

## GERSTEL AppNote 227

# Aroma Office Version 7: A New Data Analysis Workflow for Aroma/Flavor compounds in combination with Deconvolution and Multivariate Analysis Software.

## Application to Premium Gins

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### Keywords

Aroma Office (Ver. 7), Gin, DHS, Multi-volatile Method (MVM), GC-MS, Automated Data Analysis Workflow, Data Handling Software Integration, Unknowns Analysis.

### Abstract

Aroma Office 2D (Gerstel K.K.) is an integrated software approach for simultaneous processing of retention index (RI) and mass spectra (MS) for rapid and improved identification of flavor/aroma compounds. The previous Ver. 6 (and earlier) of this software ran on the Agilent ChemStation platform and the current Ver. 7 upgrades to integration into MassHunter Unknowns Analysis. After MassHunter deconvolution and mass spectral library search, the data is processed by Aroma Office with cross searching of deconvoluted library search results and RI values. The Aroma Search module allows an entire Total Ion Chromatogram (TIC) to be processed automatically. Search results can be further transferred to multivariate analysis software such as Agilent Mass Profiler Professional (MPP). The linkage and integration of these various operations offers a unique data analysis workflow for automated detection and characterization of aroma/flavor compounds and for sample classification in combination with authentication.

### Introduction

Two previous Application Notes provide an appreciation of the development and application scope of Aroma Office.

Gerstel AppNote 183 [1] outlines a key function of Aroma Office in which a GC-MS library search for a compound will display a retention index (RI) value in addition to the normal Probability Based Matching (PBM) ranking and CAS Registry Number (CAS RN). Both can be transferred to the Aroma Office database for cross searching. Only when there is a match between the CAS RN and RI value can the identity of the compound be accepted. A PBM hit list can often be composed of candidates with remarkably similar mass spectra and the added security with RI values is therefore critical for identification. The Aroma Office searchable data base contains retention index information on >10,000 compounds from greater than 100,000 entries and from a wide range of literature references. In addition to RI values the database contains compound names, analytical conditions, literature references and provides a novel odor character search for flavor compounds. Automated cross searching with two RI values only (i.e. after GC-O) from different columns is also available for compound identification.

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GERSTEL AppNote 198 [2] describes the application of Aroma Office technology to a complex matrix-heavy beverage after extraction by both standard Stir Bar Sorptive Extraction (SBSE) and Solvent-Assisted SBSE (SA-SBSE), the latter based on a solvent swollen polydimethylsiloxane stir bar for extraction and enhanced recovery of polar compounds. This AppNote expands further on the Aroma Search option, where cross searching and transfer to the database can be extended to a full TIC. In this operation, after TIC integration and PBM library searching all CAS RNs together with RI values are sent to the database. Aroma Office then reports detailed information (average RI, CAS RN, formula, odor characteristics etc.) on flavor compounds satisfying the CAS-RI requirement.

The current Version 7 of Aroma Office discussed here retains all the functionality and applicability of the earlier version but offers a significant expansion in integration with other contributory software modules. In particular Aroma Office can be integrated into Agilent GC-MS MassHunter Unknowns Analysis. Information rich library search data after deconvolution can be batch transferred to Aroma Office for RI cross searching, and Aroma Search automatically extracts aroma/flavor compounds. These results can further be batch transferred to a multivariate analysis software such as Agilent Mass Profiler Professional (MMP) or exported as an Excel file.

### Experimental

#### Samples

Three premium commercial Gin samples were purchased for analysis. These are described as follows.

#### Gin A

"Anise, coriander, fresh floral, gorse flower, juniper, lavender, zesty citrus".

#### Gin B

"citrus, coriander, lemon, nutmeg, angelica, red berry, juniper, meadowsweet".

#### Gin C

"lemon, citrus, ginger, gorse flower, herbal".

