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Laboratory Experiment

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Analysis of Whiskey by Dispersive Liquid−Liquid Microextraction Coupled with Gas Chromatography/Mass Spectrometry: An Upper Division Analytical Chemistry Experiment Guided by Green Chemistry

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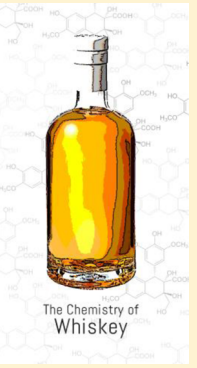
Supporting Information

ABSTRACT: Analysis of whiskey samples prepared by a green microextraction technique, dispersive liquid−liquid microextraction (DLLME), before analysis by a qualitative gas chromatography−mass spectrometry (GC/MS) method, is described as a laboratory experiment for an upper division instrumental methods of analysis laboratory course. Here, aroma compounds in whiskey samples (n = 11) were extracted using ultrasound-assisted DLLME with chloroform (as extraction solvent). The chloroform extract was analyzed by GC/MS with data manipulation by AMDIS (automated mass spectral deconvolution and identification system) to allow for comparisons between whiskey samples. Aroma compounds commonly reported in the literature (furfural, isoamyl acetate, 5-methyl furfural, ethyl esters, phenylethyl alcohol, whiskeylactone, and vanillin) were tentatively identified based upon the match to the MS library. This unique laboratory allows students to engage in a real-world analysis of a high-value product and to explore the use of AMDIS to tentatively identify compounds and compare chromatographic profiles of various whiskey samples for identification of common and unique constituents. Students also use the literature to provide sensory information for these identified semivolatile compounds.



KEYWORDS: *Upper-Division Undergraduate, Laboratory Instruction, Analytical Chemistry, Green Chemistry, Natural Products, Gas Chromatography, Mass Spectrometry*

INTRODUCTION

The spirits/distillation industry accounts for a $40 billion share in the United States with craft-distilling making up a very small (∼1%) market share,[1] with 54 craft distilleries in Colorado,[2] the third-highest in the nation.[1] While the production processes of whiskey are tightly regulated,[3] the distillation and cask or barrel aging impart many of the important compounds responsible for the complex flavor that make each mature whiskey unique in taste and aroma.[4] Methods for the determination of organoleptic or aromatic compounds in foods or beverages, including honeys,[5] wines,[6] and whiskey[3,7] (or wood products[8] used in the production of these commodities) have been well described. Preparation of these sample types for analysis has included liquid−liquid extraction[7,8] with concentration[7] or solid-phase microextraction (SPME)[5,6] prior to gas chromatographic analyses (with flame ionization or mass spectrometric (MS) detection)[6,7] or liquid chromatography−tandem MS analysis.[3] Aroma compounds in bourbon whiskey were quantified in a recent study with only slight concentration differences observed for the majority of concentrations of odorants in the whiskies included in that study.[7] In particular, the ethyl esters identified in the study were of particular importance to the overall whiskey aroma.[7]

Laboratory experiments involving food or beverage analyses, including whiskey profile analysis,[9] have been described as ways to better engage undergraduate students within the laboratory by employing these "real-life" scenarios.[9−12] As well, students in this present laboratory exercise completed the whiskey profile analysis by preparing their samples using a microextraction technique, dispersive liquid−liquid microextraction (DLLME), which uses small amounts of solvent.[13] DLLME relies upon a ternary phase system of the aqueous sample matrix and addition of a dispersive solvent (which is water-miscible) and extraction solvent (water-immiscible) to improve extraction efficiency of analytes into a microvolume extraction solvent layer (Figure 1). The development of greener analytical laboratory experiments is an important area of study[14] given that many analytical procedures, including those used in the teaching laboratory, rely on large amounts of solvents or derivatizing reagents.[15] In this *Journal*, there are just a few experiments that focus on solvent microextraction techniques, though development of such procedures to enhance student understanding of green chemistry and sustainability is of interest.[16] While the DLLME procedure is well-described and well-known in the literature, this experiment is novel for its use in this undergraduate laboratory experiment for the analysis of whiskey.

