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Analysis of selected aldehydes in packaged beer by solid-phase microextraction (SPME)-gas chromatography (GC)-negative chemical ionization mass spectrometry (NCIMS)

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Abstract

Unambiguous identification and quantification of certain aldehydes in packaged beer measured at low to sub-ppb levels is challenging due to the vast number of compounds that can interfere with the measurement of the target aldehydes. This difficulty in resolution has hindered the quantitation and subsequent connection with sensory data. Successful techniques to overcome the challenges of sensitivity and selectivity have been established, particularly utilizing SPME-GC-Electron Ionization mass spectrometry EIMS, derivatization with O-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) and using selected ion monitoring (SIM) at m/z 181. This work builds on earlier methods using this technique to improve both sensitivity and selectivity. Resolution improvement can be achieved by operating the mass spectrometer in the negative chemical ionization (NCI) mode, as opposed to the EI mode. Firstly, by taking advantage of the derivatization reagent PFBHA, which serves to magnify the signal intensity by virtue of the five fluorine atoms accepting the electrons produced from the interaction of the ion filament and the reagent gas methane. This is classified as the electron capture mechanism, often referred to as high-pressure electron capture mass spectrometry (HPECMS) and is responsible for the increased sensitivity compared to operating in EI mode. Secondly, the production of characteristic fragment ions inherent to NCI mode, results in improved selectivity of the target aldehydes. This is especially useful when attempting to measure aldehydes at the sub-ppb levels. Even at these levels, many aldehydes can impart significant undesirable flavor characteristics. Other aldehydes provide information related to the specific degradation reactions and metric levels surrounding fresh beer profiles. Changes in the levels of these selected aldehydes during controlled storage conditions over time can be used to improve brewing practice's, make better comparisons with flavor characteristics, and assess ingredient/contact material changes. As an example of this technique and improved analytical method, a fresh batch of lager beer was profiled at selected times over a 12 week period and changes in target aldehydes were reported.

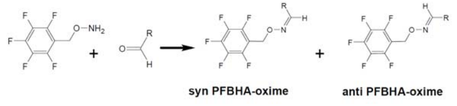
Introduction

Aldehydes contribute to the flavor of packaged beer. With increasing time and temperature their contribution becomes more significant. Various chemical reactions produce aldehydes: Enzymatic oxidation of lipids and release from adducts, e.g. (E)-2-octenal, (E)-2-nonenal, (E,E)-2,4-decadienal; Maillard reactions, e.g. 2-furfural, 5-methylfurfural; Strecker degradation of amino acids, e.g. 2-methylpropanal, 3-methylbutanal, methional, phenylacetaldehyde. These and other aldehydes deteriorate the fresh taste because they possess undesirable flavor characteristics, e.g. vinous, bitter, rancid, astringent, stale, paper, cardboard, cucumber and oily. Lager or Pilsner type beers are especially prone due to lack of masking ability, i.e. low levels of alcohol, extract and hops.

A sensitive and selective method of analysis for aldehydes is needed to overcome the difficulties of measuring this class of compounds. Major difficulties include, efficient and selective extraction at trace levels, reactive functional group, disproportionate competing volatility from non-targeted compounds, matrix effects and resolution power of the detector.

This SPME-GC-NCIMS method incorporates analytical techniques that work synergistically to overcome these challenges. On-fiber derivatization by reaction of PFBHA with the aldehydes on the SPME fiber phase polydimethylsilozane/divynylbenzene (PDMS/DVB), efficiently and selectively separates the aldehydes from the complex beer matrix. Additionally, this produces a molecularly stable PFBHA-oxime compared to the aldehyde in its free form, which enables it to maintain its molecular structure as it passes through the components of the GC. The phase of the fiber is formulated for optimal adsorption of amines (i.e. PFBHA-oximes). The derivatization reaction produces two PFBHA-oxime geometrical isomers (syn & anti) from each aldehyde, see Figure 1. Only negative ions will be detected when operating the MS in NCI mode, consequently the PFBHA-oximes need to be converted to negative ions. This is accomplished when a fluorine atom of the pentafluorobenzyl group captures a low energy electron. These low energy electrons are generated continuously when high energy electrons emitted from the ion source filament bombard the reagent gas methane, which in turn releases low energy electrons. NCI mode also produces a less energetic process of ionization which results in abundant characteristic negative ions, unique to the target aldehydes. The salting-out technique is utilized to increase the volatility of the aldehydes, especially for those in the low to sub-ppb range. This increases the amount of aldehydes adsorbed onto the phase of the SPME fiber. To overcome matrix effects, calibration was based on the technique of standard additions along with an internal standard (3-Fluorobenzaldehyde) to incorporate the ratio of peak area of aldehyde standard to peak area of internal standard into the calculations. An aliquot of internal standard was added to each sample and the concentrations were calculated based on area ratio.

