Complete Separation and Quantitation of Fusel Oils by Capillary GC

Application

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Abstract

Fusel oils are of great importance in the alcoholic beverages industry, since they affect the flavor and aroma of the beverage. Thus, their accurate quantitation is essential in assuring consistent quality of alcoholic beverages. Traditionally, packed GC columns were used for this analysis. Capillary columns do not offer the specific stationary phases that were available, and necessary, for packed columns. This application note describes the successful separation of all fusel oils, including other compounds typically found in alcoholic beverages, in a single run on a common stationary phase. In particular, baseline separation of methanol/acetaldehyde and isoamyl/active amyl alcohol was achieved. Examples include standards as well as real samples of fermented and distilled spirits, with quantitative data provided for a number of spirits.

Introduction

Fusel oils are small alcohols that typically include 1-propanol, 1-butanol, isobutanol, as well as isoamyl alcohol (3-methyl-1-butanol) and active amyl alcohol (2-methyl-1-butanol). They are formed through transamination of carbohydrates by amino acids:

Glucose → α-Keto-Acids → Decarboxylation and Reduction → Alcohols

Amino acid	α-Keto acid	Fusel alcohol
Leucine	α-Isocaproate	3-Methyl-1-butanol
Isoleucine	α-Keto- β-methyl valerate	2-Methyl-1-butanol
Valine	α-Ketoisovalerate	2-Methyl-1-butanol
Threonine	α-Ketobutyrate	1-Propanol
2-Phenylalanine	α-Phenyl-2-ketopropionate	2-Phenylethanol

Fusel oils are important flavor constituents in alcoholic beverages. As a group they contribute "fusel/diesel-like" character. Individual aromas range from "banana" (isoamyl acetate) to "rose-like" (phenylethanol). At high levels they are considered undesirable; however, low to moderate levels contribute to the complexity of the beverage. Analysis of fusel oils is used to monitor distillation processes, malfunctions in distillation apparatuses, as well as confirming fermentation substrate authenticity. Thus, their accurate quantitation is essential in assuring consistent quality of alcoholic beverages.

Separation of all fusel oils and the low boiling components on a single capillary column can be problematic. In particular, baseline separation of isoamyl alcohol (3-methyl-1-butanol) and active amyl alcohol (2-methyl-1-butanol) present some challenges. Traditionally, packed GC columns were used for this analysis. Capillary columns do not offer the plethora of different stationary phases that were available, and necessary, for packed columns to accomplish specific separations. In general, isoamyl- and active amyl alcohol will not

separate on polar columns typically used for separation of alcohols (Figure 1). By contrast, their separation is easily achieved on a DB-MTBE (Figure 2), one of the least polar columns with respect to polar analytes. Unfortunately, resolution of other analytes typically found in alcoholic beverages, such as methanol and acetaldehyde, behaves in just the opposite manner (Figures 1 and 2). It would then stand to reason that a compromise of the two, that is, a mid-polarity column, should separate all 4 compounds. Complete baseline resolution of all fusel oils in about 13 minutes (Figure 3) was achieved with a DB-624.

Materials and Methods

Samples

All alcoholic beverage samples were purchased from commercial sources.

Standards

All standards were purchased commercially and were of the highest grade available. A list of analytes is given in Table 1. Standard solutions containing 0, 10, 50, 100, 250, and 500 ppm (vol/vol) of each analyte were prepared in 20% (vol/vol) aqueous ethanol.

Internal Standards

Two internal standards were evaluated, 2-propanol and 3-pentanol. Separate solution of each internal standard were prepared by diluting 25 mL of the neat IS component to a final volume of 250 mL with absolute ethanol.