In this experiment, upper-division analytical chemistry students (typically 12 students are enrolled in the course in spring semesters) utilized DLLME for the preparation of a suite

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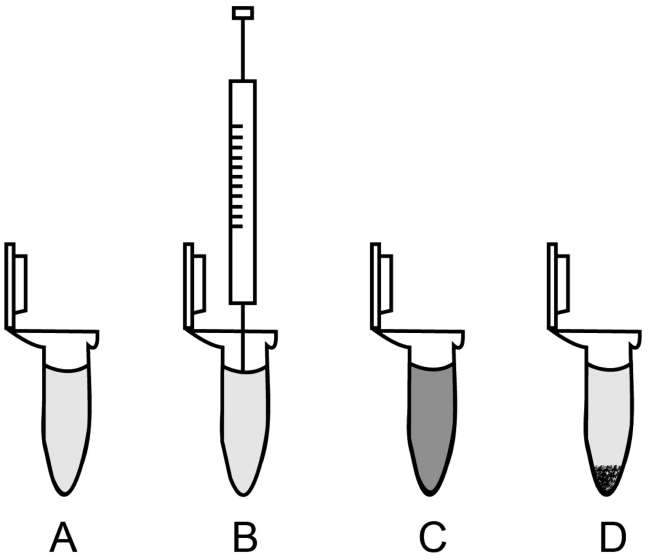

Figure 1. Schematic representing the process of DLLME for preconcentrating analytes in a GC-compatible solvent. Here, the (A) 500 μL of the whiskey sample with 500 μL of 18 MΩ DI water is treated with (B) chloroform as the extraction solvent. After (C) sonication, a cloudy solution is obtained. The extraction layer (bottom layer) is removed after (D) centrifugation for GC/MS analysis.

of whiskey samples made at a local Colorado distillery as well as whiskey samples from other national and international distillers. Upon DLLME preparation, the students analyzed their whiskey samples by gas chromatography−mass spectrometry (GC/MS). Compounds were tentatively identified based upon their match to the NIST05 library after data file manipulation through AMDIS (automated mass spectral deconvolution and identification system). Significant aroma compounds were identified by students with reporting of the chemical name, odor type, and description as well as taste description through CAS number search.

This experiment was designed to cover three laboratory sessions of 2.75 h, but it can be shortened or lengthened to accommodate other schedules. In the first laboratory meeting for this analysis, students (working in pairs) performed the DLLME preparation of the whiskey samples and analyzed the samples by GC/MS. After the sample runs had completed, students were expected to determine if their initial analyses were adequate in terms of separation and number of discrete compounds that could be potentially attributed to the whiskey sample and not background solvent. Subsequent days (two and three, if needed) in the laboratory experience were spent using AMDIS program to report putative identification of compounds with chemical name, probability of correct match (quality factor), and CAS number. AMDIS was also used to compare chromatograms of the analyzed whiskey samples to determine those compounds that were common between them or to identify unique semivolatiles. Students were then expected to analyze each compound for taste and odor components by using literature as well as chemical scent and flavor Web sites.[17]

MATERIALS AND METHODS

Chemicals and Reagents

Chloroform (ACS reagent grade) and methylene chloride (GC/MS grade) were from Fisher Scientific (Fairlawn, NJ) as were all other chemicals unless otherwise specified. Only 18 MΩ DI water produced using a Barnstead E-pure filtration system (Thermo Scientific, Asheville, NC) was utilized in experiments.

Whiskey Sample Description

Whiskey samples (20 mL of each) were obtained from a local distillery (Distillery 291) in Colorado Springs, Colorado. Nine of the 11 samples were from the distillery and included finished, aged whiskeys as well as immature whiskeys (i.e., those that were sampled during their barrel aging process). The remaining two samples were whiskeys from a national and international distiller.