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*Instrumentation*

50 µl of each gin in a 10 ml headspace vial was analyzed in triplicate by a previously published 2-Step Multi-Volatile method [3]. This involves two Dynamic Headspace (DHS) sampling parameter sets which are performed sequentially to highlight both volatile

compounds and hydrophilic and/or polar compounds, while minimizing or eliminating ethanol and water. All samples were analyzed in triplicate.

**Table 1:** 2-step MVM analytical conditions.

Sample	
Sample Volume	50 µL in a 10 mL HS vial

DHS Method BX (1 <sup>st</sup> Sampling)			
Incubation Temp.	25 °C (w/UPC+)	Incubation Time	5 min
Purge Volume	650 mL	Purge Flow Rate	100 mL/min
Dry Volume	300 mL	Dry Flow Rate	50 mL/min
Trap Adsorbent	Carbopack B/X (015954-005-00)	Trap Temp.	30 °C

DHS Method FEDHS (2 <sup>nd</sup> Sampling)			
Incubation Temp.	80 °C	Incubation Time	0 min
Purge Volume	3000 mL	Purge Flow Rate	100 mL/min
Dry Volume	n.a.	Dry Flow Rate	n.a.
Trap Adsorbent	Tenax TA (013741-005-00)	Trap Temp.	40 °C

**Table 2:** TD-GC-MS analytical conditions.

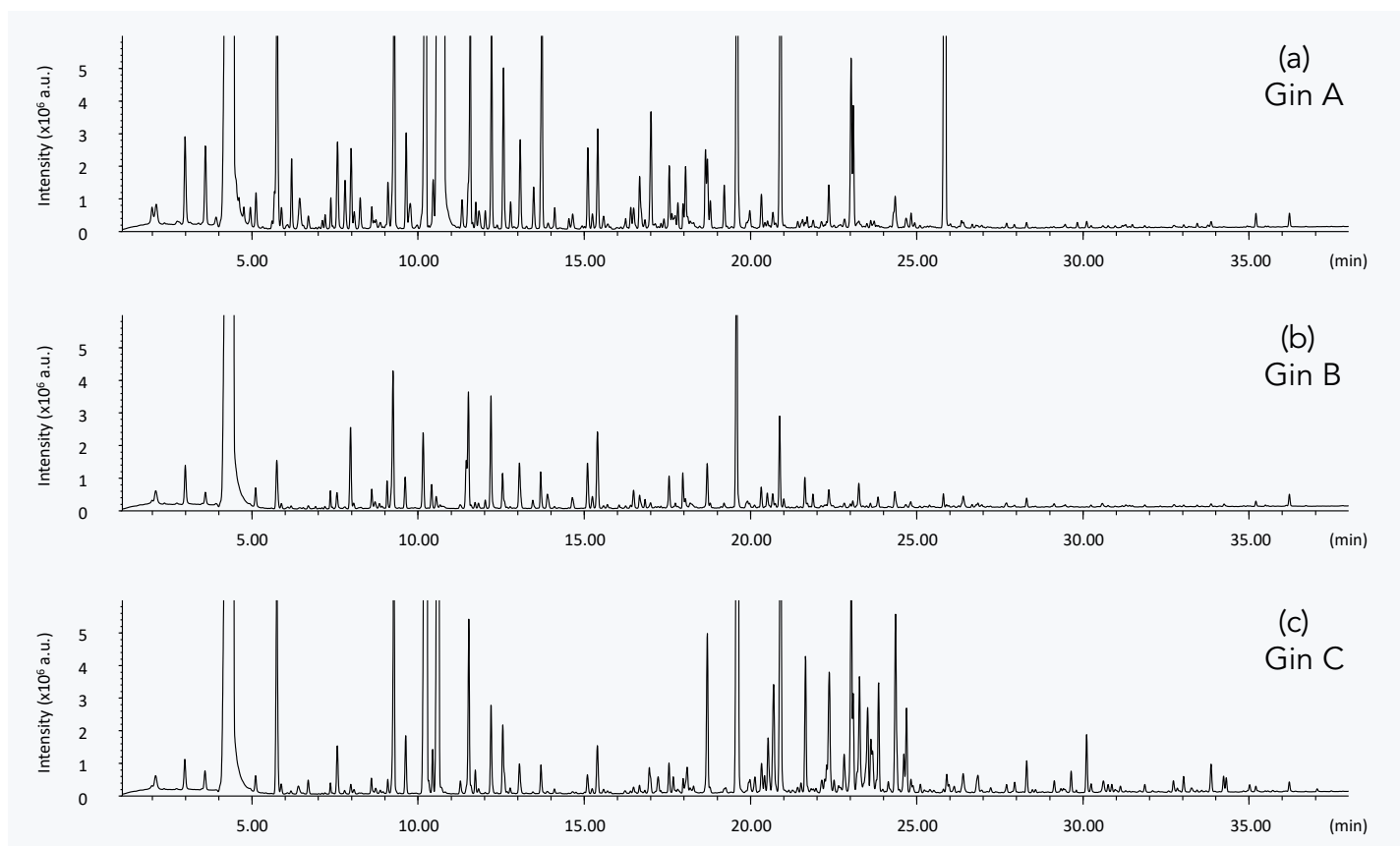
2-Step MVM-DHS@/Thermal Desorption: GERSTEL MPS2/TDU/CIS4			
Desorption Temp.	1. Method FEDHS: 30 °C (0.3 min) - 210 °C/min - 240 °C (3 min) 2. Method BX: 30 °C (0.3 min) - 270 °C/min - 300 °C (3 min)		
Desorption Flow	100 mL/min (@ 25 kPa)	Desorption Mode	Splitless
CIS 4 Temp.	1. Method FEDHS: 20 °C (hold) 2. Method BX: -40 °C (3.5 min) - 12 °C/min - 240 °C (hold)		
CIS Liner	Tenax TA packed liner	Injection Mode	Low Split 1:3