GC Conditions

GC:	Agilent 6890 Gas Chromatograph;
	ChemStation Software
Autosampler:	Gerstel Model MPS2
Column:	DB 624 60 m × 0.25 mm id × 1.4 µm
Carrier gas:	Helium at 35 cm/s at 40 °C (1.9 mL/min)
Oven:	40 °C for 5 min;
	10 °C/min to 250 °C
Injector:	250 °C
	Splitless; Split vent open at 5.00 min
	at 17.7 mL/min
Detector:	Agilent 5973 MSD; Interface 280 °C

SPME Fibers

Polyacrylate 85um (Supelco, Inc.)
Carbowax-Divinylbenzene 65 um (Supelco, Inc.)

Sampling Conditions

Beverage samples were diluted to 20% ethanol with deionized water prior to sampling. A 10 mL aliquot of standard or diluted sample was placed into a 20 mL headspace vial and 100 mL of IS was added prior to closing with a PTFE lined crimp top seal. The SPME fiber was inserted into the headspace and allowed to equilibrate at 25 °C for 30 min. The fiber was then inserted into the GC inlet and desorbed at 250 °C for 5 min.

Statistical Analyses

All samples and standards were analyzed a minimum of two times. Means, standard deviations, and relative standard deviations (%RSD) were calculated where appropriate. Linear calibration curves were prepared and used for quantitation of unknown samples.

Results and Discussion

SPME Fiber Choice

Two different fibers were evaluated for their response to the analytes monitored. Peak areas for early eluting analytes (methanol, acetaldehyde, ethanol, 2-propanol) were approximately two times greater using the carbowax-divinylbenzene fiber compared to the polyacrylate fiber. Response of the later eluting analytes (amyl alcohols, 1-hexanol, phenylethanol) was slightly higher using the polyacrylate fiber compared to the Carbowax fiber. The Carbowax fiber was used for all quantitative analyses.

Internal Standards

Both 2-propanol and 3-pentanol were evaluated for use as internal standards. The retention time for 2-propanol was close to that of ethanol. 3-Pentanol was well resolved from all other analytes (Table 1; Figure 3). Standard curves calculated using peak area ratios for both internal standards gave similar results in terms of linearity and reproducibility. 3-Pentanol was chosen as the internal standard for quantitation in this study.

Standard Curves

Table 1. Standard Curve Equations. Analyte Concentration vs. Peak Area Ratio for Analyte and Internal Standard

Analyte	Retention time (min)	Range (ppm)	Equation	r^2
Acetaldehyde	4.6	10–500	Y = 6x 10–5(x) + 0.0048	0.9954
Methanol	4.9	10–500	Y = 0.0001(x) – 0.0033	0.9938
Acetone	7.3	10–500	Y = 0.0007(x) – 0.0006	0.9994
1-Propanol	9.7	10–500	Y = 0.0013(x) + 0.05	0.9637
Ethylacetate	10.7	10–500	Y = 0.0049(x) + 0.0412	0.9983
2-Methyl-1-propanol	11.9	10–500	Y = 0.0037(x) + 0.0728	0.9716
1-Butanol	13.1	10–500	Y = 0.0038(x) + 0.0184	0.9772
3-Pentanol (IS)	14.0			
3-Methyl-1-butanol	15.2	10–500	Y = 0.0094(x) + 0.0607	0.9994
2-Methyl-1-butanol	15.3	10–500	Y = 0.013(x) + 0.2127	0.9928
1-Hexanol	18.5	10–500	Y = 0.029(x) + 0.7569	0.9907
Phenyethanol	24.5	10–500	Y = 0.0497(x) + 1.5034	0.9918

All standards prepared in 20% (vol/vol) ethanol.

