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Short Chain Fatty Acid Headspace Analysis Optimization for GC Using Alcohol Esterification

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Introduction

Short chain fatty acids can be difficult to analyze by GC due to their highly acidic and polar nature affecting activity within the GC system, as well as affinity for polar solvents such as water when performing headspace analysis. Esterification is a common solution to reducing the activity of these compounds and improving detection. However, these derivatizations typically involve multiple steps in the sample preparation. In-vial derivatization using headspace analysis is investigated as a easy single step analysis method to address these issues.

In addition to the issues caused by polarity and activity, if FID detection is used, short chain acids like formic acid give exhibit low sensitivity due to the low number of C-H bonds. Esterification with alcohols is a technique used to reduce polarity and activity, and increase sensitivity. Typically, these derivatizations involve several sample preparation steps including: reaction, neutralization, extraction, and drying prior to GC analysis. Performing this in-vial derivatization by headspace analysis eliminates the need for these multiple sample preparation steps by allowing the reaction to occur in the headspace vial, then injecting directly from the headspace. In addition to the reduced sample preparation, the derivatization by headspace aids in improving the partition ratio of the analyte from the liquid phase to the headspace by decreasing the polarity of the analytes.

Within this technique there are several options in selecting the appropriate alcohol and ester to optimize analysis of the compounds of interest for different applications. Several alcohol / fatty acid combinations will be used with a USP-467 based GC method on a Shimadzu GCMS-QP2010 Ultra with an AOC-5000 Plus Autosampler to show suitability of headspace analyses of formic and acetic acid using different esterification reagents.

Experimental

Instrumentation

- Shimadzu GCMS-QP2010 Ultra
- AOC-5000 Plus Autosampler
- GC-MS Solution Workstation

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Table 1: Instrument Operating Conditions and Method Parameters

Head Space	AOC-5000 Plus
Sample	5-mL sample volume 20-mL headspace vial
Equilibration	See Table 2
Syringe Temperature	150 °C
Gas Chromatograph	**GCMS-QP2010 Ultra**
Injection	Split injection at 150 °C with 10:1 split ratio.
Column	ZB-624, 30 m x 0.25 mm x 1.4 µm Helium carrier gas Constant linear velocity, 35 cm/second
Oven Program	40 °C, hold 20 min. Ramp 10 °C/min to 240 °C, hold 20 min.
Detector	**GCMS-QP2010 Ultra**
Operating Mode	Scan mode 29-450 m/z
Ion Source	200 °C, EI mode
Solvent Cut Time	2.0 min
MS Interface	240 °C

