LabWorks APPNOTE

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Identifying Key Odor Compounds in Bourbon using Solvent-free Aroma Dilution Analysis and a Novel Software for Interpreting GC-O Data

Laurel Vernarelli, John Stuff, Jaqueline Whitecavage, and Fred Foster

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA

Keywords

LabWorks Platform, Stir Bar Sorptive Extraction, Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS/O), Aroma Dilution Analysis, Aroma Extract Dilution Analysis, Olfactometry

Abstract

Stir Bar Sorptive Extraction (SBSE) coupled with gas chromatography-mass spectrometry and olfactory detection (GC-MS/O) allows for separation and identification of aroma compounds in complex sample matrices with minimal sample preparation time. Aroma Dilution Analysis (ADA) is a solvent-free approach of Aroma Extract Dilution Analysis (AEDA) which employs a GC inlet system to split the carrier gas flow and thereby the injected sample to a desired ratio. The approach of ADA has been applied to direct immersion SBSE of bourbon samples for determination of flavor dilution (FD) factors and identification of key aroma-active compounds. The GERSTEL LabWorks Platform with a Thermal Desorption Unit (TDU 2) and Cooled Injection System (CIS 4) allowed two independent split ratios to be set. The product of the split ratios determines the overall dilution factor. The developed method allowed the determination of FD factors in a range from 1-201. Data generated by ADA was evaluated using a novel software that allows for the handling of GC-O intensity data along with the GC/MS data for identification of key odorants in bourbon.

Introduction

Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS/O) is a robust technique that allows for the screening of aroma-active components of a complex sample matrix and provides instrumental and sensory analysis simultaneously. It is commonly applied to food, beverage and consumer products to focus on the identification of key aroma impact odorants. Many analytical techniques have been developed for the determination of relative odor potency of aroma-active compounds in a product. One of these methods is Aroma Extract Dilution Analysis (AEDA) which involves stepwise dilutions of an extract, with the diluted extracts being evaluated by GC-O to provide the flavor dilution (FD) factor. FD factors are defined as the maximum dilution of an extract at which the compound can be detected [1]. AEDA is commonly applied to liquid-liquid extraction (LLE), solvent-assisted flavor evaporation (SAFE) and simultaneous distillation/extraction (SDE). The solvent-free approach of Aroma Dilution Analysis (ADA) involves the serial dilution of the sample by adjusting the GC inlet split ratio following the thermal desorption process. The dilution factor of the sample corresponds to the overall split ratio of the system, determined as the product of the two independent split ratios of the Thermal Desorption Unit (TDU 2) and Cooled Injection System (CIS 4). It has been established elsewhere that a good linear relationship between the resulting peak area (concentration) of the extracted compound and the dilution factor is required to ensure reliability of the GC-O dilution analysis [1, 2].

For stir bar sorptive extraction (SBSE), a polydimethylsiloxane (PDMS)-coated stir bar is applied to a sample by direct immersion (DI) or headspace (HS) to extract the analytes from the sample. SBSE is highly effective for the extraction of semi-volatile compounds and has a considerably larger sorbent volume in comparison to other common microextraction techniques, such as solid phase microextraction (SPME). However, due to the nature of microextraction techniques, SBSE is typically non-exhaustive and thus dependent on the partitioning coefficients of the analytes between the phases. The traditional AEDA approach cannot reliably be applied to SBSE, as the dilution of the sample would alter the sample matrix and corresponding partitioning coefficients. The technique of ADA has previously been applied to various micro-

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extraction techniques, including SBSE [2]. The aim of this study is to demonstrate the use of aroma dilution analysis of direct immersion SBSE to identify key odor compounds of a bourbon sample.

Experimental

Instrumentation

GERSTEL LabWorks Platform with Olfactory Detection Port ODP 4 on Agilent® 7890 GC/5977 MSD.

