

Evaluation of PDMS-Based Extraction Techniques and GC-TOFMS for the Analysis of Off-Flavor Chemicals in Beer

LECO Corporation; Saint Joseph, Michigan USA

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1. Introduction

Various techniques employing polydimethylsiloxane (PDMS) as an extraction medium prior to GC-TOFMS analysis were investigated to measure off-flavors in aged beer. The techniques included stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), purge-and-trap (P&T) and closed-loop-stripping (CLS). SBSE appeared to provide the most accurate quantitation and was capable of detecting the most odor-active compounds. The peak deconvolution capability of the LECO Pegasus® GC-TOFMS was found critical to the detection and accurate quantitation of key off-flavor chemicals. Compared to fresh control beer, increases in furfural, furfuryl ethyl ether, furyl hydroxymethyl ketone, 2,4-dodecadienal, (E,E), benzeneacetic acid ethyl ester, β -damascenone and 3-pyridinecarboxylic acid ethyl ester (a.k.a. nicotinic acid ethyl ester) were observed in beer samples incubated 12 wks at 30°C and increases in dimethyl disulfide, dimethyltrisulfide and benzeneacetaldehyde occurred in beer exposed to sunlight for 8 hours.

Carbonyl compounds (particularly aldehydes), furfuryl derivatives and other types of organic chemicals are considered to play a role in the development of off-flavors in aged beer samples. 3-Methyl-2-butene-1-thiol and other thiols and organic sulfur compounds have been shown to contribute skunky off-notes to light-exposed beer. Numerous GC-MS studies have been conducted to study off-flavor problems in beer. Sample preparation/extraction techniques that have commonly been used in the past for studying beer off-flavors include steam distillation [1], purge-and-trap on Tenax GR [2], extraction with XAD-2 resin [3], solid-phase microextraction [4], solid-phase microextraction with on-fiber derivatization [5] and Freon extraction [6].

The important advantages of polydimethylsiloxane phase as an extraction medium have been previously described [7]. Polydimethylsiloxane (PDMS)-coated magnetic stir bars (GERSTEL Twister®) can be used to extract flavor compounds and applied in different formats, including placement in the beer sample (stir bar sorptive extraction or SBSE) and placement in the headspace above the beer sample (headspace sorbent extraction or HSSE). PDMS foam mounted in thermal desorption tubes is a new PDMS format that has recently been introduced by GERSTEL GmbH & Co. KG and was investigated as an extraction sorbent in a purge-and-trap (P&T) technique and a closed-loop-stripping (CLS) method.

The goal of this work is to develop a solventless analytical method for studying off-flavor development in beer that is quantitative, relatively simple to perform, and capable of extracting a wide array of potentially significant flavor compounds from beer at low ppb levels. Beer contains dozens of odor active chemical components in concentrations ranging from percent to parts-per-trillion

(ppt). Besides PDMS extraction, a second critical component to the analytical strategy employed in this work is the application of GC-TOFMS incorporating sophisticated peak deconvolution algorithms.

2. Experimental Conditions

Instrumentation

Analyses were performed on a 6890 GC (Agilent Technologies) equipped with a CIS 4 inlet and MPS 2 robotic sampler with TDU option (GERSTEL) and a Pegasus® TOFMS (LECO).

Analysis Conditions

TDU:

Splitless 20°C; 60°C/minute; 260°C (3 minutes)

CIS 4:

0.05 minute solvent vent (50 mL/minute);
Splitless (1.5 minute), -120°C; 10°C/second;
300°C (3 minutes)

Column:

30 m HP-5MS (Agilent); di = 0.32 mm; df = 0.25 μ m

Pneumatics:

He; Pi = 1.6 psi; constant flow = 1.5 mL

Oven:

40°C (1 minute); 10°C/minute; 270°C (6 minutes)

TOFMS:

40 to 300 amu; 10 spectra/second;
S/N (data processing) 50.0

Beer Samples Analyzed

(a) Control Beer:

American lager beer stored for 12 weeks at 0°C
(Sensory: fresh)

(b) Heat-Abused Beer:

Control beer stored at 30°C for 12 weeks
(Sensory: stale, chemical off-flavor)

(c) Light-Abused Beer:

Control beer subjected to 8 hours of sunlight in
a clear glass bottle (Sensory: skunky, sulfury odor,
objectionable flavor)

Sample Preparation

In all cases, 10 mL of beer or standard was extracted at room temperature.

(a) SBSE: A Twister stir bar is placed in the beer or standard in a 20 mL GC vial, sealed, and stirred for 2 hours at 900 rpm. After extraction, the Twister was rinsed in distilled water for 3 seconds and patted dry with a clean lintless towel.