Figure 1. Derivatization reaction of PFBHA with aldehydes to produce two geometrical isomers (syn) and (anti)



Materials and methods

Analysis of selected aldehydes by SPME-GC-NCIMS with PFBHA on-fiber derivatization

An improved analytical method was developed for the identification and quantification of selected aldehydes in beer, see Table 1. The volatile aldehydes were separated from the beer matrix by adsorption onto the SPME fiber coated with PFBHA for the selective on-fiber derivatization reaction. The resultant aldehyde PFBHA-oximes were subsequently desorbed into and separated by GC and detected by NCIMS.

Auto-sampler

To maximize the reproducibility, an Agilent GC 120 Multipurpose auto-sampler was utilized. Key features include automated heated/agitated on-fiber derivatization reaction, injection, a fiber bake out station utilized after every injection to ensure no carry over and a refrigerated sample tray to maintain sample stability.

SPME fiber and sample preparation

A 65 um PDMS\DVB, fused silica, 23 Ga fiber was used. The sample preparation and auto-sampler parameters were as follows:

- Removal of CO2 from sample – 100 mL of sample in a baffled flask placed on rotary platform at 175 rpm for 5 min
- Sample vial – 10 mL of beer + 3.5 g NaCl + 10 uL internal standard in 20 mL vial
- Derivative reagent (PFBHA) vial – 10 mL of H_2O in 20 mL vial + 100 uL of 6 g/L PFBHA
- PFBHA vial – incubated for 5 min at 50 ºC
- Sample vial – incubated for 15 min at 50 ºC
- Fiber exposed to headspace of PFBHA vial for 10 min at 50 ºC
- Fiber subsequently exposed to headspace of sample vial for 60 min at 50 ºC

GC

An Agilent 7890A gas chromatograph equipped with a septumless head injection port and fitted with an Agilent J&W DB-5, 60 m x 0.250 mm id, 0.50 um film thickness, capillary column was used for separating the aldehyde PFBHA-oximes. The GC was set to the following parameters:

- Split/splitless inlet with 1 mm glass inlet liner at 250 ºC in splitless mode
- Helium carrier gas at a flow rate of 1.2 mL/min in constant flow mode
- Oven temperature program of 40 ºC for 0 min, then 10 ºC/min to 140 ºC for 0 min, then 7 ºC/min to 250 ºC for 14 min
- Interface (transfer line) temperature 280 ºC

MS

An Agilent 5975C mass selective detector equipped with a performance turbomolecular pump was set to the following parameters:

- Negative chemical ionization, selected ion monitoring
- CI gas methane flow rate, 40% (2 mL/min)
- Source temperature 150 ºC
- Quadrupole temperature 150 ºC

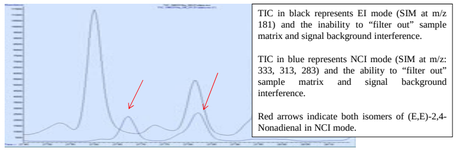
Table 1. Characteristic ions of the NCI mass spectra for target aldehyde PFBHA-oxime derivatives

PFBHA-oxime	[M]·⁻	[M-HF]·⁻	[M-HF-30]·⁻
2-Methylpropanal	267	247	217
2-Methylbutanal	281	261	231
3-Methylbutanal	281	261	231
Pentanal	281	261	231
Hexanal	295	275	245
Furfural	291	271	241
Heptanal	309	289	259
Methional	299	279	249
5-Methylfurfural	305	285	255
Octanal	323	303	273
Benzaldehyde	301	281	251
(E)-2-Octenal	321	301	271
Phenylacetaldehyde	315	295	265
(E)-2-Nonenal	335	285	315
Decanal	351	331	301
(E,E)-2,4-Nonadienal	333	313	283
(E,E)-2,4-Decadienal	347	327	297

