Analysis of Flavoring Agents in Alcohol Based Flavors

Vanessa R. Kinton; Edward R. Limowski; Tomika L. Moore & Janet M. Scalese

Alcohol and Tobacco Tax and Trade Bureau (TTB), Nonbeverage Products Laboratory (NPL), U. S. Treasury Department, National Laboratory Center, Beltsville, MD 20705

Abstract

Several flavoring agents were analyzed in different alcohol solutions using gas chromatography (GC), mass spectrometry (MS) and flame ionization detection (FID) techniques. The same agents were analyzed using a retention time locking (RTL)[1] method.

Due to differences in alcohol concentration, solvent composition and solids content in the different flavors several sampling techniques and different capillary columns were tested. These included, headspace, solid-phase microextraction and liquid injections.

The use of a deuterated internal standard (IS) was examined for quantitation along with two other internal standards (non-deuterated). The best correlation coefficients were found using a wax column along with three IS and detection with either MS or FID.

Introduction

The Department of the Treasury's Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for regulating the use of ethanol in products manufactured in the United States, and collecting revenue generated from such use. One area that falls within TTB's jurisdiction is the regulation of nonbeverage products under Title 27 of the Code of Federal Regulations[2]. Nonbeverage products are medicines, medicinal preparations, food products, flavors, flavoring extracts, and perfumes which are manufactured using taxpaid distilled spirits, and which are unfit for beverage purposes. To be unfit for beverage purposes a product must be such that an ordinary person would not consume it as an alcoholic beverage.

Manufacturers who use distilled spirits in manufacturing nonbeverage products are eligible for drawback of most of the Federal excise tax paid on those spirits. A manufacturer wishing to receive drawback, in most instances, must first obtain formula approval from TTB. One such way to ensure that products are unfit for beverage purposes and eligible for drawback is for the manufacturer to add specific flavor agents to the ethanol base at levels determined by TTB.

It is TTB's responsibility to verify that these flavors and flavoring extracts are indeed nonbeverage products through examination of formulas and analyses of samples. When this examination reveals that a product is fit for beverage purposes, the formula is disapproved. Thus drawback of tax is denied. Denial of drawback can amount the loss of thousands of dollars for manufacturers.

In the absence of materials that will make the product more palatable (sugar, glycerin, high fructose corn syrup, etc.), TTB has published commonly used favoring agents that when present in the stated amounts will make a product unfit for beverage purposes. Expansive lists of flavoring agents that make a formula unfit at 1% by weight are presented on the lists for artificial and natural flavors. For a list of these compounds see TTB website: http://www.ttb.gov/ssd/drawbacktutorial.shtml.

The goal of this study is to investigate the application of 4 different methodologies for the quantitative determination of 27 commonly used flavoring agents in alcohol based flavors. By developing analytical methods such as the ones described here, we can verify the quantity required of a substance was added to an alcoholic flavor to make it unfit for beverage use.

Experimental:

Table 1. Flavoring agents†

Name	Structure	FEMA #	CAS #
2-Methyl Butyrate		2693	623-42-7
2-Methyl Butyric Acid		2695	116-53-0
Acetoin		2008	513-86-0
Acetophenone		2009	98-86-2
Anisic Aldehyde		2670	123-11-5
Benzaldehyde		2127	100-52-7
Benzyl Alcohol		2137	100-51-6
Butyric Acid		2221	107-92-6
Cinnamic Aldehyde		2286	104-55-2
cis-3-Hexenol		2563	928-96-1
Citral (Geranial)		2303	5392-40-5
Cyclotene		2700	765-70-8
Decanal		2362	112-31-2
Ethyl Benzoate		2422	93-89-0
Ethyl Butyrate		2427	105-54-4
Ethyl Isobutyrate		2428	97-62-1
Ethyl Lactate		2440	687-47-8
Furaneol		3174	3658-77-3
Hexanal		2557	66-25-1
Limonene		2633	5989-27-5
Linalool		2635	78-70-6
Myrcene		2762	123-35-3
Thymol		3066	89-83-8
trans-2-Hexenal		2560	6728-26-3
α-Ionone		2593	127-41-3
β-Ionone		2595	79-77-6
γ-Decalactone		2360	706-14-9

† All the above chemicals were purchased from Sigma Aldrich, food grade.

A. Standards preparation-liquid injections

1% wt/wt individual solutions of each of the 27 chemicals, plus 3 internal standards were prepared in 200 proof ethanol (Pharmo, Connecticut, USA).

100 ppm individual solutions were prepared by diluting the 1% wt/wt individual solutions (described above) with methanol (Fisher Scientific, Fair Lawn, NJ). For liquid injections, 1 µl of these solutions was injected to obtain retention times, target ions and qualifiers.

Three calibration levels, 10 ppm, 50 ppm and 100 ppm of all combined chemicals were prepared using the 1% wt/wt individual solutions, and spiked with the internal standards at 50 ppm. These solutions were placed in 2 mL GC vials.