Precision

Table 2. Relative Sstandard Deviation (%) for Replicate Analyses (n>3) at 5 Concentrations on Different Days

Analyte	500 ppm	250 ppm	100 ppm	50 ppm	10 ppm
Acetaldehyde	9.5	15.9	7.3	14.6	22.0
Methanol	6.9	13.2	20.9	4.6	18.6
Acetone	8.2	15.4	8.4	15.1	11.9
1-Propanol	13.5	11.7	15.7	12.2	22.3
Ethylacetate	5.3	12.4	3.3	11.7	10.2
2-methyl-1-propanol	4.6	3.0	9.6	6.6	10.8
1-Butanol	2.4	4.0	8.8	12.5	17.6
3-Methyl-1-butanol	7.1	3.0	7.8	10.3	11.2
2-Methyl-1-butanol	4.6	4.5	8.4	8.1	12.1
1-Hexanol	7.2	8.3	13.4	7.0	10.9
Phenylethanol	6.7	10.9	11.0	9.6	15.9

Note that %RSD is slightly greater at the low concentrations, particularly for the low molecular weight analytes. Volatilization from the standards between days and during analysis can occur and further work is needed to minimize this variation at low concentrations.

Analysis of Alcoholic Beverages

Chromatograms of selected alcoholic beverages are presented in Figures 4–6. Quantitative results for individual analytes are given in Table 3. Analyte concentrations are typical of those reported for these beverages (Nykanen, 1986).

Table 3. Fusel Alcohol, Methanol, Acetaldehyde, Acetone, and Ethylacetate Concentrations (ppm) in Commercial Beverages*

Analyte	Brandy A (80 proof)	Brandy B (80 proof)	Vodka (80 proof)	Gin (80 proof)	Scotch (80 proof)
Acetaldehyde	14.7	58.9	<10	85.6	<10
Methanol	<10	<10	<10	<10	<10
Acetone	<10	<10	<10	<10	<10
1-Propanol	54.4	74.5	<10	<10	107.9
Ethylacetate	78.2	44.4	<10	<10	74.3
2-methyl-1-propanol	142.8	192.4	<10	<10	261.4
1-Butanol	<10	<10	<10	<10	<10
3-Methyl-1-butanol	<10	<10	<10	<10	<10
2-Methyl-1-butanol	571.9	764.8	<10	<10	87.2
1-Hexanol	153.3	215.9	<10	<10	40.9
Phenylethanol	<10	<10	<10	<10	<10

*Concentrations reported in ppm (vol/vol).

Summary

Resolution of all analytes of interest was achieved using a mid-polarity stationary phase (DB-624). Further optimization of column length and film thickness provided baseline resolution in under 30 minutes for all components quantified. Use of SPME headspace sampling combined with either FID or MSD detection was easy, rapid (~30 min per sample) and readily automated. Volatility of the low molecular weight analytes requires careful sample preparation and temperature control to ensure reproducible results between days. This technique holds promise for the routine analysis of alcoholic beverages in order to monitor distillation processes, malfunctions in still operations, and fermentation substrate authenticity.

References

L. Nykanen, Am. J. Enol. Vitic., 37, 84 (1986).

Column: **DB-WAX**
30 m × 0.25 mm id, 0.25 µm
P/N: **122-7032**
Carrier: Hydrogen at 45 cm/s
Oven: 30 °C for 5 min
30–80 °C at 1°/min
Injector: Split 1:50, 1.5 µL
Detector: FID, Nitrogen makeup gas
at 30 mL/min