GC-MS: Agilent 7890GC-5975MSD	
Column	DB-WAX UI (20 m x 0.18 mm i.d., 0.30 µm thickness)
Column Temp.	40 °C (3 min) - 5 °C/min - 240 °C (10 min) + Back Flush 10 min
Acquisition mode	Scan
Scan range	m/z 28.7 ~ 400

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## Results &amp; Discussion

Figure 1 depicts the TIC's of the three gin samples after 2-Step MVM extraction.

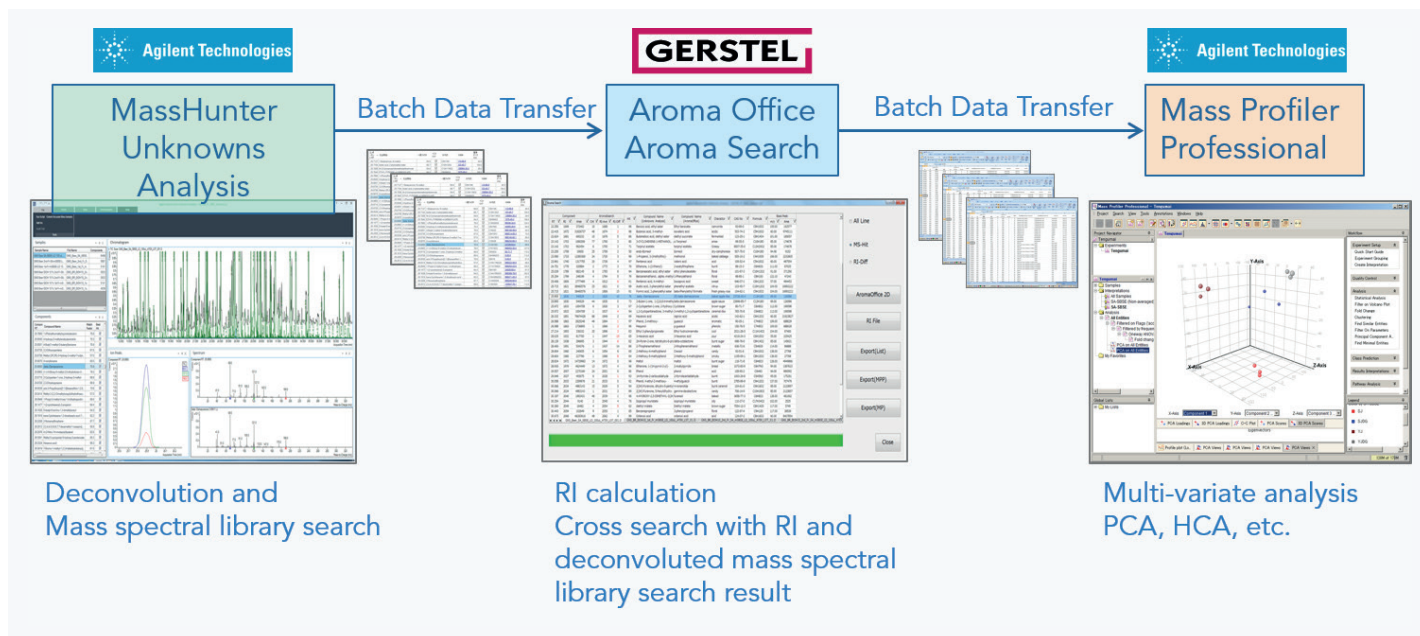


**Figure 1:** Comparison of total ion chromatograms of three types of gin samples. (a) sample gin A, (b) sample gin B, (c) sample gin