Sample Preparation

For each whiskey, 500 μL was pipetted into a 1.5 mL solvent-compatible microcentrifuge tube. After addition of the whiskey, 500 μL of 18 MΩ DI water and 200 μL of chloroform (as the extraction solvent) were added by gastight glass syringes (Figure 1). Whiskey already contains significant ethanol content, and ethanol is an excellent dispersive solvent, so the addition of a solvent with these characteristics was not required. After addition of the water (which aids in separation of the aqueous and organic layers) and chloroform to the whiskey samples, the microcentrifuge tubes were gently shaken before sonication (Fisher Scientific FS20D sonicator) for 5 min. After a cloudy dispersed liquid was achieved in each microcentrifuge tube, the samples were centrifuged at 10,500 × g (Abbott Laboratories TDX centrifuge) for 3 min. The aqueous and extraction solvent layers were well separated upon centrifugation. A 50 μL aliquot of the chloroform layer was transferred to a 100 μL PolySpring insert housed in an amber glass autosampler vial. The samples were then analyzed by GC/MS.

Instrumental Analysis

Analysis was completed using a Hewlett-Packard 6890N series gas chromatograph with a 5973A Mass Selective Detector fitted with a DB-5MS column (60 m × 0.25 mm id × 0.25 μm film; Agilent Technologies, Santa Clara, CA). Ultrapure helium was used as the carrier gas (1 mL/min). Sample injection volume was 1 μL in splitless mode at 250 °C, and analytes within the chloroform extract were separated using the temperature program as follows: initial column temperature of 60 °C (hold for 8 min) with a ramp of 10.0 °C/min to a final temperature of 300 °C (hold for 3 min) for a total run time of 35.00 min per sample. The mass spectrometer was operated in full scan mode (m/z 35−350 with 4.45 scans/sec) for positive ion detection with a 5.00 min solvent delay. The transfer line was held at 280 °C, electron ionization source at 230 °C, and quadrupole at 150 °C. Methylene chloride was used for autosampler needle wash vials.

Spectral Deconvolution and Peak Identification

Data files were manipulated using AMDIS,[18] which is freely available and capable of manipulating raw data files collected by different instruments (not just Agilent, as was described here). AMDIS deconvolutes the raw data file to find separate components using default or user-defined parameters. The program works by extracting pure component spectra to determine if the component can be identified as one of the compounds found in the mass spectral library.[19] The default deconvolution settings (found in *Analyze* tab > *Settings* > *Deconv.*) are optimal for most users. For the deconvolution, the default number of scans across a peak (at half-height) is 12.[19] For the chromatographic data shown here, our scan speed (at 4.45 scans/s for m/z 35−350) allowed for an average of 13.6

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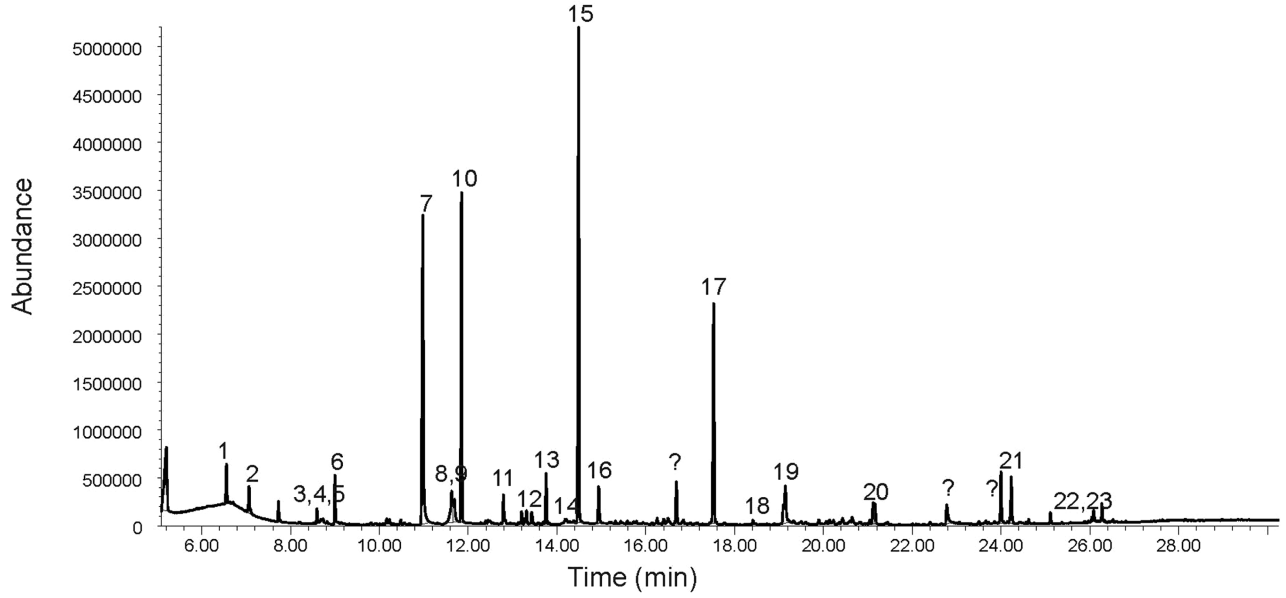

