206.1176	$[M+H]^+$	0.26	0.61
9	Etrogol	4.58	207.1380	$[M+H]^+$	0.27	0.56
10	Juglone	5.87	175.0391	$[M+H]^+$	0.63	1.12
11	Mosesin	11.50	641.4292	$[M+H]^+$	5.00	1.30
12	3,5-Dimethoxy-8,8-dimethyl-2-phenyl-4H,8H-benzo[1,2-b:3,4-b']dipyran-4-one	5.59	365.1362	$[M+H]^+$	−2.31	0.65
13	Oxacillin	6.71	402.1102	$[M+H]^+$	−4.01	0.78
14	Jasmolone glucoside	2.67	365.1572	$[M+Na]^+$	2.16	0.56
15	Albendazole-γ-hydroxysulphone	5.52	314.0822	$[M+H]^+$	1.49	0.67
16	8-Methyldihydrochelerythrine	6.72	364.1544	$[M+H]^+$	0.24	0.88
17	8-Deoxypumiliotoxins	11.10	314.2456	$[M+Na]^+$	0.63	0.63
18	Destomycin	8.06	545.2649	$[M+NH_4]^+$	−2.25	0.84
19	Mitomycin	2.26	335.1340	$[M+H]^+$	−1.29	0.64
20	Amritoside	6.23	649.1011	$[M+Na]^+$	−1.70	0.81
21	Propantheline	7.82	368.2218	$[M]^+$	−1.94	0.80
22	Glucosyl-4,4'-diaponeurosporenoate	7.30	298.1844	$[M+2H]^{2+}$	−2.47	0.57

No.	Tentatively Identified Compound	RT (min)	Accurate Mass (*m/z*)	Adduct	Error (ppm)	VIP Value
Binh Phuoc Black Pepper						
23	Isocycloneosamadaridine	10.03	346.2377	$[M+H]^+$	0.10	1.12
24	N1,N5,N10-Tris-*trans*-*p*-coumaroylspermine	10.09	641.3338	$[M+H]^+$	0.74	0.70
25	26,27-Dinor-cholest-5-en-23-yn-3beta-ol	12.46	372.3263	$[M+NH_4]^+$	1.08	1.41
26	2'-Norberbamunine	7.46	583.2805	$[M+H]^+$	1.41	0.82
27	7-Ethyl-10-(4-N-aminopentanoic acid)-1 piperidino) carbonyloxycamptothecin (APC)	10.33	619.2789	$[M+H]^+$	4.53	1.30
28	Demethoxycurcumin	4.67	384.1784	$[M+H]^+$	−3.48	1.23
30	14'-Apo-β-Carotenal	11.52	328.2612	$[M+H]^+$	−2.26	1.06
DakLak Black Pepper						
31	Isolobinine	6.42	288.1960	$[M+H]^+$	0.70	0.52
32	Anandamide	9.42	348.2899	$[M+H]^+$	0.53	0.44
33	Momordicilin	13.53	558.4882	$[M+H]^+$	−0.14	0.70
34	(R)-3,4-Dihydro-2-methyl-2-(4,8,12-trimethyl-3,7,11-tridecatrienyl)-2H-1-benzopyran-6-ol	10.98	400.3195	$[M+H]^+$	−3.94	0.77
35	2,6-Dimethyl-4-hydroxybenzaldehyde	5.32	151.0753	$[M+H]^+$	−0.32	0.94
36	(11Z)-8,18-Methano-retinal	10.96	314.2457	$[M+H]^+$	−2.09	0.76
37	Cassyfiline	5.68	342.1339	$[M+H]^+$	4.36	1.11

Conclusion

Metabolic profiling coupled with chemometrics was successfully used to determine the narrow geographic origins of black pepper. A comprehensive solution comes from Agilent Technologies including instruments and data analysis software. This solution helps users to easily develop methods of classification, traceability, and adulterated detection of commercial products with a metabolomic profiling approach. The robustness and stability of the Agilent 1290 Infinity II LC system (UHPLC) and Agilent 6546 quadrupole time-of-flight mass spectrometer (LC/Q-TOF) were illustrated to help obtain high-quality raw data. Agilent MassHunter Profinder (MP) combined with Mass Profiler Professional (MPP) is a powerful tool to extract features, do multivariate data analysis, and build discriminant models. For routine analysis, the Classifier software simplifies and quickly classifies new data.

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