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Establish an analytical model for chemical preservatives using QTOF and MPP software

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Introduction

Kathon, one of the chemical preservatives, is an excellent fungicide commonly used in cosmetic products, shampoos, and conditioners. It is also widely used in textile, paper, oil refining, oil field water injection, sewage treatment and in areas of sterilization and anti-corrosion treatments.

Kathon is a mixture of isothiazolinone derivatives and inorganic stabilizers. The main compounds of isothiazolinone derivatives include CIT/MIT, MIT, OIT, DCOIT, BIT, etc. Kathon products got to the market in the early 1970s, developed and patented by the Rohm&Haas. With the continuous development of MIT,OIT and other new derivative ingredients, the excellent properties of such products in bactericidal performance and wide range use were gradually recognized.

The major analytical methods for Kathon are based on high performance liquid chromatography coupled with diode array detector (HPLC-UV). This method is limited for its lack of capability in product quality determination. The impurities may affect the performance of the product. They are difficult to detect by HPLC-UV because of their low concentrations.

QTOF MS system, with better mass accuracy and higher resolution, was powerful in identification of unknowns in complex matrixes at trace level. MPP software has comprehensive statistical algorithm and pattern recognition method. After data is acquired by QTOF and imported to MPP, a class prediction model is built inside MPP.

Figure 1 Agilent 1290 UHPLC/6545 QTOF System

Experimental

Sample Preparation

6 Kathon samples were purchased from different manufacturers. Each sample group has 3 duplicates and total 18 samples were tested in this study. 1.0mg (±0.1mg) of sample was transferred to a 2mL vial and dissolved with 1mL MEOH. The solutions were vigorously shaken and centrifuged for 5 min at 12000rpm. Supernatant followed by filter was directly analyzed with LC/MS

Methods

Agilent UHPLC 1290 System

Column	Eclipse Plus SB-C18 3.0*100mm,1.8u
Mobile phase	A: 0.1%FA H2O B: ACN Gradient Elution from 95 to 0 A in 25min
Flow Rate	0.4ml/min
Column Temperature	40°C
Inject	1ul

Agilent 6545 LC/Q-TOF Mass Spectrometer

Ion source	AJS
Polarity	Positive
Ion Spray Voltage	4000V
Nozzle Voltage	0V
Dry gas Temperature	300°C
Nebulizer Pressure	45psi
Sheath Gas Flow	7L/min
Sheath Gas Temp	350°C
Fragmentor	100V
Acquisition Speed	2Hz

Data Processing.

This UV and total ion chromatograms of different sample groups are shown in Fig.1.Kathon sample have two main compounds, one is MIT(RT=3.81min) and the other is CIT(RT=7.05min). It is difficult to find the differential compounds between samples from TIC. MassHunter software is a productive tool for processing multiple samples in profiling analyses, allowing the user to visualize, review, and edit results by compounds across many samples. Chromatographic peak extraction was done using the Profinder software by molecular feature extraction features and imported to MPP software for analysis.