Table 2. Method validation

PFBHA-oxime	Linearity		Precision	Accuracy	LOD	LOQ
	Range (ug/L)	R^2	(% RSD)	(% Recovery)	(ug/L)	(ug/L)
2-Methylpropanal	1.0 - 49	0.9991	5.1	97 - 103	0.35	1.1
2-Methylbutanal	1.0 - 32	0.9992	3.5	97 - 103	0.15	0.46
3-Methylbutanal	1.0 - 20	0.9976	5.3	88 - 96	0.39	1.2
Pentanal	0.1 - 2.0	0.9995	3.8	98 - 114	0.015	0.046
Hexanal	0.1 - 3.0	0.9991	2.2	98 - 120	0.033	0.101
Furfural	15 - 390	0.9991	2.2	96 - 108	0.90	2.7
Heptanal	0.1 - 1.0	0.9993	3.4	100 - 107	0.011	0.034
Methional	0.5 - 16	0.9880	6.8	99 - 101	0.94	2.8
5-Methylfurfural	0.5 - 14	0.9980	4.5	88 - 104	0.24	0.72
Octanal	0.1 - 2.0	0.9880	22.5	38 - 106	0.062	0.19
Benzaldehyde	0.3 - 11	0.9785	15.8	92 - 115	0.15	0.45
(E)-2-Octenal	0.04 - 1.0	0.9978	6.0	98 - 100	0.005	0.014
Phenylacetaldehyde	2.0 - 50	0.9984	2.9	95 - 108	0.70	2.1
(E)-2-Nonenal	0.03 - 0.6	0.9976	7.3	101 - 117	0.010	0.030
Decanal	0.1 - 2.0	0.9959	8.3	98 - 117	0.024	0.073
(E,E)-2,4-Nonadienal	0.02 - 0.2	0.9978	5.8	100 - 113	0.004	0.012
(E,E)-2,4-Decadienal	0.04 - 0.4	0.9933	12.0	102 - 107	0.007	0.024

TIC 1. Illustration comparing selective capability of NCI versus EI for the detection of (E,E)-2,4-Nonadienal

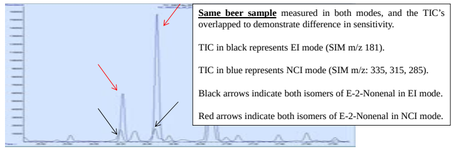


TIC in black represents EI mode (SIM at m/z 181) and the inability to "filter out" sample matrix and signal background interference.

TIC in blue represents NCI mode (SIM at m/z: 333, 313, 283) and the ability to "filter out" sample matrix and signal background interference.

Red arrows indicate both isomers of (E,E)-2,4-Nonadienal in NCI mode.

TIC 2. Illustration comparing sensitive capability of NCI versus EI for the detection of E-2-Nonenal



Same beer sample measured in both modes, and the TIC's overlapped to demonstrate difference in sensitivity.

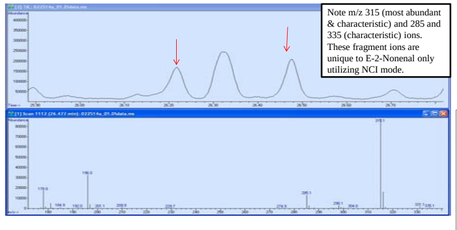
TIC in black represents EI mode (SIM m/z 181).

TIC in blue represents NCI mode (SIM m/z: 335, 315, 285).

Black arrows indicate both isomers of E-2-Nonenal in EI mode.

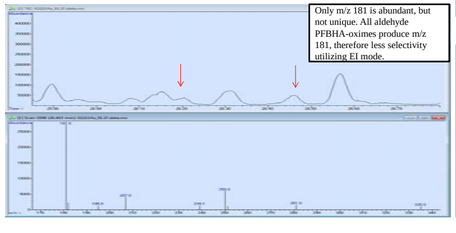
Red arrows indicate both isomers of E-2-Nonenal in NCI mode.

TIC with mass spectra 3. E-2-Nonenal isomers in American light lager 'X' utilizing NCI scan mode



Note m/z 315 (most abundant & characteristic) and 285 and 335 (characteristic) ions. These fragment ions are unique to E-2-Nonenal only utilizing NCI mode.

TIC with mass spectra 4. E-2-Nonenal isomers in American light lager 'X' utilizing EI scan mode



Only m/z 181 is abundant, but not unique. All aldehyde PFBHA-oximes produce m/z 181, therefore less selectivity utilizing EI mode.

Table 3. Changes in levels (ppb) of aldehydes during storage for 12 weeks at 24 ºC compared to 4 ºC: American light lager 'X'