Analysis Conditions LabWorks Platform

TDU 2	
Pneumatics mode	splitless
Temperature	40 °C (0 min), 720 °C/min to 280 °C (3 min)
CIS 4	
Liner	glas beads
Pneumatics mode	solvent venting, splitless
Vent flow	50 mL/min until 0,01 min
Split flow	20 mL/min @ 1.2 min
Split	10:1, 25:1, 50:1, 100:1 or 200:1
Temperature	-120 °C (0 min), 12 °C/sec to 280 °C (3 min)
ODP 4	
Transferline	280 °C
Mixing chamber	150 °C
Split ODP:MSD	2:1

Analysis Conditions GC

GC Agilent 7890	
Column	30 m Rxi-5ms (Restek), d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He, constant flow, 1.0 mL/min
Temperature	40 °C (1 min), 10 °C/min to 280 °C (3 min)

Analysis Conditions MS

MSD Agilent 5977A	
Scan	30 to 350 amu

Sample

Bourbon was purchased at a local store. A 1 mL aliquot of bourbon was diluted with 9 mL of bottled water in a 10 mL screw-capped vial. A conditioned Twister stir bar was placed into the 10 mL vial. The vials were screw capped, and the samples stirred at 1000 RPM at room temperature for 90 minutes. Twister stir bars were rinsed with water, blotted dry and placed into conditioned TDU tubes for analysis.

Sample Introduction

Samples were desorbed in splitless mode under a 50 mL/min helium flow at 280 °C for 3 minutes. Analytes were cold trapped in the CIS 4 inlet at -120 °C on a glass bead liner. When desorption was complete, analytes were transferred to the column in splitless or split (10:1, 25:1, 50:1, 100:1 or 200:1) mode by heating the inlet rapidly to 280 °C.

Olfactometry

GC-O analysis was performed on a GC/MS equipped with an Olfactory Detection Port (ODP 4). The column effluent was split 2:1 between the ODP 4 and mass spectrometer respectively. The ODP transfer line was heated to 280 °C. The mixing chamber was heated at 150 °C and purged with humidified nitrogen to prevent olfactory fatigue by dehydration of the nasal mucous membranes.

Results and Discussion

The FD factor was determined for each aroma-active compound by identifying the highest split ratio at which each aroma-active compound could be detected at the ODP. Olfactory analysis was carried out by one trained analyst and repeated in triplicate for each split ratio. The FD factor was defined as the split ratio at which a compound was detected 2 out of 3 times by the analyst.

ADA SBSE was performed by varying the split ratios (SR) of the carrier gas flow at the CIS via the GC pneumatics, while the TDU was operated in splitless mode. The CIS was operated in solvent vent mode, and the purge flow to split vent was started at 0.01 minutes to split the flow at the inlet or 1.2 minutes to operate the CIS in splitless mode. An FD factor of 1 was assigned to the compounds detected 2 out of 3 times in splitless mode. The split ratios within the CIS are given by the following equation:

$$SR_{CIS} = \frac{\text{purge flow to split vent + column flow}}{\text{column flow}}$$

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It has been shown elsewhere that varying SRCIS resulted in high r^2 values and slopes closest to the ideal value of -1 for ADA SBSE, thus varying the split ratio at the CIS inlet was the focus of this study [2].

Figure 1 shows the linearity of the regression plots for ethyl hexanoate, ethyl octanoate, phenylethyl alcohol and cis-oak lactone with an FD factor of 201, and isoamyl alcohol with an FD factor of 101.