1 Acetaldehyde
2 Ethyl acetate
3 Methanol
4 Ethanol
5 1-Propanol
6 3-Pentanol
7 2-Methyl-1-butanol
8 3-Methyl-1-butanol

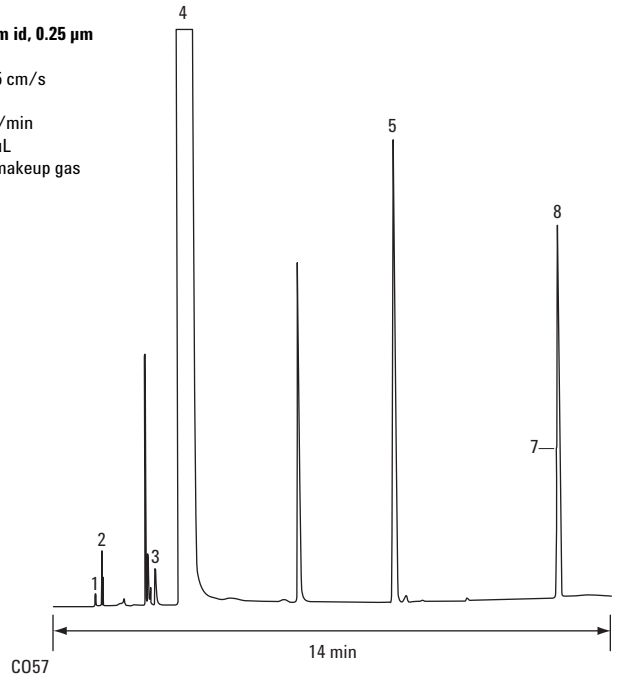

Figure 1. Scotch whiskey.

Column: **DB-MTBE**
30 m × .45 mm id × 2.55 µm
Inlet: 250 °C, split
FID: 300 °C
Carrier: H2, 50 cm/s
Oven: 40 °C for 5 min.
10 °C/min to 250 °C

1 Acetaldehyde
2 Methanol
3 3-Methyl-butanol (isoamyl alcohol)
4 2-Methyl-butanol (active amyl alcohol)

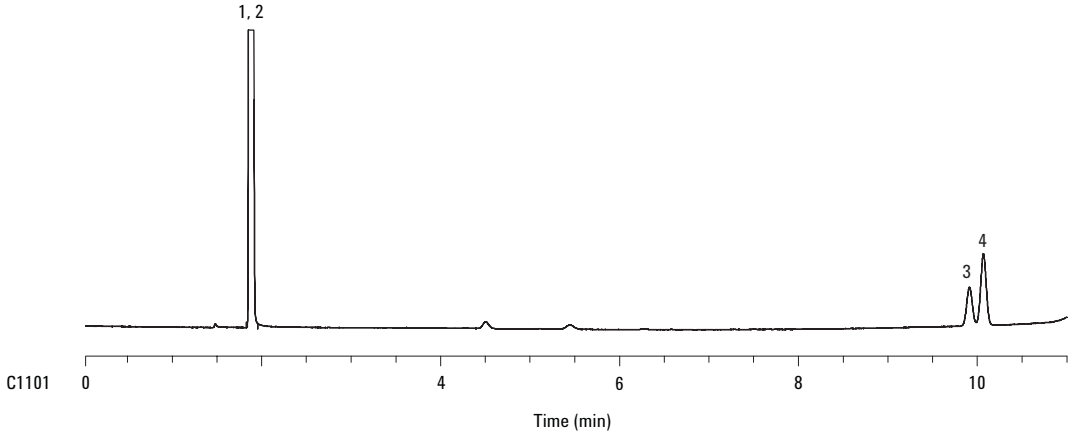

Figure 2. Fusel oil simple standard.

Column:	**DB-624**	1	Acetaldehyde	8	1-Butanol
	60 m × .25 mm id × 1.4 µm	2	Methanol	9	3-Pentanol (IS)
Inlet:	250 °C, split	3	Ethanol	10	3-Methyl-butanol (isoamyl alcohol)
FID:	300 °C	4	Acetone	11	2-Methyl-butanol (active amyl alcohol)
Carrier:	H2, 50 cm/s	5	1-Propanol	12	Hexanol
Oven:	40 °C for 5 min.	6	Ethyl acetate	13	Phenylethanol
	10 °C/min to 250 °C	7	Isobutanol		