C

Figure 2 gives a pictorial overview of the data analysis workflow that operates on a data file after GC-MS analysis. Aroma Office Ver. 7 offers a new aroma/flavor data analysis workflow ranging from an Agilent MassHunter Unknowns Analysis to a multi-variate analysis software such as Mass

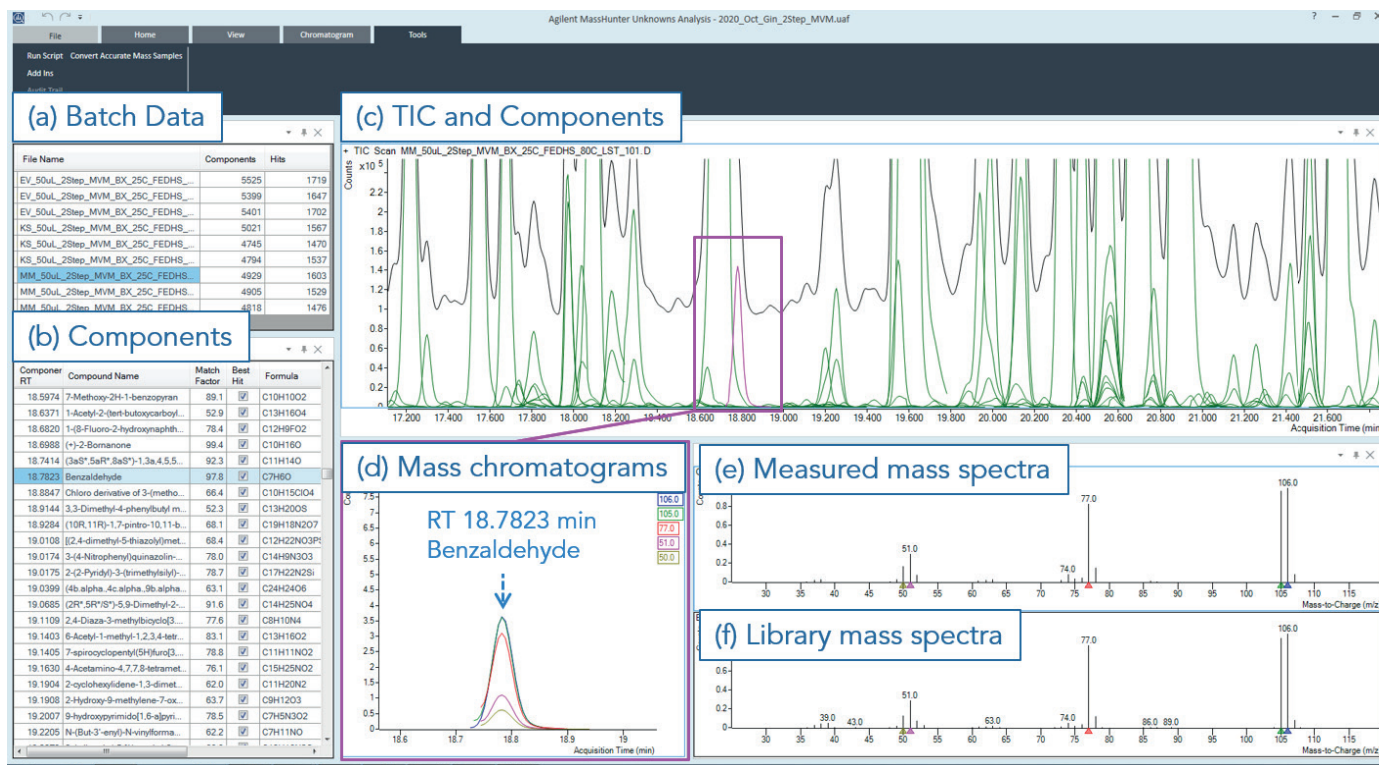
Profiler Professional (MPP) by seamless transfer of batch data files. The Aroma Search process automatically extracts aroma/flavor compounds from the deconvolution results, while excluding non-aroma matrix compounds.