(b) HSSE: A Twister is suspended in the headspace above the sample with a paper clip. HSSE is conducted for 2 hours while stirring with a micro stir bar at 900 rpm.

(c) P&T: The sample is placed in a Scientific Instruments Inc. (SIS, Ringoes, NJ) purge vessel and purged with nitrogen (30 mL/minute) for 20 minutes into a GERSTEL TDU desorption tube containing a PDMS foam trap. A second stream of dry nitrogen (25mL/minute) is used to purge the PDMS foam tube to prevent condensation of water.

(d) Dynamic headspace CLS (DHSCLS): Ten milliliters of sample is placed in a 250 mL gas washing bottle, and a GERSTEL TDU desorption tube with a PDMS foam trap is attached to the outlet of the wash bottle. The inlet to the bottle is attached to the outlet of an oil-free pump, while the PDMS foam trap is attached to the inlet of the pump (Figure 1). Air in the washing bottle is recirculated (120 mL/minute) through the system for 5 minutes while the sample is stirred (500 rpm).

Note: The total volume of purging gas is 600 mL; the same for both P&T and DHSCLS.

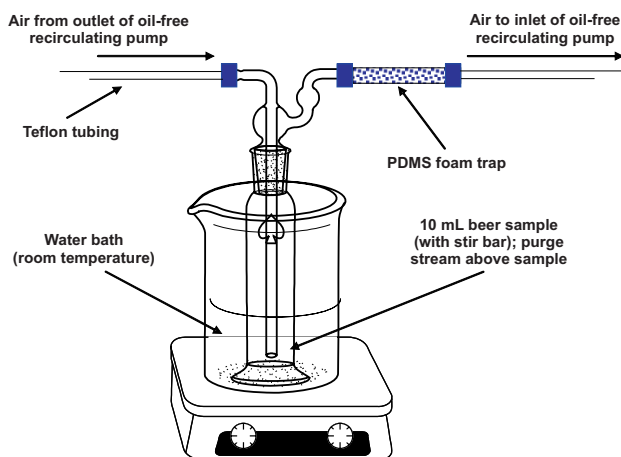


Figure 1. Dynamic headspace closed-loop-stripping with PDMS foam trap.

Time-Course Studies and Standard Calibration Curves

The first step in developing and evaluating the analytical methods was to study the recovery behavior of a variety of flavor compounds known to occur in beer as a function of extraction time. Time-course extraction studies were conducted with a mid-range working standard solution. These studies revealed that 2 hour extraction times for SBSE and HSSE were sufficient. For P&T with the PDMS foam trap, 20 minutes extraction time provided good recovery of volatiles under the conditions used. DHSCLS sampling times greater than 10 minutes resulted in a reduced analyte recovery from the PDMS foam. Since the amount of volatiles extracted at 5 minutes and 10 minutes were essentially the same, 5 minute extraction times were employed for DHSCLS.

A stock solution of 14 flavor compounds known to occur in beer plus 2-undecanone as IS at 55 ppb was prepared in ethanol (Table 1). This stock solution was added to an imitation beer system (5% ethanol/water solution adjusted to pH 4.5 with phosphoric acid) at 5, 10, 25, 100 and 200 ppb for all standards except isoamyl acetate and phenyl ethyl alcohol. For these two standards, working standard concentrations of 50, 100, 250, 1000, and 2000 ppb were prepared. The solutions were analyzed by each

of the four sample preparation methods. The linear least squares correlation coefficients were determined and used as a measure of test accuracy for each of the sample preparation methods (Table 1). Table 2 shows the quantitation of these 14 analytes in the control, heat-abused and light-abused samples.

Table 1. Linear least squares correlation coefficients (R²) for 14 analyte standards spiked in 5% ethanol/water adjusted to pH 4.5 with phosphoric acid solution for four different sample preparation procedures at five levels of spikes from 5 to 200 ppb.