	4 ºC	24 ºC					
Aldehyde	12 w	1 w	2 w	4 w	8 w	10 w	12 w
2-Methylpropanal	1.83	2.30	2.95	4.45	6.48	8.31	9.21
2-Methylbutanal	0.89	0.95	1.09	1.41	1.76	2.02	2.25
3-Methylbutanal	4.24	4.48	4.97	6.08	7.10	7.92	8.58
Pentanal	0.14	0.15	0.16	0.20	0.21	0.28	0.29
Hexanal	0.41	0.42	0.47	0.61	0.67	0.90	0.96
Furfural	12.5	14.1	21.0	33.5	55.4	68.7	79.2
Heptanal	0.15	0.074	0.080	0.10	0.11	0.20	0.32
Methional	3.06	2.43	2.95	3.50	3.84	4.03	4.53
5-Methylfurfural	1.99	1.01	0.91	1.04	1.20	1.28	1.59
Octanal	0.14	0.21	0.28	0.22	0.28	0.28	0.38
Benzaldehyde	2.99	2.71	3.17	3.17	3.46	3.25	2.80
(E)-2-Octenal	0.039	0.046	0.051	0.056	0.058	0.071	0.086
Phenylacetaldehyde	4.17	4.46	5.36	6.87	8.34	10.1	11.5
(E)-2-Nonenal	0.174	0.215	0.272	0.303	0.338	0.416	0.453
Decanal	0.092	0.076	0.10	0.13	0.12	0.18	0.15
(E,E)-2,4-Nonadienal	0.015	0.017	0.021	0.021	0.024	0.029	0.030
(E,E)-2,4-Decadienal	0.029	0.040	0.050	0.046	0.059	0.082	0.094

Table 4. Changes in levels (ppb) of aldehydes during storage for 12 weeks at 24 ºC compared to 4 ºC: American light lager 'Y'

	4 ºC	24 ºC					
Aldehyde	12 w	1 w	2 w	4 w	8 w	10 w	12 w
2-Methylpropanal	1.88	2.98	3.48	4.70	6.90	8.39	9.47
2-Methylbutanal	0.77	1.04	1.14	1.39	1.89	2.05	2.34
3-Methylbutanal	4.35	5.45	5.78	6.55	8.21	8.81	9.88
Pentanal	0.077	0.096	0.10	0.12	0.14	0.15	0.17
Hexanal	0.19	0.25	0.26	0.33	0.41	0.45	0.51
Furfural	8.71	16.0	19.3	31.1	51.7	62.1	70.7
Heptanal	0.045	0.062	0.064	0.074	0.083	0.16	0.27
Methional	N.Q.	N.Q.	N.Q.	N.Q.	2.76	3.32	3.48
5-Methylfurfural	N.Q.	N.Q.	N.Q.	N.Q.	0.80	0.92	1.06
Octanal	N.Q.	0.20	0.21	0.29	0.24	0.26	0.31
Benzaldehyde	0.96	0.99	1.05	1.14	1.16	1.04	0.86
(E)-2-Octenal	N.Q.	0.015	0.016	0.018	0.020	0.022	0.028
Phenylacetaldehyde	3.27	4.55	4.85	5.74	7.09	8.66	8.92
(E)-2-Nonenal	0.031	0.049	0.051	0.062	0.075	0.077	0.084
Decanal	N.Q.	0.083	0.095	0.104	0.096	0.10	0.10
(E,E)-2,4-Nonadienal	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
(E,E)-2,4-Decadienal	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

N.Q.: not quantifiable

Results and discussion

Application of the mass spectrometer in negative chemical ionization mode

In NCI mode, the objective is to convert neutral sample molecules into negative ions suitable for the detector. PFBHA-oximes, which contain 5 fluorine atoms, are ideal for accepting electrons into their atomic orbitals. Upon doing so, they become negative ions, amenable to NCIMS. The polarities of all the MS analyzer voltages are reversed to allow detection of negative ions. Within the ion source of the analyzer, the reagent gas methane is bombarded with high energy electrons emitted from the filament for the production of low energy (thermal) electrons.

$CH_4 + e^-_{(230eV)} \rightarrow CH_3^+ + 2e^-_{(thermal)}$

The thermal electrons are subsequently captured by sample molecules containing electronegative atoms via various mechanisms.

Electron capture

$MX + e^-_{(thermal)} \rightarrow MX^{\cdot-}$ (capture of electron without fragmentation of molecule)

Dissociative electron capture

$MX + e^-_{(thermal)} \rightarrow M + X^-$ (capture of electron but fragmentation of molecule occurs)

No negative reagent gas ions are formed which greatly reduces background noise, resulting in a quieter baseline and lower detection limits achieved. See the designated section of the Total ion chromatogram (TIC) 1 and 2 for comparative illustration of electron ionization mode versus negative chemical ionization mode.