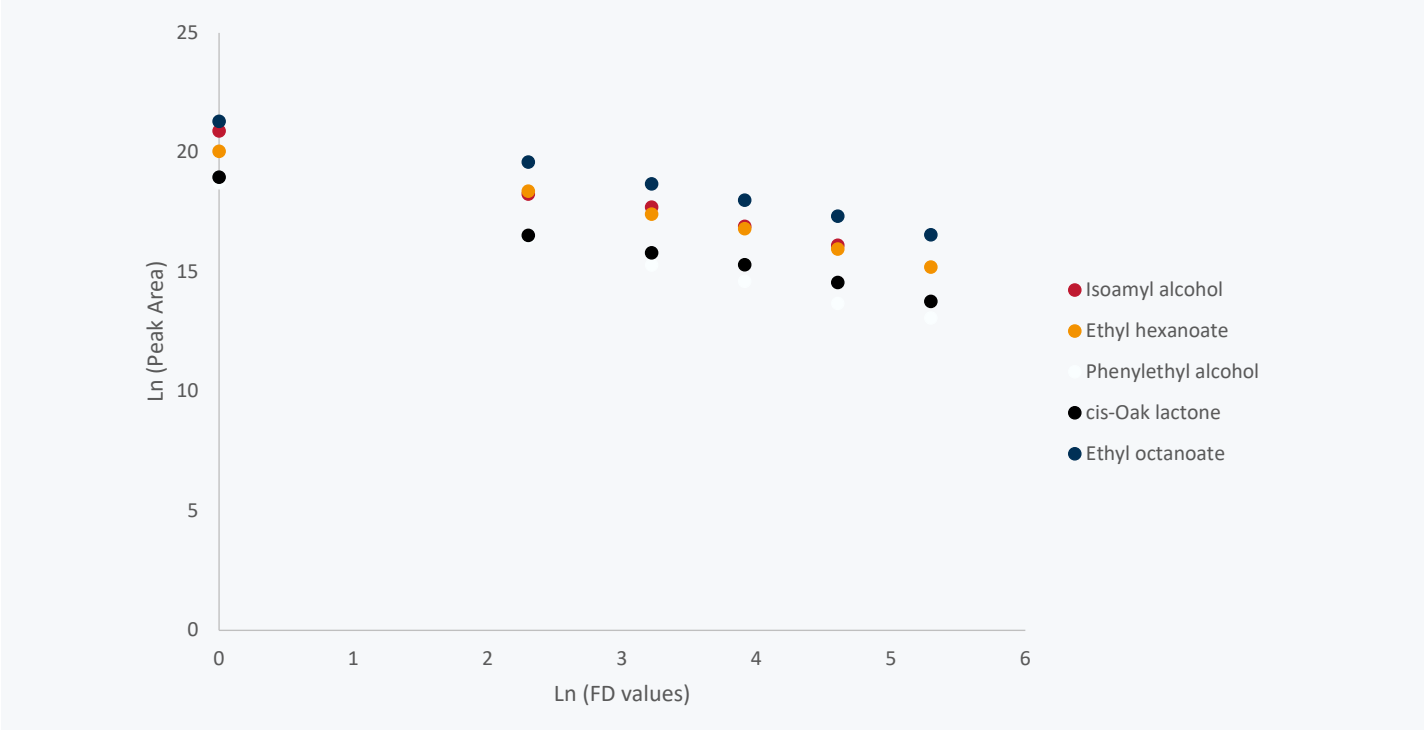

Figure 1: Regression plots of Ln (peak area) versus Ln (FD values) for selected aroma-active compounds in the SBSE-GC-MS/O analysis of bourbon.

The m/z quantification ion, regression equations and r^2 values corresponding to the plot in figure 1 for isoamyl alcohol, ethyl hexanoate, phenylethyl alcohol, ethyl octanoate and cis-oak lactone are shown in table 1.

Table 1: Regression equations and r^2 values of plots in figure 1.

Compound	m/z	m	t	r^2
Isoamyl alcohol	55	-1.0176	20.834	0.9936
Ethyl hexanoate	88	-0.9129	20.242	0.9883
Phenylethyl alcohol	91	-1.0853	18.800	0.9980
Ethyl octanoate	88	-0.8938	21.453	0.9928
cis-Oak lactone	99	-0.9562	18.899	0.9963

Aroma-active compounds were identified by the NIST17 standards reference database within the ODI software. Descriptors were assigned to aroma-active compounds by comparing the retention time in the mass spectrum and olfactogram. The FD factors, descriptors and log $K_{(o/w)}$ values for each of the identified aroma-active compounds are shown in table 2.

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Table 2: FD factors, aroma descriptors and log $K_{(o/w)}$ values for aroma-active compounds in bourbon by DI-SBSE-GC/MS-O.