Standard Compound	CLS	P&T	HSSE	SBSE
Pentanal	0.9998	0.9930	0.9172	0.9997
Hexanal	0.9572	0.9779	0.9959	0.9426
Furfural	0.8664	0.9952	NL	0.9423
1-Butanol, 3-methyl-, acetate	0.7875	0.9532	0.9973	0.9929
Methional	0.8582	ND	ND	0.9943
Hexanoic acid, ethyl ester	0.9936	0.9911	0.9252	0.9964
Octanal	0.9954	0.9885	0.9991	0.9972
Benzeneacetaldehyde	0.9985	0.9977	0.9962	0.9996
Nonanal	0.9971	0.9898	0.9989	0.9995
Phenyl ethyl alcohol	NL	NL	NL	0.9796
2-Nonenal, (E)-	0.9916	0.9906	0.9993	0.9997
Ethyl octanoate	0.9980	0.9833	0.9695	0.9559
Decanal	0.9090	0.8598	0.9995	0.9837
Ethyl decanoate	0.8601	0.9571	0.9858	0.9935
Average R ² :	0.9394*	0.9731**	0.9803***	0.9841

ND= None detected; NL=Non-linear

*Excluding phenyl ethyl alcohol

**Excluding methional and phenyl ethyl alcohol

***Excluding furfural, methional and phenyl ethyl alcohol

Table 2. Concentrations of 14 flavor chemicals in control beer, control beer abused by heat (12 weeks storage at 30°C), and the control beer abused by exposure to sunlight for 8 hours (Analytical method: SBSE with GC-TOFMS).

Compound	R.T. (seconds)	Quant Mass (amu)	Control (ppb)	Heat-Abused (ppb)	Light-Abused (ppb)	Average % Std. Dev.*
Pentanal	150	58	<2.5	<2.5	<2.5	—
Hexanal	215	56	<2.5	<2.5	<2.5	—
Furfural	242	96	89	1,007	369	8.3
1-Butanol, 3-methyl-, acetate	272	43	535	608	569	5.4
Methional	298	48	<2.5	<2.5	<2.5	—
Hexanoic acid, ethyl ester	378	88	66.8	74.1	68.7	3.2
Octanal	382	57	3.17	6.25	3.04	3.6
Benzeneacetaldehyde	419	91	2.33	2.54	3.80	4.9
Nonanal	472	57	2.59	5.18	3.10	6.5
Phenyl ethyl alcohol	487	91	5,491	5,917	6,268	11.0
2-Nonenal, (E)-	523	83	0.43	0.39	0.37	4.9
Ethyl octanoate	555	70	169.0	162.2	141.0	2.1
Decanal	566	112	0.71	0.88	0.81	3.9
Ethyl decanoate	720	88	42.3	37.1	18.8	4.2

*std dev = $(\sum d^2/2n)^{1/2}$

3. Results and Discussion

Figure 2 shows TIC results for the control beer sample analyzed by SBSE and HSSE based on the GERSTEL Twister. Figure 3 shows TIC results for the P&T and DHSCLS which used PDMS foam trapping. Chromatograms for HSSE, P&T, and DHSCLS are the most similar.

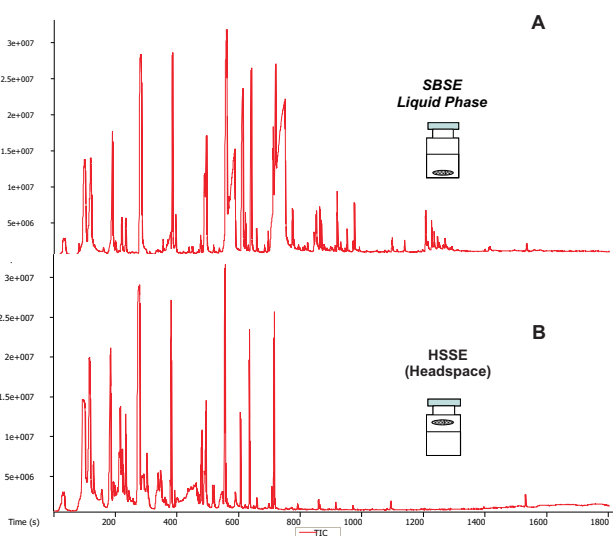


Figure 2. SBSE (A) and HSSE (B) TIC plots with Twister (control beer).

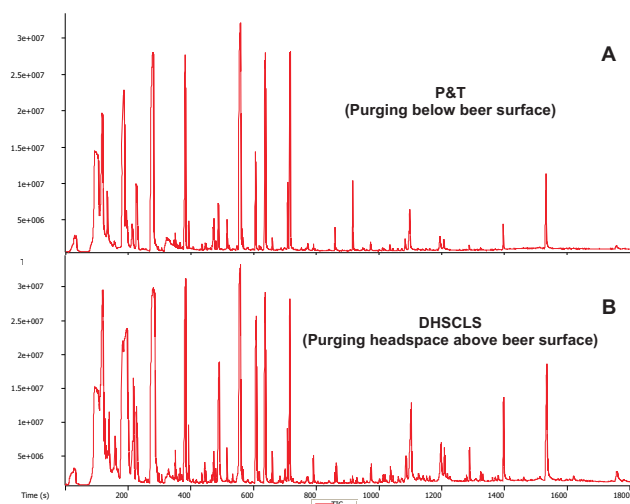


Figure 3. P&T (A) & DHSCLS (B) TIC plots with PDMS foam traps (control beer).