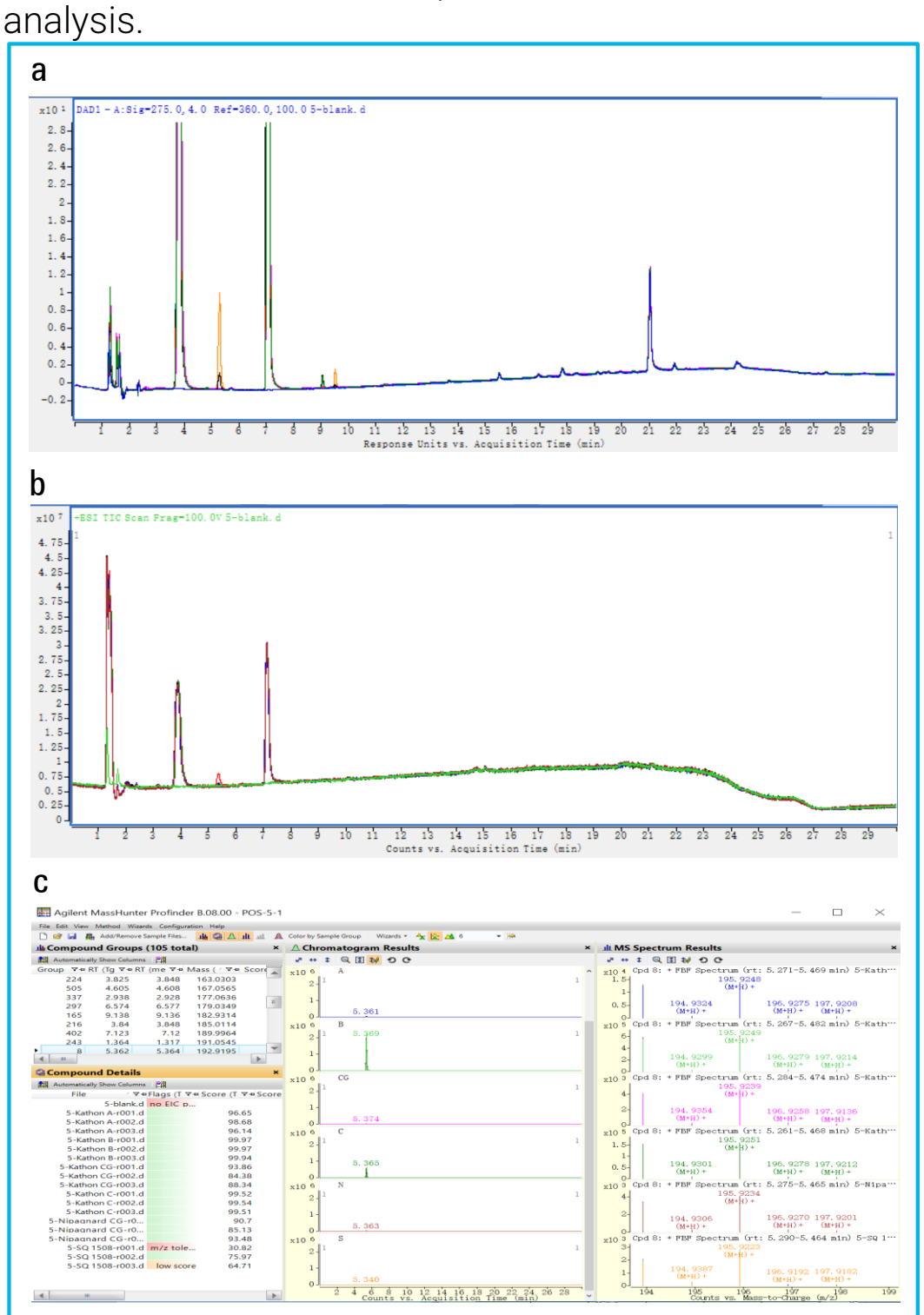


Figure 2 (a) The 275nm UV chromatograms of six groups of Kathon samples;

(b) The total ion chromatograms of six groups of Kathon samples, 3 replicate samples for each groups;

(c) The main view of the MassHunter Profinder software and identified features;

Mass Profiler Professional (MPP)

Principle Component Analysis(PCA) is a commonly used unsupervised statistical method to reduce the dimensionality of large data sets to reveal the differences among samples.