B. Standards preparation-headspace injections

Retention times, target ions and qualifiers for each compound were obtained by sampling 20 µL of the 1% wt/wt individual solutions (in ethanol, described in A.) placed in 20 mL headspace vials.

Three calibration levels, 0.5%, 1.0% and 1.5% wt/wt of all combined chemicals were prepared using the neat chemicals and spiked with the internal standards at 1% using ethanol as a solvent. 20 uL of these solutions were placed in 20 mL headspace vials.

C. Internal Standards

Three internal standards were used for quantitation:

- Deuterated Ethyl Butyrate-4,4,4,-d3 (C/D/N Isotopes Inc, Pointe-Claire Quebec, Canada).
- 2-Nonanol (CAS # 628-99-9 Sigma Aldrich, 99% purity).
- 3',4'-(Methylenedioxy)acetophenone (CAS # 3162-29-6; Sigma-Aldrich, 98% purity.

D. Solid phase microextraction (SPME)

Preliminary data obtained by sampling 20 µL of neat flavors indicated heavy overload of the GC/MSD system. Therefore, no further analyses were carried out using SPME as a sampling technique.

Table 2. Experimental conditions for the 4 methods used in this study

Method	MSD-Wax	FID-Wax	MSD-HP5 [1]	MSD-Rtx 200
Gas chromatograph	Agilent 6890	Agilent 6890	Agilent 6890	Agilent 6890
Autosampler	Agilent 7673	Gerstel MPS 2	Gerstel MPS 2	EST Analytical (HS 9000)
Autosampler mode	Liquid	Liquid	Liquid	Headspace
Introduction individual compounds	1 µL of 100 ppm	1 µL of 100 ppm	1 µL of 100 ppm	20 µL of 1%
Introduction combined compounds	1 µL- 10, 50 & 100 ppm	1 µL- 10, 50 & 100 ppm	1 µL- 10, 50 & 100 ppm	20 µL- 0.5, 1.0 & 1.5 %
Inlet	250 ºC; 5:1 split	250 ºC; 5:1 split	250 ºC; 5:1 split	250 ºC; 25:1 split
Oven profile	40 ºC 1 min; 3 ºC/min 200 ºC; 1 min	40 ºC 1 min; 3 ºC/min 200 ºC; 1 min	60 ºC; 3 ºC/min 200 ºC; 1 min	40 ºC 1 min; 3 ºC/min 200 ºC; 1 min
Post run	250 ºC for 5 min	250 ºC for 5 min	None	250 ºC for 5 min
Column	Restek Stabilwax	JW DB-Wax	Agilent HP-5	Restek Rtx-200
Column dimensions	30 m × 0.25 mm × 0.25 µm	30 m × 0.32 mm × 0.50 µm	30 m × 0.25 mm × 0.25 µm	30 m × 0.25 mm × 0.50 µm
Mode	Constant flow 1 mL/min	Constant flow 1.6 mL/min	Constant pressure 10.74 psi	Constant flow 1 mL/min
Detector	Agilent MSD 5973	300 ºC, H2 30 mL/min, air 400 mL/min	Agilent MSD 5975	Agilent MSD 5973
Solvent delay	3.90 min	None	2.0 min	2.0 min
Scan	30-300 amu	N/A	30-300 amu	35-195 amu

Table 3. Headspace autosampler-HS 9000 parameters

Constant heat mode	Enable
Vial type	20 mL
Pressure check	Continue
Sample vial temperature	110 °C
Sample equilibrium time	15.0 min
Mixing	Agitate On
Speed	Medium
Post mix stabilization time	5 s
Line temperature	120 °C
Valve/loop temp	115 °C
Standby flow	40 ml/min
Sample mode	1N on Column
Vial pressurization	15.0 psi
Pressure equilibration time	20 s
On column inject time	0.1 s
Multiple headspace extraction	Off
Inject solenoid temperature	100 °C

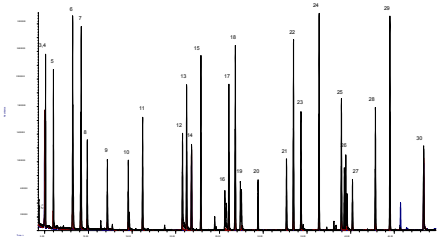

Figure 1. Overlay of TIC of 10, 50 and 100 ppm using the MSD- wax method. **1** Ethyl Isobutyrate; **2** 2-Methyl Butyrate; **3** Deuterated Ethyl Butyrate; **4** Ethyl Butyrate; **5** Hexanal; **6** Myrcene; **7** Limonene; **8** trans-2-Hexenal; **9** Acetoin; **10** Ethyl Lactate; **11** cis-3-Hexenol;**12** Decanal; **13** Benzaldehyde;**14** 2-Nonanol;**15** Linalool; **16** Butyric Acid;**17** Acetophenone;**18** Ethyl Benzoate;**19** 2-Methyl Butyric Acid; **20** Citral (Geranial); **21** Cyclotene; **22** α-Ionone; **23** Benzyl Alcohol; **24** β-Ionone; **25** Anisic Aldehyde; **26** Cinnamic Aldehyde; **27** Furaneol; **28** γ-Decalactone; **29** Thymol; **30** 3',4'-(Methylenedioxy)acetophenone