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**Figure 2:** A new data analysis workflow for secure identification and multi-variate analysis of aroma/ flavor compounds.

In Figure 3 additional detail is displayed for one of the triplicate gin C samples

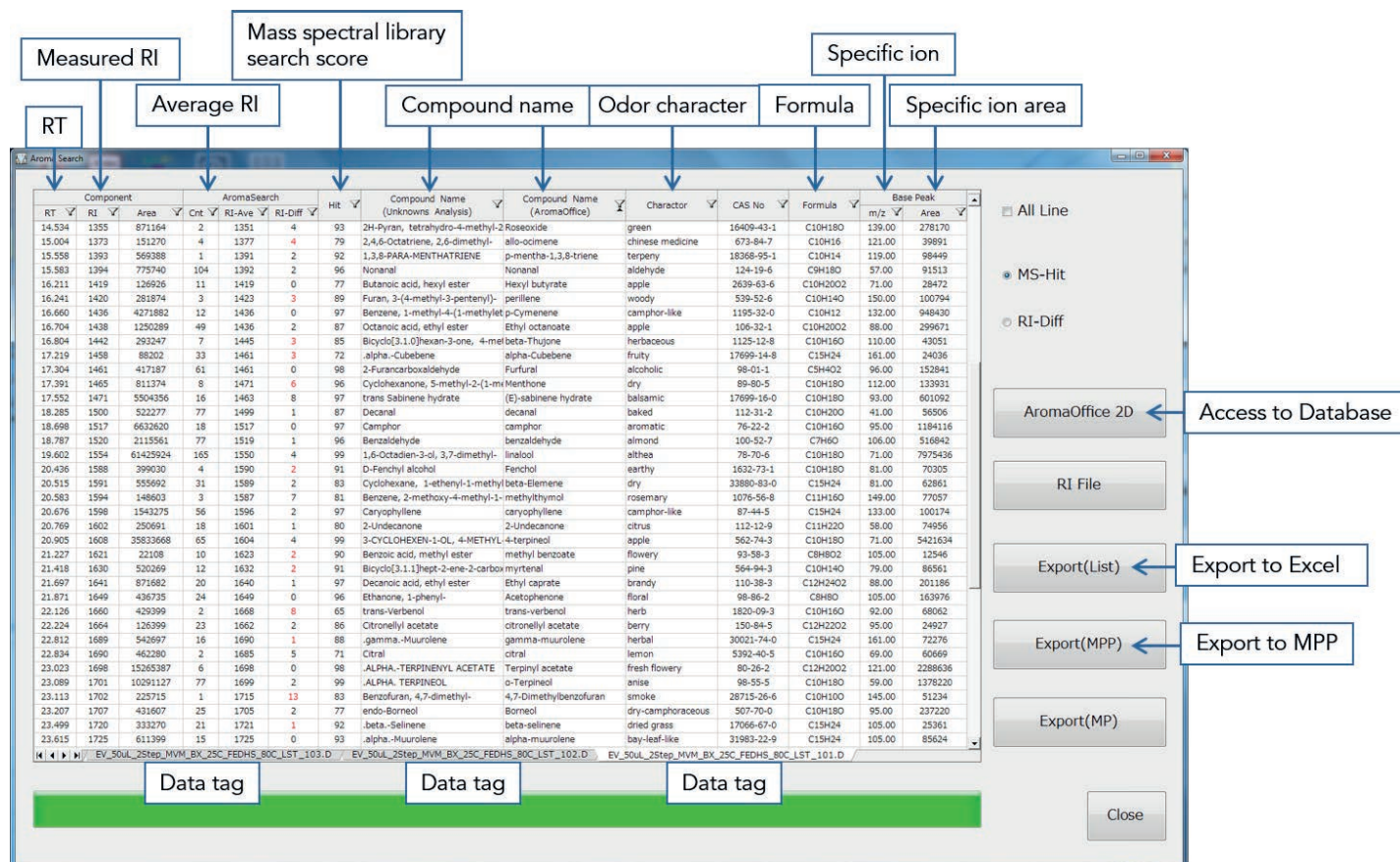


**Figure 3:** Deconvolution and mass spectral library search results with an Agilent MassHunter Unknowns Analysis. (a) Batch data (triplicate analyses for three gin samples), (b) components obtained from the deconvolution of the 1st analysis data for the sample gin C, (c) total ion chromatogram (TIC, black trace) and the components (green trace), (d) mass chromatograms at RT 18.7823 min (m/z 50, 51, 77, 105, and 106 for benzaldehyde), (e) measured mass spectra (benzaldehyde), (f) library mass spectra (W9N11.1).

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In addition to searching for characteristic aroma compounds it is important to obtain the "aroma profile" of a sample by detecting several hundred compounds using GC-MS. This can be achieved

when using the Aroma Search function in the data analysis workflow. This is illustrated in Figure 4.



**Figure 4:** Aroma Search results for triplicate analyses of the three gin samples using the deconvoluted mass spectra and RI.

The numbers of aroma/flavor compounds tentatively identified by the combination of Unknowns Analysis and Aroma Search were 116 for sample Gin A, 96 for sample Gin B, and 106 for sample Gin C, respectively. The compound lists for all batch data can be exported as an excel file using the "Export (List)" button. In addition to qualitative information about aroma compounds, Aroma Search also provides the specific ion (base peak) area of the components for all batch data, which can then be processed by multivariate analysis software such as Agilent MPP. The specific

