

Quantitation of Flavoring Agents in Complex Matrices

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Abstract

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. Nonbeverage products are medicines, medicinal preparations, food products, flavors, flavoring extracts, and perfumes which are manufactured using tax-paid distilled spirits, and which are unfit for beverage purposes. One such way to ensure that products are unfit for beverage purposes is for the manufacturer to add specific flavor agents to the ethanol base at levels determined by TTB.

A quantitative method was developed to determine the concentration of various flavoring agents commonly used in alcohol based flavors using gas chromatography (GC) coupled to both a mass spectrometer (MS) and a flame ionization detector (FID). Analytes studied include anisyl alcohol, benzyl alcohol, cinnamyl alcohol, ethyl benzoate, ethyl butyrate, ethyl isobutyrate, cis-3-hexenol, limonene, linalool, menthol, 2-methyl butyrate, myrcene, and thymol. Deuterated ethyl butyrate was used as an internal standard (IS) as well as two non-deuterated internal standards: 2-nonanol and 3',4'-(Methylenedioxy)-acetophenone. Accuracy and precision were also determined, with recoveries above 90% for most of the compounds and the relative standard deviations less than 4%. The method was successfully applied to determine chemical concentrations in fifteen different flavor concentrates.

Introduction

Domestic 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.

It is TTB's responsibility to verify that these flavors and flavoring extracts are indeed nonbeverage products through examination of formulas and analysis 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 result in the loss of thousands of dollars for manufacturers.

The goal of this study is the quantitative determination of 13 commonly used flavoring agents in alcohol based flavors. We investigated the use of a microfluidic two-way splitter. The splitter divides the effluent from the chromatographic column between two different detectors, a mass selective detector (MSD) and a flame ionization detector (FID). The quantitation of 2 compounds that elute before or close to the MSD solvent delay was investigated using the FID signal. This splitter also allows backflush which prevents high-boiling contaminants from entering the detectors by reversing the column flow in the post run.

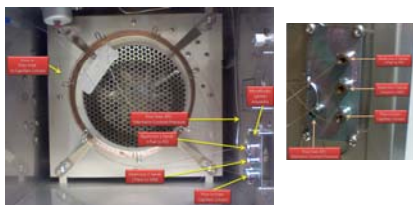


Figure 1. Two-way splitter for GC/MS/FID. Effluent split between 2 detectors and backflush allowed

Table 1. Flavoring agents[†]

Name	Structure	FEMA #	CAS #	% RSD [‡]	Correlation coeff (r ²)
Anisyl Alcohol	<chem>Oc1ccc(OCC)cc1</chem>	2099	105-13-5	0.66%	0.998
Benzyl Alcohol	<chem>Oc1ccc(OCC)cc1</chem>	2137	100-51-6	0.57%	1.000
Cinnamyl Alcohol	<chem>Oc1ccc(OCC)cc1</chem>	2294	104-04-6	0.68%	0.999
cis-3-Hexenol	<chem>OCC=CCCC</chem>	2563	928-96-1	0.65%	0.999
Ethyl Benzoate	<chem>CCOC(=O)c1ccccc1</chem>	2422	93-89-0	0.74%	1.000
Ethyl Butyrate	<chem>CCOC(=O)CCC</chem>	2427	105-54-4	0.85%	0.999
Ethyl Isobutyrate	<chem>CCOC(=O)CC(C)C</chem>	2428	97-62-1	1.80%	0.978
Limonene	<chem>C1=CC=CC=C1</chem>	2633	5989-27-5	1.02%	1.000
Linalool	<chem>C1=CC=CC=C1</chem>	2635	78-70-6	0.86%	1.000
Menthol	<chem>C1=CC=CC=C1</chem>	2665	1490-04-6	0.72%	1.000
2-Methyl butyrate	<chem>CCOC(=O)CC(C)C</chem>	2693	623-42-7	3.90%	0.994
Myrcene	<chem>C1=CC=CC=C1</chem>	2762	123-35-3	0.85%	1.000
Thymol	<chem>C1=CC=CC=C1</chem>	3066	89-83-8	0.57%	1.000
Deuterated Ethyl Butyrate-4,4,4-d3	<chem>C1=CC=CC=C1</chem>	113435-99-7	ISTD	NA	NA
2-Nonanol	<chem>C1=CC=CC=C1</chem>	3315	628-99-9	ISTD	NA
3',4'-(Methylenedioxy)-acetophenone	<chem>C1=CC=CC=C1</chem>	3162-29-6	ISTD	NA	NA

[†] All chemicals purchased from Sigma Aldrich (St. Louis, MO) except Deuterated Ethyl Butyrate-4,4,4-d3 (C/D/N Isotopes Inc, Pointe-Claire Quebec, Canada).

[‡] RSD calculated with MSD data only, except for Ethyl Isobutyrate and 2-Methyl Butyrate (FID), n=7

Table 2. Experimental conditions

Gas chromatograph	Agilent 6890
Autosampler	Gerstel MPS 2
Autosampler mode	Liquid
Injection volume	1 µL
Inlet	250 °C; 5:1 split
Oven profile	40 °C 2'; 3 °C/min 240 °C; 1.0'
Post run	240 °C for 5 min
Backflush inlet flow	0.1 mL/min
Backflush pressure splitter	60 PSI
Column	Phenomenex ZB-WAXplus
Column dimensions	30 m x 0.25 mm x 0.25 µm
Pressure at splitter	20 PSI
Restrictor 1 (to FID)	0.18 µm ID; 2.128 m
Restrictor 2 (to MSD)	0.18 µm ID; 2.886 m
Mode	Constant flow 1 mL/min
MSD Detector	Agilent MSD 5975 Inert
FID Temperature	300 °C
FID Detector flows	H ₂ 30 mL/min, air 400 mL/min
Solvent delay-(MSD)	4.67 min
Scan-(MSD)	30-300 amu

A. Standards Preparation

Each analyte in Table 1 was diluted with 200 Proof ethanol to a concentration of 1% (wt/wt). A stock solution containing each analyte at 750 ppm was created by diluting the 13 individual 1% solutions with methanol. Seven calibration levels (5, 10, 25, 50, 75, 100 and 125 ppm) were prepared by diluting the stock solution with methanol. Retention times, target ions and qualifiers had been previously determined. (1)

B. Internal Standards Preparation

A stock solution containing three internal standards at 300 ppm each was prepared by diluting Deuterated Ethyl Butyrate-4,4,4-d3; 2-Nonanol; and 3',4'-(Methylenedioxy)acetophenone with methanol.