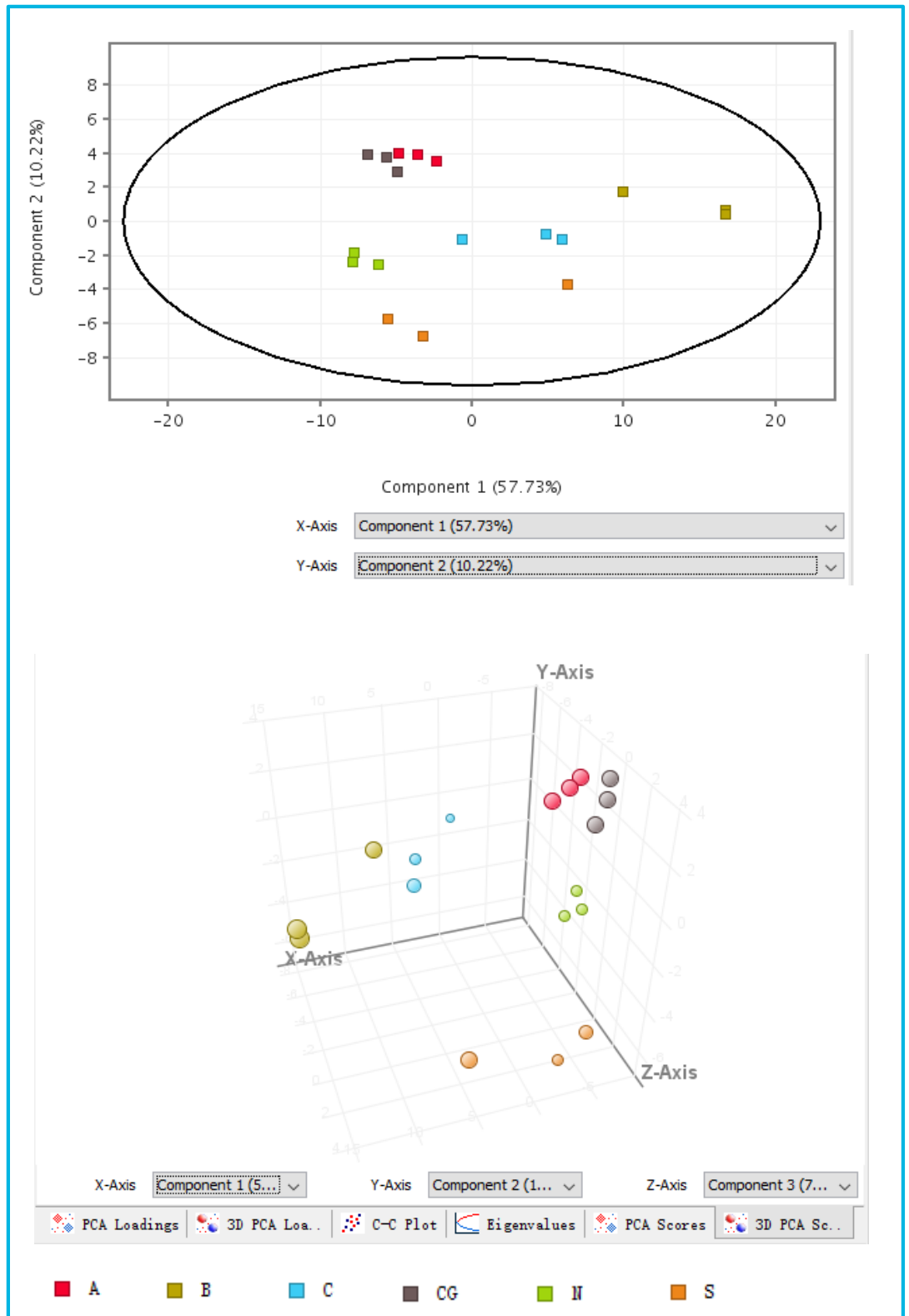
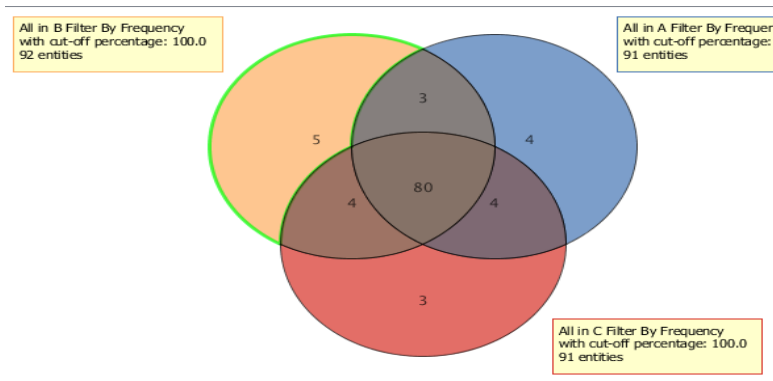


Figure 3 2-D and 3-D principle component analysis(PCA) of six groups samples.

Venn Diagram is typically used to compare entities in common or unique among different groups. It allows to compare up to 4 entity lists to isolate compounds that are in common or unique to each group. You can select the section and export to ID Brower.

Results and Discussion

a



b

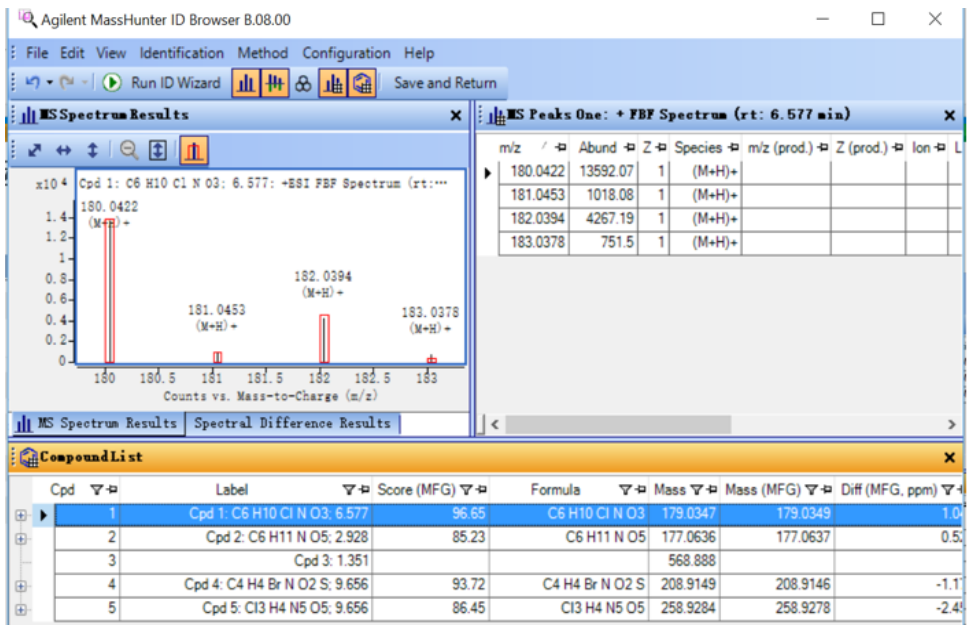


Figure 4 (a)Venn Diagrams allow you to compare up to 4 entity lists to isolate compounds that are in common or unique to each list.(b) ID Browser function in MPP for compound identification.