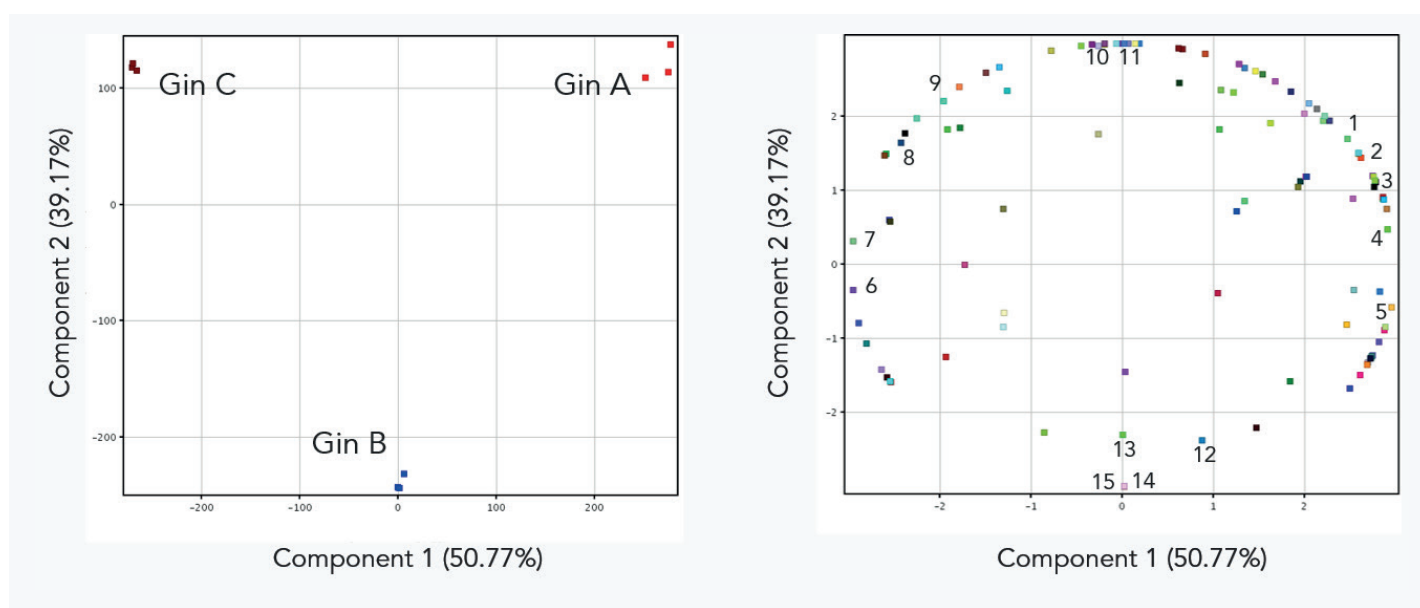
ion area values can be exported as an excel file using the "Export (MPP)" button. This excel file can be directly loaded as a generic file in the MPP software.

Principal component analysis (PCA) was applied to the three gin samples (in triplicate analysis) to obtain a simplified view of the classification and the key aroma/flavor compounds.

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Figure 5 shows the PCA score plot using PC1 and PC2 and the corresponding loading plot for the three gin samples. The two principal components (PC) account for 89.94% of the total variance of the data (PC1: 50.77%, and PC2: 39.17%, respectively). This score

plot clearly differentiates all gin samples. From the loading plot, several aroma/flavor compounds can be characterized according to the strength of the contribution to each PC.



**Figure 5:** PCA score plot using PC1 and PC2 and the corresponding loading plot for sample gin A, sample gin B, and sample gin C. 1. Benzaldehyde, 2. Ethyl hexanoate, 3. p-Anisaldehyde, 4. Diacetyl, 5. Roseoxide, 6. Geraniol, 7.  $\beta$ -Elemene, 8. Spatulenol, 9. Coumarin, 10.  $\beta$ -Ocimene, 11.  $\alpha$ -Terpineol, 12. Menthol, 13. Hexa-4-olide, 14.  $\beta$ -Selinene, 15. Salicylaldehyde.

Benzaldehyde (bitter almond), ethyl hexanoate (fruity), p-anisaldehyde (anis), diacetyl (creamy), and roseoxide (rose) all show higher contribution to the positive PC1 and would correlate with the complex aroma of the sample gin A.