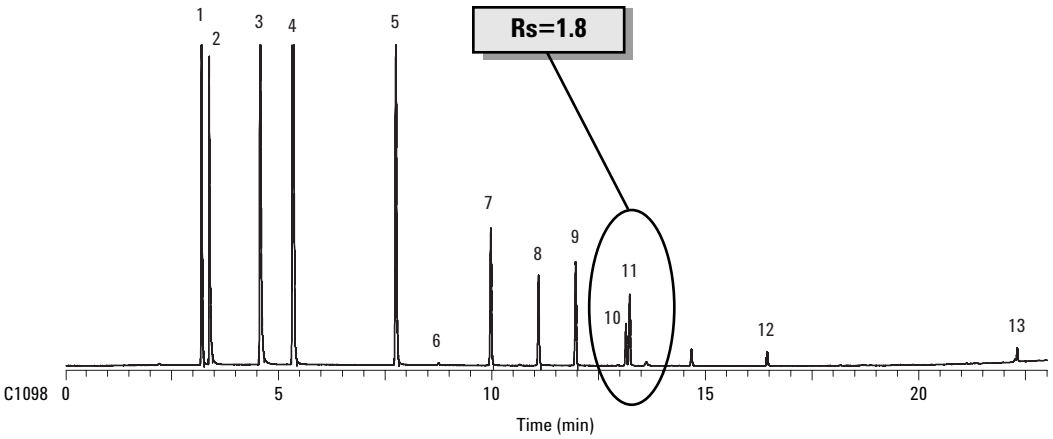

Figure 3. Fusel oil standard.

Column:	**DB-624**	1	Acetaldehyde	8	1-Butanol
	60 m × .25 mm id × 1.4 µm	2	Methanol	9	3-Pentanol (IS)
Inlet:	250 °C, split	3	Ethanol	10	3-Methyl-butanol (isoamyl alcohol)
FID:	300 °C	4	Acetone	11	2-Methyl-butanol (active amyl alcohol)
Carrier:	H2, 50 cm/s	5	1-Propanol	12	Hexanol
Oven:	40 °C for 5 min.	6	Ethyl acetate	13	Phenylethanol
	10 °C/min to 250 °C	7	Isobutanol	14	Acetic acid

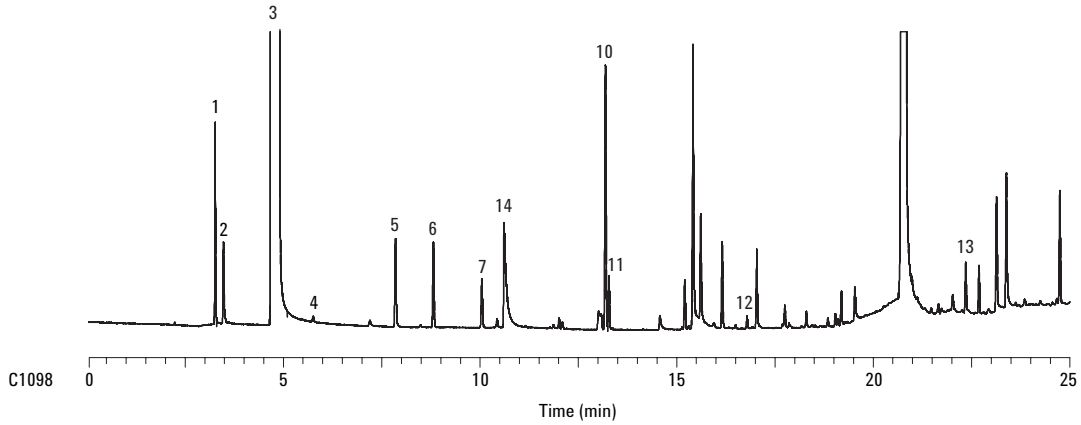

Figure 4. Sherry sample.