Table 4. Retention times and correlation coefficients for each method

Compound Name	Target ion*	liq inj-MSD 60' method-Wax column RT (min)	r2	liq inj-MSD RTL method-HP5 column RT (min)	r2	headspace inj-MSD 60' method-Rtx-200 column RT (min)	r2	liq inj-FID 60' method-Wax column RT (min)	r2
Deuterated Ethyl Butyrate (internal standard, IS)	74	5.04	N/A	not found		***	N/A	8.33	N/A
Ethyl Isobutyrate	43	4.18	0.946	2.41	0.911	5.67	0.983	not found	****
2-Methyl Butyrate	74	4.29	0.946	2.14	0.920	4.91	0.984	not found	****
Ethyl Butyrate	71	5.09	0.978	not found		7.00	0.982	8.40	0.999
Hexanal	56	5.98	0.998	2.82	0.946	8.19	0.982	9.82	0.997
Myrcene	93	8.19	0.999	6.65	0.983	9.92	0.983	12.80	0.999
Limonene	68	9.16	1.000	7.86	0.966	10.98	0.985	14.21	0.999
trans-2-Hexenal	41	9.86	1.000	3.55	0.990	***		14.95	0.997
Acetoin	45	12.16	1.000	not found		6.87	0.969	17.61	0.994
Ethyl Lactate	45	14.55	1.000	2.99	0.998	8.82	0.976	20.08	0.990
cis-3-Hexenol	67	16.20	1.000	3.60	0.999	7.66	0.982	21.90	0.999
2-Nonanol (IS)	45	21.79	N/A	10.50	N/A	17.33	N/A	27.47	N/A
Decanal	57	20.76	0.998	14.81	0.995	25.04	1.000	26.55	0.986
Benzaldehyde	106	21.23	1.000	5.84	0.959	15.49	0.989	27.24	0.998
Linalool	71	22.86	1.000	10.43	0.991	16.76	0.999	28.47	0.999
Butyric Acid	60	25.78	0.974	2.64	0.999	***		31.38	0.977
Acetophenone	105	26.06	1.000	9.18	0.958	21.08	0.997	32.15	0.999
Ethyl Benzoate	105	26.80	1.000	13.31	1.000	***		32.83	0.972
2-Methyl Butyric Acid	74	27.37	0.986	not found		***		33.46	1.000
Citral (Geranial)	69	29.39	1.000	not found		29.25	0.989	32.29	0.883
3',4'-(Methylenedioxy)acetophenone (IS)	149	48.36	N/A	24.62	N/A	37.17	N/A	54.37	N/A
Cyclotene	112	32.65	1.000	not found		18.85	0.999	38.59	0.976
α-Ionone	121	33.45	0.998	21.46	0.646	36.57	0.984	39.51	0.999
Benzyl Alcohol	79	34.31	1.000	8.03	0.987	15.96	0.997	40.18	0.998
β-Ionone	177	36.38	0.999	26.99	0.944	***		42.48	0.999
Anisic Aldehyde	135	38.94	1.000	16.86	0.998	30.50	0.996	44.94	1.000
Cinnamic Aldehyde	131	39.44	1.000	17.57	**	31.47	1.000	45.39	0.992
Furaneol	128	39.58	1.000	not found		31.30	0.986	45.12	0.712
γ-Decalactone	85	42.83	0.998	26.14	0.986	42.67	0.275	48.81	0.973
Thymol	135	44.49	0.999	18.58	0.992	24.51	0.618	49.94	0.996

*target ions for MSD methods only (3 qualifier ions/ compound) ** 10 ppm solution not detected ; *** compounds not added, not available at the time of experiment **** compounds eluting under solvent peak.

Conclusions and future work

- From the data collected, excellent correlation coefficients were obtained when a wax column was used for the separation.
- The use of either FID or MSD as detectors provided good linearity.
- Headspace sampling compared to liquid injections provided good linearity but sampling of complex flavors could be cumbersome.
- The use of the retention time locked (RTL) method did not provide the best results due to the high initial temperature (60 ºC) as opposed to our method (40 ºC).
- We intend to use a splitter that will allow splitting the column effluent to two detectors, MSD and FID.
- Flavors made in-house with different matrices (propylene glycol, citric acid and glycerin) will be spiked with the flavoring agents from this study and their recoveries evaluated.

References

1. *David, F. et al. Analysis of Essential Oil Compounds Using Retention Time Locked Methods and Retention Time Databases. Agilent Application Note 5988-6530 EN, May 2002.*
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