SBSE provided the most accurate quantitation (based on linear least squares correlation coefficients) and was capable of detecting the most odor-active compounds (Table 1). SBSE was the only method that could detect all 14 standard analytes quantitatively. HSSE calibration curves for furfural were non-linear. Methional was not detected by P&T or HSSE in any of the standards. Phenyl ethyl alcohol calibration curves were non-linear with CLS, P&T and HSSE. Standard deviations of replicate analyses were less than 10% for all analytes analyzed by SBSE except for phenyl ethyl alcohol (PEA). The poor precision for PEA may be due to the fact that it is present in all beer samples at levels outside the range of the PEA standard calibration curve.

Several interesting flavor chemicals were created or increased in concentration in the beer sample stored at 30°C for 12 weeks compared to the beer control sample, as shown in Figure 4. These included furfural, furfuryl ether, furfuryl hydroxymethyl ketone, 2,4-dodecadienal, (E,E), benzeneacetic acid ethyl ester, β -damascenone, 3-pyridinecarboxylic acid ethyl ester, octanal, and nonanal. The increases in β -damascenone and furfuryl ethyl ether may be most significant to off-flavor development considering their extremely low taste threshold levels.

Chemical Changes in Beer Caused by Heat Exposure

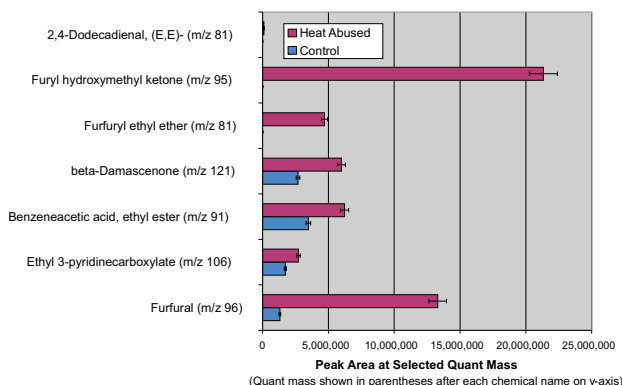


Figure 4. Chemicals generated in beer after aging at 30°C for 12 weeks; analysis by SBSE/GC-TOFMS.

Increases in aldehyde levels were relatively insignificant considering the flavor threshold levels of most of the alkyl aldehydes. Trans-2-Nonenal, which has been implicated as a major off-flavor contributor in aged beer and has a low flavor threshold in beer of 0.11 ppb, was at relatively constant levels in control, heat-abused, and light-abused beers.

The most significant changes in the light-exposed beer sample vs. the control were the formation of dimethyl disulfide and dimethyl trisulfide in the light-exposed beer (Figure 5). Benzene-acetaldehyde concentration increased significantly in the light-exposed sample. 3-Methyl-2-butene-1-thiol (MBT), a compound widely known to be a major contributor to the skunky odor of light-damaged beer, was not detected in the light-exposed sample. It is likely present in the light-abused sample at levels too low to be detected by the analytical methods used but high enough to be perceived by smell. SBSE combined with a detector more sensitive to sulfur compounds (the PFPD) has previously been reported to detect MBT in beer (8).

Chemical Changes in Beer Caused by Light Exposure

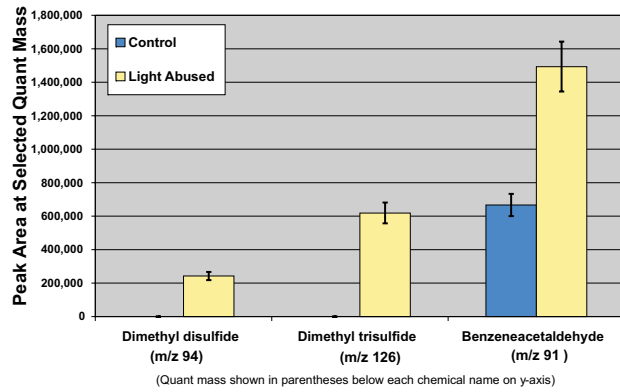


Figure 5. Chemicals generated in beer after exposure to sunlight for 8 hours; analysis by SBSE/GC-TOFMS.