Column:	**DB-624**
	60 m × .25 mm id × 1.4 µm
Inlet:	250 °C, split
FID:	300 °C
Carrier:	H2, 50 cm/s
Oven:	40 °C for 5 min.
	10 °C/min to 250 °C

1	Acetaldehyde	8	1-Butanol
2	Methanol	9	3-Pentanol (IS)
3	Ethanol	10	3-Methyl-butanol (isoamyl alcohol)
4	Acetone	11	2-Methyl-butanol (active amyl alcohol)
5	1-Propanol	12	Hexanol
6	Ethyl acetate	13	Phenylethanol
7	Isobutanol		

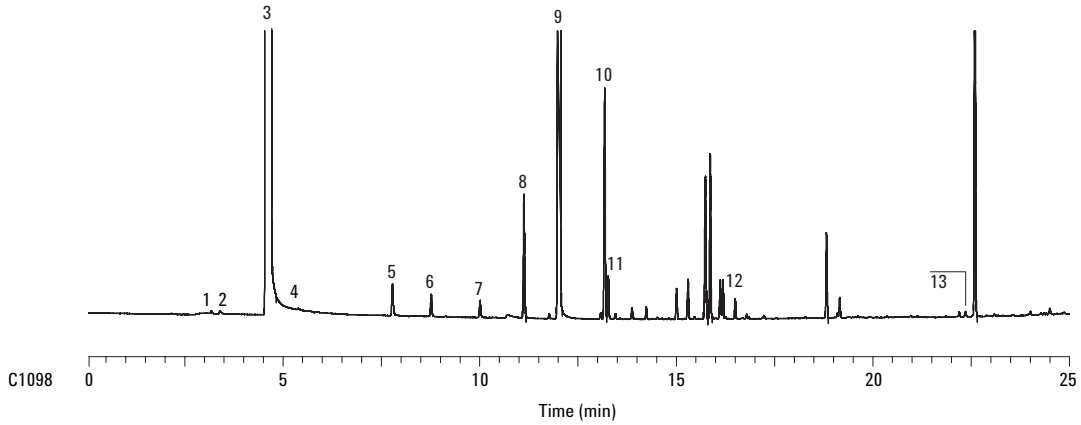

Figure 5. Brandy sample (SPME).

Column:	**DB-624**
	60 m × .25 mm id × 1.4 µm
Inlet:	250 °C, split
FID:	300 °C
Carrier:	H2, 50 cm/s
Oven:	40 °C for 5 min.
	10 °C/min to 250 °C

1	Acetaldehyde	8	1-Butanol
2	Methanol	9	3-Pentanol (IS)
3	Ethanol	10	3-Methyl-butanol (isoamyl alcohol)
4	Acetone	11	2-Methyl-butanol (active amyl alcohol)
5	1-Propanol	12	Hexanol
6	Ethyl acetate	13	Phenylethanol
7	Isobutanol		

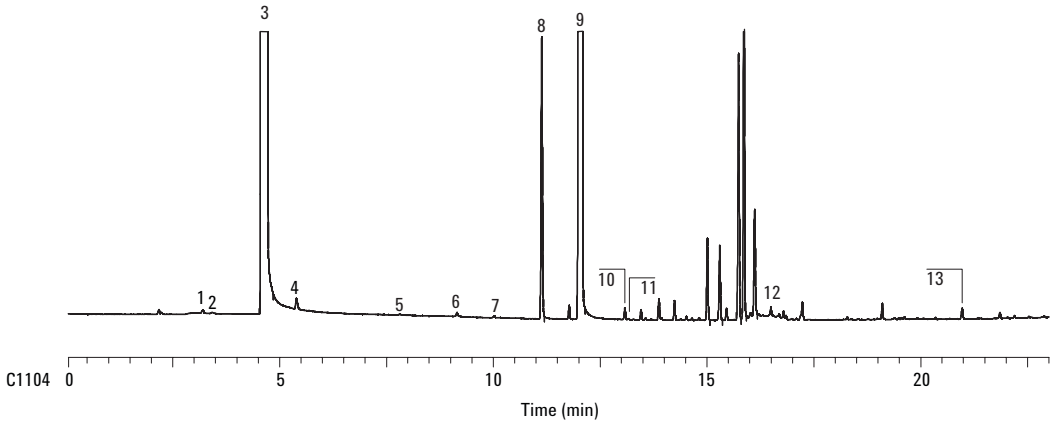

Figure 6. Vodka sample (SPME).

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