Figure 2. Total ion current chromatogram of whiskey sample A analyzed by GC/MS after DLLME sample preparation. Twenty-three aroma compounds were tentatively identified in this sample after spectral deconvolution through AMDIS. For peaks with a "?" label, multiple compounds with high probability match factors (>80) were reported, so no tentative identification of the compound is offered.

scans to be collected across a chromatographic peak. AMDIS was able to identify (and with high probability match, exceeding 90%) even compounds that were poorly resolved (see Figure 2 and Table 1 for compound 20 at 21.12 and 21.16 min). The software was utilized in two ways: first, data files were opened and processed for a *simple* analysis (meaning that individual components were identified). Second, data files were compared for the identification of matching compounds or unique compounds. Instructions for these analyses are provided in the Supporting Information.

The AMDIS program interfaces with the NIST05 mass spectral library. Thus, after the labeling of a matching or unique peak with the program upon comparing two data files, the raw mass spectrum for the peak of interest could be compared to the entries included in the NIST05 mass spectral library for tentative compound identification (please see Supporting Information for a full set of instructions). After identification of peaks through library search with the NIST05 mass spectral library, the CAS registry numbers were recorded for the compounds with the top quality match factor. Students were able to review sensory information for these compounds using Internet databases.[17]

Equivalently, there were options within the ChemStation software that allowed for identification of peaks. In the Enhanced Data Analysis software, individual peaks could be identified through the *Annotate chromatogram with PBM results* option (under the Chromatogram tab). Library search results were then exported to Excel. This exported spreadsheet contained the following information:

(1) Compound number
(2) Retention time
(3) Scan number
(4) Area
(5) Height (baseline and absolute)
(6) Peak width at half-maximum (min)
(7) The top 20 library hits.

For each hit, the compound name was listed along with the quality match factor, molecular weight, CAS number, and the search library used. Students were asked to report compounds with match factors exceeding 80.

In either case, it is critical that students realize that the compound identification results produced via AMDIS and ChemStation programs may differ. Students should critically evaluate their produced data, especially for compounds that are coeluting or are poorly resolved. Alternative experiments (that future instructors may include) are included in the Supporting Information.

HAZARDS

Safety glasses, laboratory coats, and nitrile gloves were required personal protective equipment of all students. Solvents (chloroform, methylene chloride) are toxic and flammable. Students were instructed to not consume or taste any of the whiskey samples.

RESULTS AND DISCUSSION

Production and sale of whiskey, especially by local or craft distillers, is growing rapidly with as many as 700 small craft distilleries in the United States today and sales of bourbon whiskey up by 36% in the last five years.[20] Recent news concerning the theft and distribution of stolen Kentucky bourbon[21] suggests that rapid methods for chemical analysis for authentication and provenance are important for this high-value product. This described laboratory experiment, which relies on the green chemistry technique DLLME for rapid screening, may find importance in future authentication studies given the ability to extract semivolatile components commonly found in whiskey (Figures 2 and 3; Tables 1 and 2).

The chromatograms for two whiskey samples are provided in Figures 2 and 3 (all other chromatograms and tabular information on tentatively identified compounds for other whiskeys are provided in Supporting Information) with the data reported in Figure 2 from an international distiller (Scotland,

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Table 1. Tentatively Identified Aroma Compounds Present in Whiskey Samples Included in the Study[a]