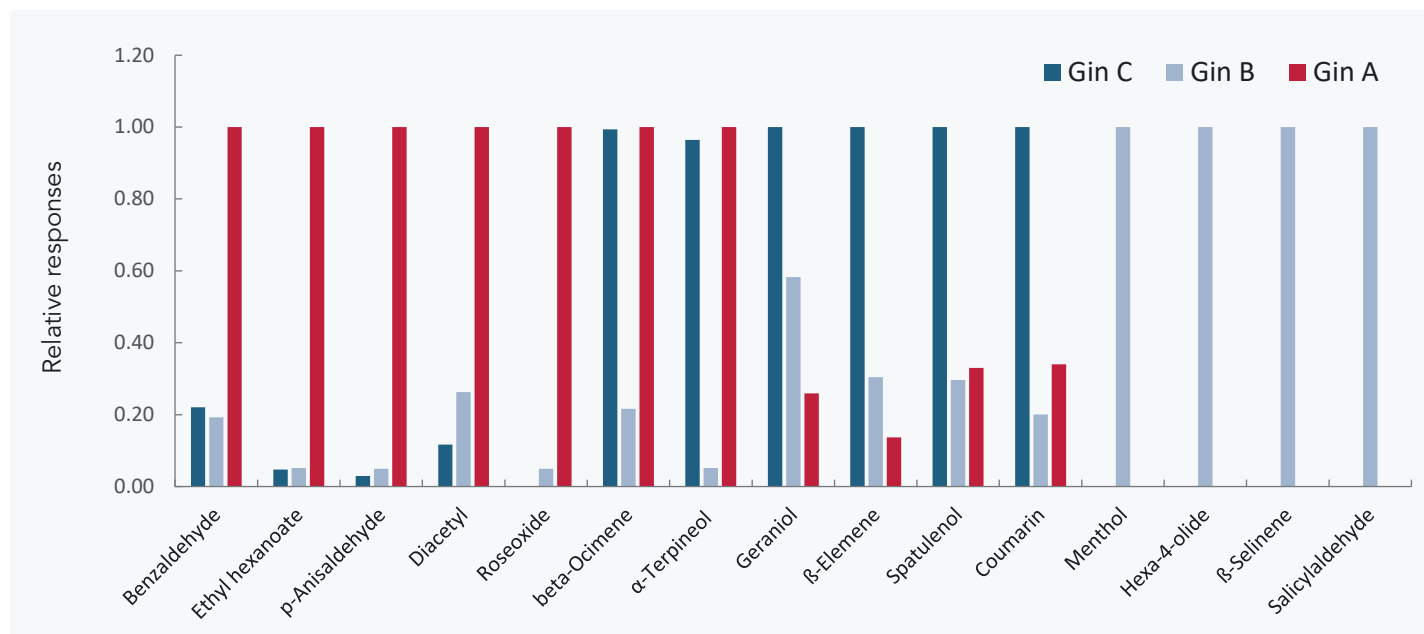
Geraniol (floral),  $\beta$ -elemene (herbal), spatulenol (woody), and coumarin (fresh floral) show higher contribution to the negative PC1 and would correlate with herbal notes of the sample gin C.

$\beta$ -Ocimene (floral) and  $\alpha$ -terpineol (floral) show higher contribution to the positive PC2 and would correlate with the floral notes of both sample gin A and gin C.

Menthol (mint), hexa-4-olide (coconut),  $\beta$ -selinene (herbal), and salicylaldehyde (pungent) show higher contribution to the negative PC2 and would correlate with the sample gin B's complex personality.

The MPP file of Aroma Search results lists the area values of the reference (base) peaks (in this case, in triplicate), making it easy to compare detection responses among samples. Fig.6 shows the average relative responses for the selected compounds (No. 1-15 in the Fig. 5) which contribute to classification of the three gin samples. The average relative responses were normalized with the highest one.

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**Figure 6:** Comparison of the average relative responses for the selected compounds

## Conclusions

The latest Version 7 of Aroma Office is shown to be a particularly powerful tool for GC-MS investigation and characterization of aroma compounds. Version 7 retains the core technology of matching CAS and Retention Index parameters for compound identification but also allows incorporation of an initial deconvolution step with mass spectral library searching as an aide to prior simplification of complex data files. After deconvolution and Aroma Office/Aroma Search processing and cross searching, data can be further transferred for multivariate analysis.

Applying this data analysis workflow to three different Gin samples succeeded in a highly efficient differentiation. Based on results obtained here this procedure could be applied to other different beverages (whiskey etc.) and has the potential to be developed as a more cost-effective candidate for a product authentication approach than use of high-resolution MS [4,5]. With high-resolution MS a skilled specialist is likely to be required and this in turn detracts from its usefulness for routine analysis of complex food related samples.

## References

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