Identification of characteristic ions for each aldehyde PFBHA-oxime were selected based on acquiring mass spectra of each standard in full scan mode. See TIC with mass spectra 3 and 4. Three unique and characteristic ions were observed for each aldehyde:

- $(M)^{\cdot-}$ Electron capture (molecular ion), where M = PFBHA-oxime
- $(M\text{-}HF)^{\cdot-}$ Dissociative electron capture ion
- $(M\text{-}HF\text{-}30)^{\cdot-}$ Dissociative electron capture ion

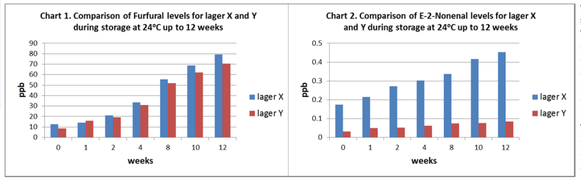
Along with this mass spectral information for each aldehyde peak, their corresponding ion chromatogram retention times were used as the qualifying parameters to identify each aldehyde measured in the beer samples. To verify all ion peaks were the product of the reaction of PFBHA and the target aldehydes only, beer sample was processed on the instrument in measurement mode but without reaction with PFBHA. No peaks were observed with the exception of benzaldehyde, which did exhibit an interference peak, consistent in size, but small relative to benzaldehyde peaks produced by derivatization with PFBHA.

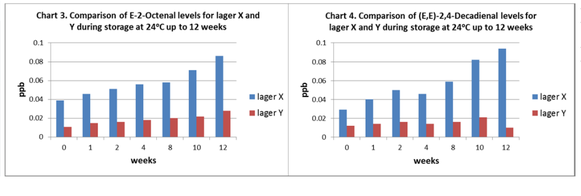
Consequently, all three characteristic ions were monitored in SIM mode, see Table 1. Additionally, the peak area of each aldehyde PFBHA-oxime isomer were summed together and the ratio (aldehyde area/internal standard area) were used for calibration and method validation, see Table 2. Based on the method validation data, all compounds demonstrated good to excellent statistical parameters with the exception of octanal, which demonstrated less than satisfactory parameters.

Application of analytical method

Two different brands of American light lager beers (designated X and Y), were collected and compared by measuring the change in levels of the selected aldehydes. Samples of each brand were stored at 4 ºC for 12 weeks, while other samples were stored at 24 ºC for 12 weeks. Samples of each brand were subsequently removed from the 24 ºC storage at 1, 2, 4, 8, 10 and 12 weeks into aging and transferred to the 4 ºC storage until 12 weeks were reached. At 12 weeks all samples were measured and reported. See Tables 3 and 4, along with Charts 1 – 4 for aldehyde level changes and comparisons of both brands throughout the 12 weeks.

By comparing the data, an obvious difference is revealed, i.e. the relatively large difference in levels of E-2-Octenal, E-2-Nonenal, (E,E)-2,4-Nonadienal, and (E,E)-2,4-Decadienal. With regards to beer flavor, these particular aldehydes possess undesirable flavor notes with very low flavor thresholds and can cumulatively create off flavors such as stale, paper, cardboard, rancid, oily. It's known that these aldehydes originate principally from the malt, and the levels can be exacerbated by brew house practices. To improve the flavor stability, steps can be taken to reduce the levels of these aldehydes by changes in brew house practices and raw materials. Subsequently, the packaged beer can be confidently measured utilizing this improved analytical methodology to reflect these changes.





Conclusion

The combination of various analytical techniques incorporated into this SPME-GC-NCIMS method of analysis produces superior sensitivity and selectivity, enabling unambiguous identification and quantification of the selected aldehydes in beer.

The sensitivity is accomplished by:

- Target aldehydes adsorbed and concentrated onto SPME fiber.
- Saturation of the sample with NaCl to increase the volatility of the target aldehydes within the GC vial.
- Energetically favorable capture of thermal electrons by the fluorine atoms of the aldehyde PFBHA-oxime to produce abundant negative ions.
- Very low background noise, i.e. low signal interference (quieter baseline) inherent to NCI.

The selectivity is accomplished by:

- Selective on-fiber reaction of PFBHA with aldehydes, fiber phase is ideal for adsorption of aldehyde PFBHA-oximes.
- Selective production of negative ions based on sample molecules with electronegative atoms, i.e. fluorine.
- Selected ion monitoring of 3 characteristic fragment ions from each aldehyde PFBHA-oxime.

The statistical method validation data demonstrates success of the methodology:

- Simple sample work up minimizes sample handling.
- Fully automated auto-sampler processing, i.e. temperature equilibration, agitation, adsorption, on-fiber derivatization, injection and fiber bake out.
- Ideal technique of calibration and quantitation based on standard addition and use of an internal standard.
- Ideal application of mass spectrometer mode (NCI) based on derivatized sample molecular composition, i.e. five fluorine atom group of the aldehyde PFBHA-oxime.

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