Compound	Analyst Descriptor	Descriptor - Literature[1]	Log $K_{(o/w)}$ value[2]	Compound	FD Factor
1	alcoholic	fermented, fusel, alcoholic	1.16	Isoamyl alcohol	101
2	fruity	fruity	1.65	Ethyl isobutyrate	51
3	fruity	fruity	2.16	Ethyl 2-methylbutyrate	101
4	fresh	aldehydic, fresh, floral	1.89	1,1-Diethoxy-2-methylpropane	26
5	fruity	fruity	2.16	Ethyl isovalerate	201
6	banana	banana	2.26	Isoamyl acetate	101
7	fruity	fruity, fatty	2.39	Isovaleraldehyde diethyl acetate	51
8	fruity	-	2.18	Acetaldehyde ethyl isoamyl acetal	101
9	fruity	fruity, pineapple	2.40	Ethyl hexanoate	201
10	fruity	fruity, rose, orange	3.43	Octyl formate	1
11	fruity, wine	fermented, cognac, pear	3.06	Hexanal diethyl acetal	26
12	musty	-	2.80	Ethyl (E)-2-heptenoate	11
13	green	green	3.33	Ethyl heptanoate	101
14	fruity, fresh	green, fresh, rummy, fruit	3.33	Heptyl acetate	1
15	floral	floral, fresh, clean	3.77	1-Nonanol	51
16	fruity	fruity, apple, cooked	1.26	Diethyl succinate	11
17	floral	floral, rose	1.36	Phenylethyl alcohol	201
18	waxy	waxy, sweet, fruity, winey	3.84	Ethyl octanoate	201
19	sweet	fatty, waxy, floral, sweet	4.57	1-Decanol	26
20	fruity	-	3.50	Ethyl 3-nonenoate	26
21	green	green, fruity, waxy, cognac	4.63	Ethyl trans-4-decenoate	11
22	waxy	waxy, rose, fruity, rummy	4.35	Ethyl nonanoate	26
23	coconut	spicy, sweet, coconut	2.63	cis-Oak lactone	201
24	spice, clove	spicy	2.20	3-Allyl-6-methoxyphenol	26
25	fatty	fatty	4.09	n-Decanoic acid	51
26	fruity	waxy, sweet, fruity	4.86	Ethyl decanoate	51
27	sweet	fruity, sweet, oily, soapy	5.22	Isoamyl octanoate	1
28	floral	floral, fresh, green	4.13	Geranyl acetone	1
29	waxy	waxy, clean, grassy	6.95	1-Hexadecanol	11
30	fatty	fatty, coconut, bay oil	4.60	Dodecanoic acid	51
31	floral	floral, green, wazy	4.68	Nerolidol	1
32	fruit	fruity, melon, quince, winey	3.92	Diethyl decanedioate	11
33	waxy	waxy, sweet, violet	6.89	Ethyl myristate	11

1. Literature descriptors were obtained from The Good Scents Company (http://www.thegoodscentscompany.com/index.html)
2. Log$K_{(o/w)}$ values were obtained from National Institute of Health PubChem (https://pubchem.ncbi.nlm.nih.gov/)

The compounds found to have the highest aroma impact with an FD factor of 201 were ethyl isovalerate, ethyl hexanoate, phenylethyl alcohol, ethyl octanoate, and cis-oak lactone.

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The Olfactory Data Interpreter (ODI) software was used to visualize and evaluate GC-O data. The ODI software overlays the chromatogram and GC-O data obtained by the GERSTEL ODP recorder. An enhanced view of an overlay of a representative olfactogram and chromatogram within the ODI software is shown in figure 2.

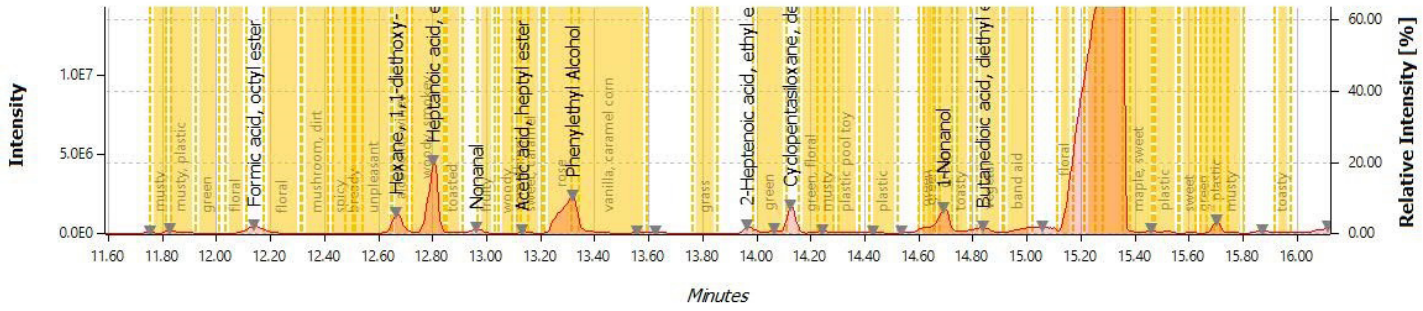

Figure 2: GC/MS-O data set visualized with ODI for FD factor of 1.