Peak Number	Retention Time (min)	Common Name, Molecular Formula, Mass (g/mol)	Normal Boiling Point (°C)	CAS Number	Present in Whiskey A? (Figure 2)	Present in Whiskey B? (Figure 3)	Literature Comparison[b]
1	6.56	Furfural, $C_5H_4O_2$, 96.08	162	98−01−1	Y	Y	29 mg/L
2	7.07	Isoamyl acetate, $C_7H_{14}O_2$, 130.19	142	123−92−2	Y	Y	2.590 mg/L
3	8.59	5-Methyl furfural, $C_6H_6O_2$, 110.11	187	620−02−0	Y	Y	0.80 mg/L
4	8.68	Benzaldehyde, C_7H_6O, 106.12	178	100−52−7	Y	N	0.33 mg/L
5	8.73	Hexanoic acid, $C_6H_{12}O_2$, 116.16	202	142−62−1	Y	N	-[c]
6	9.00	Ethyl hexanoate, $C_8H_{16}O_2$, 144.21	166	123−66−0	Y	Y	1.990 mg/L
7	10.98	Phenylethyl alcohol, $C_8H_{10}O$, 122.16	219	60−12−8	Y	N	13.900 mg/L
8	11.63	Diethyl succinate, $C_8H_{14}O_4$, 174.19	217	123−25−1	Y	N	-
9	11.69	Octanoic acid, $C_8H_{16}O_2$, 144.21	237	124−07−2	Y	Y	-
10	11.85	Ethyl caprylate, $C_{10}H_{20}O_2$, 172.27	206	106−32−1	Y	Y	8.340 mg/L
11	12.79	2-Phenylethyl acetate, $C_{10}H_{12}O_2$, 164.02	232	103−45−7	Y	N	1.9−5.0 mg/L
12	13.27	*cis*-Whiskeylactone, $C_9H_{16}O_2$, 156.22	245	55013−32−6	Y	N	2.490 mg/L
13	13.75	*gamma*-Whiskeylactone, $C_9H_{16}O_2$, 156.22	245	39212−23−2	Y	Y	-
14	14.21	Decanoic acid, $C_{10}H_{20}O_2$, 172.27	268	334−48−5	Y	N	-
15	14.49	Ethyl caprate, $C_{12}H_{24}O_2$, 200.32	243	110−38−3	Y	Y	9 mg/L
16	14.94	Vanillin, $C_8H_8O_3$, 152.15	285	121−33−5	Y	Y	2.1−4.7 mg/L
17	17.53	Ethyl laurate, $C_{14}H_{28}O_2$, 228.37	269	106−33−2	Y	Y	5.3 mg/L
18	18.41	Isoamyl decanoate, $C_{15}H_{30}O_2$, 242.40	286	2306−91−4	Y	N	-
19	19.14	Syringaldehyde, $C_9H_{10}O_4$, 182.17	192	134−96−3	Y	Y	9.6 mg/L
20[c]	21.12	E-15-Heptadecenal	310	1000130−97−9	Y	N	-
20[c]	21.16	Ethyl myristate, $C_{16}H_{32}O_2$, 256.42	295	124−06−1	Y	N	0.2 mg/L
21	24.23	Ethyl palmitate, $C_{18}H_{36}O_2$, 284.48	303	628−97−7	Y	Y	2.4 mg/L
22	26.04	Ethyl linoleate, $C_{20}H_{36}O_2$, 308.50	388	544−35−4	Y	N	3.0 mg/L
23	26.18	Ethyl oleate, $C_{20}H_{38}O_2$, 310.51	205	111−62−6	Y	N	1.6 mg/L

[a]Please see reference list.[7,22,23] [b]Dashed line indicates that no literature value was found for previously reported concentrations for the particular compound. [c]Compounds are poorly resolved with R = 0.768.

U.K.) and the data in Figure 3 from a local Colorado distillery (Distillery 291, Colorado Springs, CO). Each peak in both chromatograms is numbered with that number corresponding to compound information (retention time, common name, molecular formula, molar mass, normal boiling point, CAS number, and comparison to literature values) in Table 1. Briefly, typical whiskey compounds that were found in these samples analyzed here (and for which there are reported concentrations available in the literature)[7,22,23] are included (Table 1):

(1) Furfural
(2) Isoamyl acetate
(3) 5-Methyl furfural
(4) Benzaldehyde
(5) Ethyl hexanoate
(6) Phenylethyl alcohol
(7) Ethyl caprylate
(8) 2-Phenylethyl acetate
(9) *Cis*-whiskey lactone
(10) Ethyl caprate
(11) Vanillin
(12) Ethyl laurate
(13) Syringaldehyde
(14) Ethyl myristate
(15) Ethyl palmitate
(16) Ethyl linoleate
(17) Ethyl oleate

Sensory information (odor and taste descriptions) is included in Table 2 for these compounds.