Accurate detection and quantitation of several key flavor compounds could not have been accomplished without the peak deconvolution capabilities of the Pegasus GC-TOFMS. With over 700 chemicals detected in each beer sample, peak deconvolution was necessary to detect and accurately quantitate β -damascenone (Figure 6), furfuryl ethyl ether (Figure 7), furyl hydroxymethyl ketone, and several other potential off-flavor compounds.

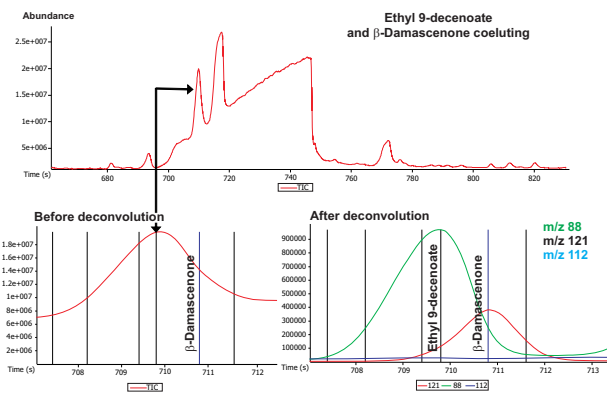


Figure 6. The importance of peak deconvolution for determination of β -damascenone in beer after aging at 30°C for 12 weeks.

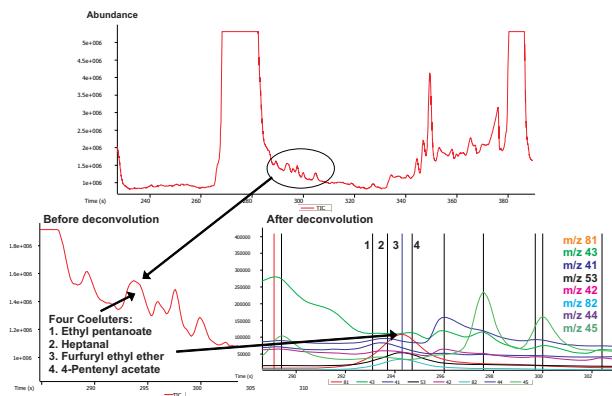


Figure 7. The importance of peak deconvolution for measurement of furfuryl ethyl ether in beer after aging at 30°C for 12 weeks.

Figure 8 shows the importance of peak deconvolution for (Z)-2-nonenal, a potential cause of stale flavor in beer. This figure shows the non-deconvoluted mass spectrum (caliper), the deconvoluted (true) mass spectrum, and the library match for (Z)-2-nonenal.

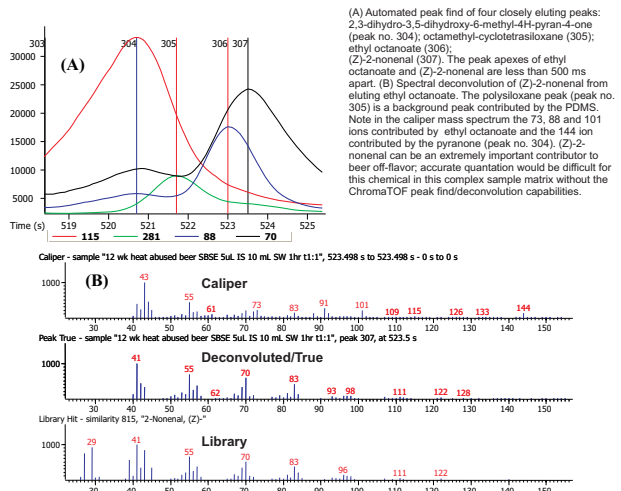


Figure 8. Peak deconvolution for (Z)-2-nonenal showing caliper, true, and library mass spectra.

4. Conclusions

All four PDMS extraction techniques provided acceptable accuracy (as measured by standard calibration curves) and precision for most analytes studied. SBSE, however, provided the best linear least squares correlation coefficients, was able to extract the most flavor chemicals, and offered the best sensitivity for β -damascenone and other flavor compounds. Results show that increases in β -damascenone and furfuryl compounds produced in Maillard reactions may be more important than aldehyde formation in influencing off-flavors in aged beer. Additional sensory studies involving spiking of fresh-tasting control beer with β -damascenone, furfuryl ethyl ether and other compounds reported in Figure 4 should be conducted to determine the contribution of these chemicals to off-flavors in beer. The significant advantage of peak deconvolution with the LECO Pegasus TOFMS was illustrated for key off-flavor chemicals in beer.

5. References

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Ray T. Marsili, Laura Laskonis, Cesar Kenaan Marsili Consulting Group, Rockford College, Starr Science Building, 5050 East State St., Rockford, IL 61108, USA

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