In figure 2, the olfactogram is represented by the yellow bars with the aroma descriptors in gray text. The descriptors are text files generated from an audio recording of the spoken descriptors during analysis. The chromatogram is represented by the red trace, and compounds identified by the integrated NIST standard reference database are denoted in black text. The user has the ability to zoom in on the chromatogram/olfactogram overlay to assist in peak identification and assignment of descriptors to compounds. A cumulative olfactogram can be constructed within the ODI software in which odor intensities from multiple GC-O runs are summed up. The cumulative olfactogram view can be utilized to gather Nasal Impact Frequency (NIF) data, another technique used to identify key odor impact compounds. The cumulative olfactogram view within the ODI software is shown in figure 3.

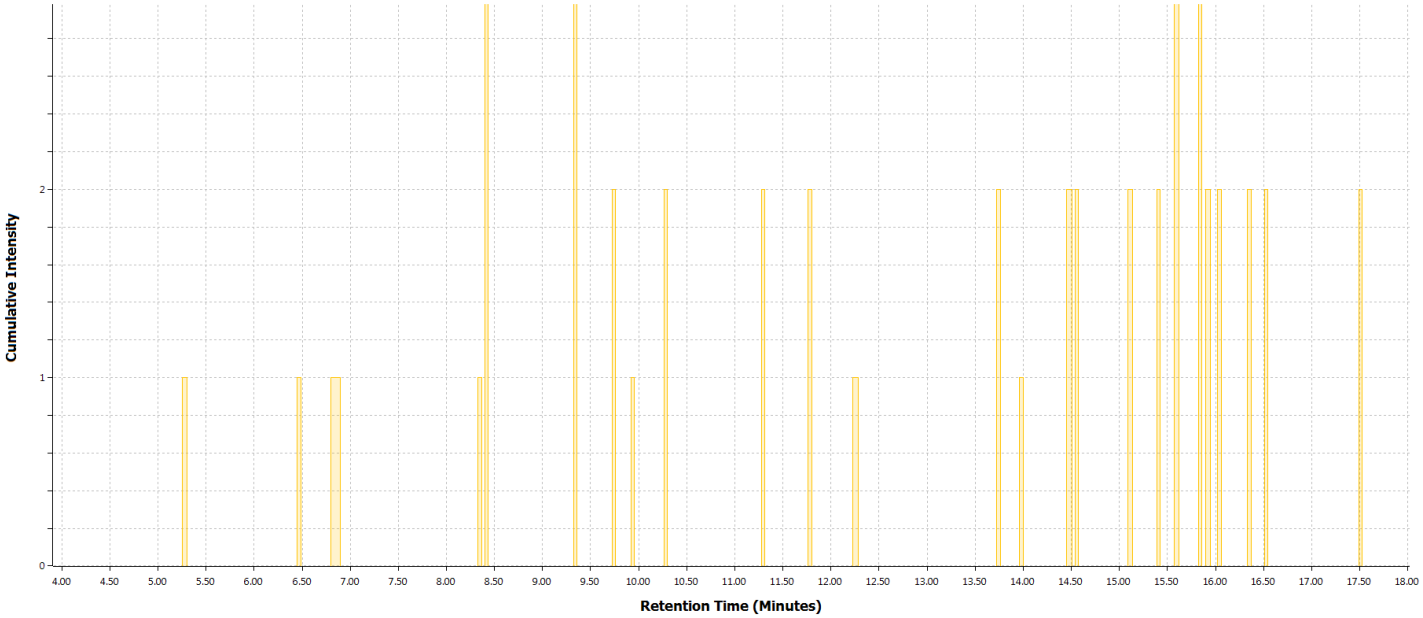

Figure 3: Cumulative olfactogram view within the ODI software.

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AEDA-like calculations can be performed directly in the software if the user applies retention time calibration, time recognition windows and performs dilutions in a log2 series. A representative ODI software layout to perform AEDA-like calculations is shown in figure 4.