C. Preparation of solutions for chromatographic analysis

1 ml of each of calibration level standard solution or sample solution was pipetted into a GC vial, followed by the addition of 200 µL Internal Standard Stock Solution. Complex Matrices were first diluted 1:100 (wt/wt) with methanol before being pipetted.

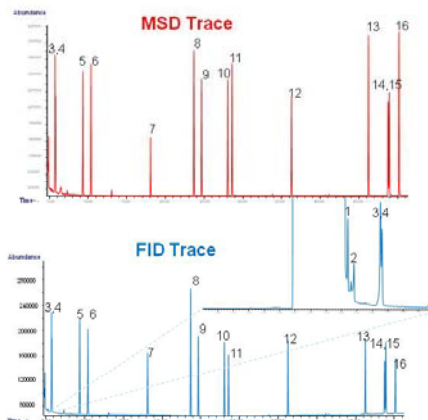


Figure 2. Total Ion Chromatogram (TIC, red trace) and chromatogram (FID, blue trace) for 125 ppm standard, 1 µl single injection. 1 Ethyl Isobutyrate; 2 2-Methyl Butyrate; 3 Deuterated Ethyl Butyrate (IS); 4 Ethyl Butyrate; 5 Myrcene; 6 Limonene; 7 cis-3-Hexenol; 8 2-Nonanol (IS); 9 Linalool; 10 Menthol; 11 Ethyl Benzoate 12 Benzyl Alcohol; 13 Thymol; 14 Anisyl Alcohol; 15 Cinnamyl Alcohol; 16 3',4'-(Methylenedioxy)-acetophenone (IS)

Table 3. Recoveries for compounds in different matrices*

Matrix	Compound #1	Compound #2	Compound #3	Compound #4	Compound #5	Compound #6	Compound #7	Compound #8	Compound #9	Compound #10	Compound #11	Compound #12	Compound #13	Compound #14	Compound #15
Matrix #1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Matrix #15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Matrices 1-12 represent industry supplied test products where the amounts listed in the present column have been disclosed by the flavor manufacturer; Matrices 13-15 represent TTB's in-house made products

Conclusions and future work

- Excellent sensitivity and reproducibility was obtained for all 13 flavor compounds in both detectors with 1 µL injections and splitting 2 parts to MSD and 1 part to FID.
- The use of an FID presented the benefit of detection of compounds close and before elution of the solvent (before solvent delay of MSD).
- All 13 compounds were detected in most of the matrices with recoveries above 90% with the exception of anisyl alcohol in Matrix # 4 and linalool in Matrix # 9.
- More studies need to be conducted to determine the shelf life of anisyl alcohol and linalool in different matrices.
- The use of deuterated ethyl butyrate as an internal standard provided excellent linearity for ethyl butyrate quantitation using the MSD.
- The use of backflushing during the post-run time eliminated carry-over issues.

References

- Kinton, V. R., Limowski, E. R., Moore, T. L., & Scalese, J. M. (2008). Analysis of Flavoring Agents in Alcohol Based Flavors. PittCon Conference. New Orleans: LA.

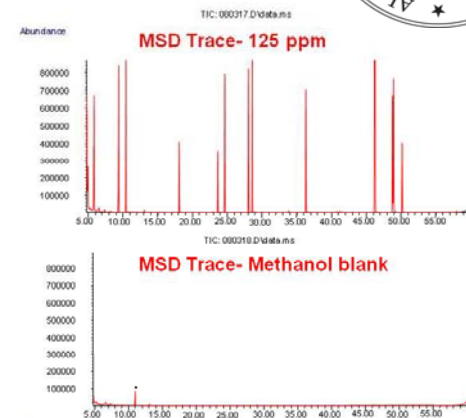


Figure 3. Total Ion Chromatograms. Top chromatogram is 125 ppm standard, the bottom chromatogram is a methanol blank run after the standard. *Peak not identified with a good match by the MSD library.

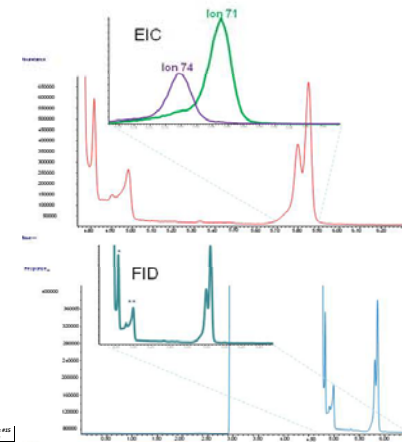


Figure 4. Chromatograms of early eluters. Top chromatogram (red trace) shows the MSD signal of 125 ppm standard with extracted ion chromatogram for deuterated ethyl butyrate (ion 74, purple trace) and ethyl butyrate (ion 71, green trace); bottom chromatogram (blue trace) shows the FID signal from 0 to 6 minutes; * Ethyl isobutyrate and ** Methyl butyrate