In all, 12 different potential markers were obtained and possible molecular formula were generated in the crude extract(Table 1). Those potential markers can be impurities related to the MIT and CIT.

Impurity	m/z	RT	Formula	MFG Score
1	193.9269	5.37	C4H4BrNOS	99.75
2	237.9533	9.66	C6H8BrNO2S	99.85
3	227.8881	9.59	C4H3BrClNOS	99.65
4	146.0813	4.60	C6H11NO3	99.91
5	122.0370	3.98	C4H8ClNO	98.68
6	183.9386	9.14	C4H3Cl2NOS	98.22
7	273.1922	1.69	C12H24N4O3	99.18
8	165.9724	5.44	C4H4ClNO2S	99.19
9	180.0422	6.69	C6H10ClNO3	97.92
10	202.1550	1.68	C9H19N3O2	98.12
11	148.0426	5.49	C5H9NO2S	96.09
12	209.9218	9.6	C4H4BrNO2S	95.74

Table 1. Compounds as potential markers for differentiating of Kathon samples.

Sample-sample correlation and clustering analysis is a powerful tool to organize compounds or entities and conditions into clusters based on the similarity of their abundance profiles. The result is displayed as a dendrogram (Fig.5). Based on their correlation coefficient values, entities are arranged on a heat map with red color showing positive correlation and blue color representing negative correlation. Sample B exhibited a positive correlation with sample C and sample SQ correlation with sample Nipagnard.

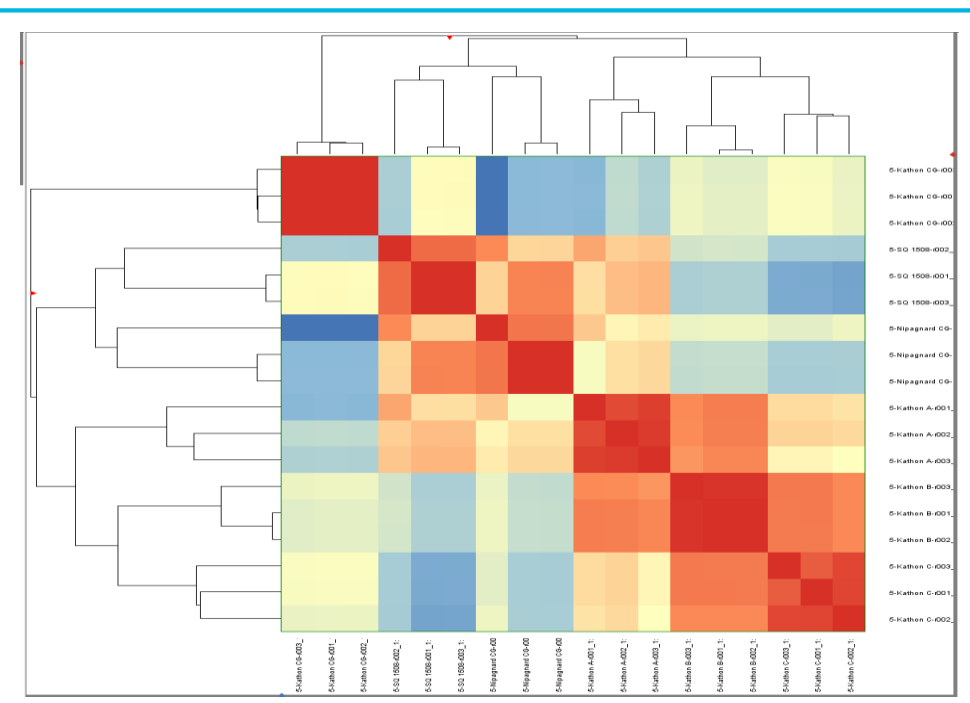


Figure 5 Sample and sample correlation analysis of 18 selected entities.

Conclusions

- A rapid method was developed for the screening of impurities of Kathon samples using UHPLC/QTOF-MS.
- The PCA results showed that the established model was capable of differentiating different manufacturer products.
- Combined with Agilent Profinder and MPP software, this workflow enabled the identification of specific markers that may help quality control .
- Those impurities are not reported, and further identification needs to be done with possible standards. We only selected 12 compounds with significant difference for analysis.

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