When all 11 of the whiskey samples from these analyses were included for comparison of compounds found (see Supporting Information for all data), it was noted that there were compounds present in most samples (labeled as *universal* constituents because they were identified in ≥ 7 whiskey

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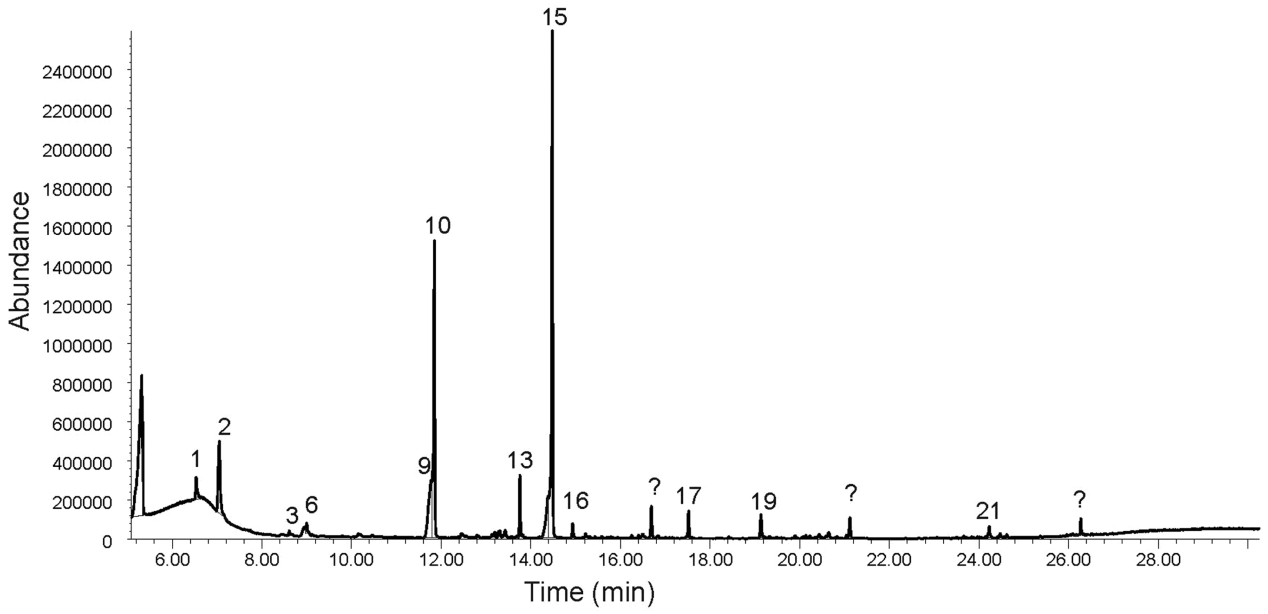

Figure 3. Total ion current chromatogram of whiskey sample B analyzed by GC/MS after DLLME sample preparation. Twelve aroma compounds were tentatively identified in this sample after spectral deconvolution through AMDIS. For peaks with a "?" label, multiple compounds with high probability match factors (>80) were reported, so no tentative identification of the compound is offered.

Table 2. Odor and Taste Descriptions of Principle Compounds Tentatively Identified in Whiskey Samples[a]