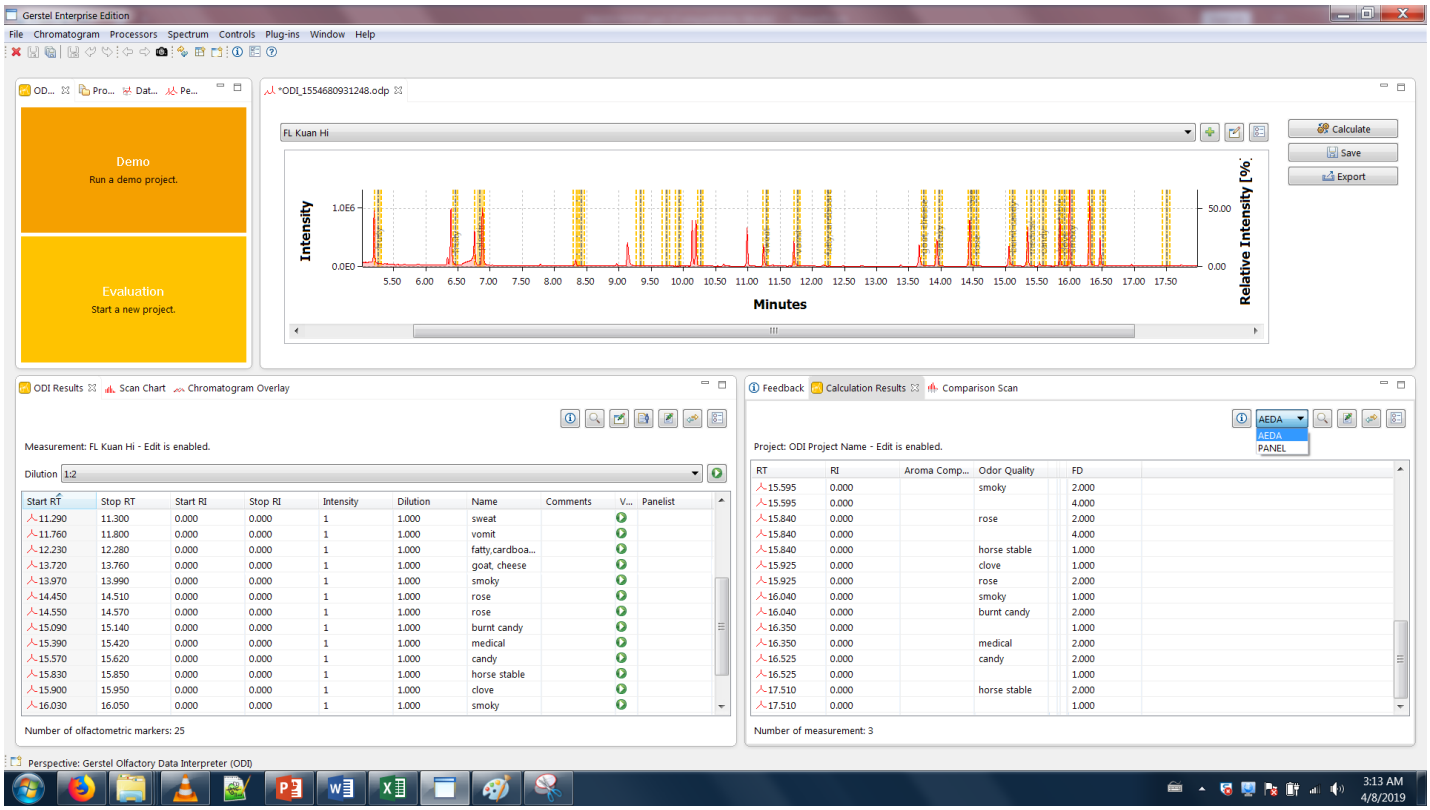

Figure 4: ODI software view of chromatogram/olfactogram overlay, odor descriptor table and AEDA report from demo data set.

ADA results are available in a dedicated report with a maximum FD factor for each of the GC-O signals.

Conclusions

ADA SBSE was applied to a Kentucky bourbon to identify the key aroma impact compounds of ethyl isovalerate, ethyl hexanoate, phenylethyl alcohol, ethyl octanoate, and cis-oak lactone with an FD factor of 201. The novel data analysis ODI software simplified the processing of combined GC-MS/O data. AEDA-like reports can be generated directly within the ODI software without further external data processing.

References

[1] Feng et al., Food Chem 187 (**2015**): 44-52

[2] Fraatz et al., Eur Food Res Technol 244 (**2018**): 949-957