Compound Number	Name	Odor Type	Odor Description	Taste Description
1	Furfural	Bready	sweet, brown, woody, bready, caramellic with a slight phenolic nuance	brown, sweet, woody, bready, nutty, caramellic with a burnt astringent nuance
2	Isoamyl acetate	Fruity	sweet, fruity, banana, solvent	sweet fruity, banana-like with a green ripe nuance
3	5-Methyl furfural	Caramellic	spice, caramel, apple	sweet, brown, caramellic, grain, maple-like
4	Benzaldehyde	Fruity	strong, sharp, sweet, bitter, almond, cherry	sweet, oily, almond, cherry, nutty and woody
5	Hexanoic acid	Fatty	sour, fatty, sweat, cheese	-[b]
6	Ethyl hexanoate	Fruity	sweet, fruity, pineapple, waxy, green, banana	sweet, pineapple, fruity, waxy and banana with a green, estry nuance
7	Phenylethyl alcohol	Floral	floral, rose, dried rose flower, rose water	floral, sweet, rosey and bready
8	Diethyl succinate	Fruity	mild, fruity, cooked apple, ylang	-
9	Octanoic acid	Fatty	fatty, waxy, rancid, oily, vegetable, cheesy	-
10	Ethyl octanoate	Waxy	fruity, wine, waxy, sweet, apricot, banana, brandy, pear	sweet, waxy, fruit and pineapple with creamy, fatty, mushroom and cognac notes
11	2-Phenylethyl acetate	Floral	floral, rose, sweet, honey, fruity, tropical	sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel
12	*cis*-Whiskeylactone	Spicy	spicy	-
13	*gamma*-Whiskeylactone	Tonka	tonka, coumarin, coconut, toasted, nutty, celery, burnt	woody, coumarinic, coconut, iactonic, creamy and nutty with a toasted nuance
14	Decanoic acid	Fatty	unpleasant, rancid, sour, fatty, citrus	-
15	Ethyl decanoate	Waxy	sweet, waxy, fruity, apple, grape, oily, brandy	waxy, fruity, sweet apple
16	Vanillin	Vanilla	sweet, vanilla, creamy, chocolate	vanilla, vanillin, sweet, creamy, spicy, phenolic and milky
17	Ethyl dodecanoate	Waxy	fruity, wine, waxy, sweet, apricot, banana, brandy, pear	sweet, waxy, fruit and pineapple with creamy, fatty, mushroom and cognac notes
18	3-Methylbutyl pentadecanoate	Fruity	waxy, banana, fruity, sweet, cognac, green	waxy, banana, fruity, green
19	Syringaldehyde	Green	mild, plastic, woody, tonka, sweet	-
20 (coeluted)	E-15-Heptadecenal	-	-	-
20 (coeluted)	Ethyl tetradecanoate	Waxy	sweet, waxy, violet, orris	sweet, waxy, creamy
21	Ethyl hexadecanoate	Waxy	mild, waxy, fruity, creamy, milky, balsam	waxy, fruity, creamy and fermented with a vanilla, balsamic nuance
22	Ethyl linoleate	-	mild, fatty, fruit	-
23	Ethyl oleate	Fatty	fatty, oil, dairy, milky, waxy, tallow	

[a]Please see reference list.[17] [b]Dashed line indicates that no descriptors for odor type, odor, or taste were offered through the resource cited.

samples), in a few samples (labeled as *common* constituents and found in 3−6 samples), or only in a few samples (labeled *unique* constituents and found in two samples of the 11). Universal constituents included those in the floral odor

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category (phenylethyl alcohol and 2-phenylethyl acetate), waxy odors (ethyl octanoate, ethyl caprylate, and ethyl caprate), and tonka (whiskey lactone). Common constituents (found in 3−6 samples) included fruity odors (isoamyl acetate, benzaldehyde, ethyl hexanoate, isoamyl decanoate), bready and caramellic odors (furfural and 5-methylfurfural), vanilla odor (vanillin), green odor (syringaldehyde), and waxy odor (ethyl myristate). Unique constituents (found in $n < 3$ samples) included a variety of odor types:[17]

(1) Roasted (2-methyl-1-butanol)
(2) Herbal (1,4-cineole)
(3) Citrus (carene)
(4) Fruity (diethyl succinate and isoamyl octanoate)
(5) Minty (ethyl benzoate)
(6) Floral (ethyl phenyl acetate)
(7) Waxy (ethyl nonanoate)
(8) Spicy (*cis*-oak lactone)
(9) Green (β-farnesene)
(10) Phenolic (ethyl vanillate)
(11) Fatty odors (ethyl oleate).

While this laboratory experiment included 11 samples (two well-known commercial whiskeys and nine from a local distillery), there was still a broad array of compounds (and hence different sensory profiles) that the students could report and evaluate for the odor contribution to the whiskey.

LEARNING OUTCOMES AND ASSESSMENT

Each student pair was randomly assigned three to five whiskey samples to analyze. Given an average of 10 students per laboratory section (or five student pairs) each with three to five samples to analyze, it thus worked out such that each whiskey sample was analyzed two or three times. Students would prepare their whiskey samples by DLLME and load the instrument autosampler with their 3−5 samples (for a total of 120−200 min of analysis time on the GC/MS). These assigned samples contained several different types of whiskey: barrel-aged whiskey (aged in an oak barrel for at least one year postdistillation—these included whiskey samples A, B, C, D, E, G, J, and K), and white whiskey (whiskey that was adjusted for alcohol content postdistillation, but not aged—these included whiskey samples F, H, and I). In subsequent laboratory meetings after their initial data collection, students were asked to determine common compounds that were present in all samples and compounds that were unique to specific samples, which could be perhaps traced back to the original whiskey type or the barrel-aging process.

Students were expected to write formal lab reports for the experiment in keeping with a standard literature format. Specifically, the reports included: abstract, theory, procedure, results, discussion, and conclusions. In their abstracts, they were to include a brief summary of findings. In the theory section, the students were expected to provide a detailed description of the instrument, both in terms of background (specifically of GC/MS) and the sample preparation techniques utilized in the experiment. In their procedure, students were asked to write a brief method, including instrumental parameters, as well as any deviations in procedure from the lab manual. In their results, students included a table of compounds that contained ten major compounds in each sample and five unique components for each whiskey. These unique components could be the same as the major components. In addition to name and structure, the students were to include probability of the match, molecular weight, and CAS number. Lastly, students had to include the results of their taste and odor findings from the literature. In the discussion, they were expected to present their findings in terms of differences and similarities in the whiskey samples. In particular, students were to analyze any differences between the white whiskey and the barrel-aged whiskey. They were to include their own scent results from smelling the samples and comment on whether their initial scent evaluation matched their results. Lastly, the students included a brief conclusion, which summarized their results.

This experiment was qualitative in nature. Because the students were simply determining compounds that were present in the samples without any quantification, they were evaluated based on the thoroughness of their literature searching postexperiment as well as their writing and presentation. The majority of the evaluation was based on the table of compounds. Students were expected to have thoroughly discussed the similarities and differences in the whiskey samples. Students were required to have citations for each taste and odor profile. In addition to their formal lab reports, students were asked to provide chromatograms of each whiskey sample as well as a mass spectrum of a unique compound from each sample. For each mass spectrum, the students identified molecular ion fragments that contributed to three significant ions. This reinforces the skill set they learned in the organic chemistry laboratory. They also had to include the carbon copies of their lab notebook pages, which included their in-lab journal and their initial notes from the AMDIS program. Students were also asked to critically evaluate the limitations of the outlined protocol, including: (1) why this method does not provide a comprehensive profile of all significant taste and odor compounds and (2) the limitations of using a nonpolar GC column and mass selective detector.

Alternative learning outcomes and additional assessment strategies that may be of interest to future instructors are provided in the Supporting Information. Additional experimental procedures could be to have students calculate the Kovats retention indices for whiskey analytes from a series of hydrocarbon standards. Other suggestions include the analysis of authentic standards to suggest to the students the amount of time and laboratory resources it would take for confirmed identification of all analytes. Students could also be asked to explain how they might make this current protocol quantitative. They should describe the current limitations of the protocol and how they might use internal standards or surrogate standards for quantitation. They may also suggest alternative applications for DLLME sample preparation with GC/MS analysis.

CONCLUSION

This exercise helped the students to develop hands-on skills of green chemistry extraction (DLLME) on "real-world" samples, and the pedagogical importance of this procedure was in the exploration of the power of MS and the postprocessing software (ChemStation and AMDIS) to determine the number of discrete compounds in the whiskey samples. The students also revisited the importance of mass spectral interpretation, a skillset often covered in organic chemistry laboratory sequences, in the analytical laboratory to critically evaluate the library match data assigned by the data analysis software.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00342.

Student laboratory procedures, notes for the instructor, and additional spectra and tabulated data (PDF, DOCX)

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Notes

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J. Chem. Educ. XXXX, XXX, XXX−XXX