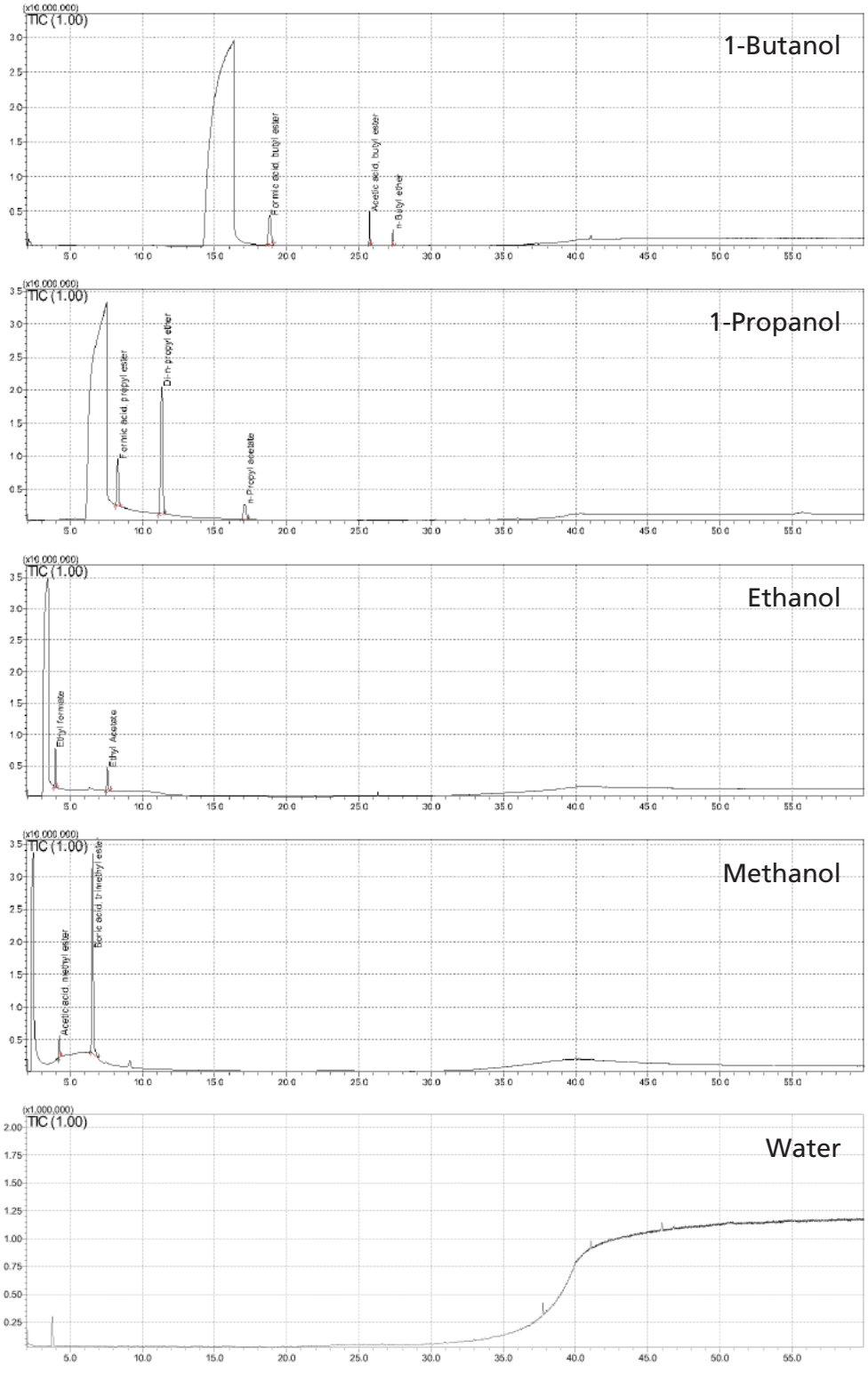
Sample Preparation

10,000 ppm standard solutions of formic and acetic acid are made in the alcohol that corresponds with the derivatization reagent to be used. 125 µL of this standard is then brought to 5 mL in a headspace vial using the appropriate BF3/alcohol derivatization reagent to give a final concentration of 250 ppm. An additional sample of 500 ppm of the acids in water was also analyzed.

Table 2: Sample concentrations and headspace conditions

Reagent	Formic and acetic acid concentration (ppm)	Incubation temperature (°C)	Incubation time
10% BF3 in methanol	250	55	10 min
10% BF3 in ethanol	250	68	
14% BF3 in 1-propanol	250	90	
10% BF3 in 1-butanol	250	90	
Water	500	90	

Results and Discussion



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Esterification with n-butanol provides the best results, with sharp chromatographic peaks and good separation of the formic acid butyl ester from the butanol solvent peak. The propanol and ethanol also provide good sharp peaks for their respective formic and acetic acid esters, however the formic acid esters move closer to the solvent peak and is affected by the end of the residual solvent peak. It is possible that this could be alleviated with a higher split flow to help sweep additional solvent from the injection port and reduce the solvent peak, however this will ultimately affect the detection limit of the target esters as well. Methanol does not work as well, particularly for formic acid where the formic acid methyl ester co-elutes with the solvent peak.

While it appears that the butanol reagent may be the best for these particular analytes, the other alcohols may provide a good alternative particularly if the solvent peak or one of the esters co-elutes with another compound of interest in an analysis.

The organic acids in water are not detected in this analysis even with a higher concentration, likely due to the combination of the low partition coefficient in water as well as the activity considerations caused by the unmasked acid group. An acid modified Wax type column is sometimes used to help with this issue, but ultimately esterification helps prevent problems caused by activity that may occur over time especially in the injection port or at the front of the analytical column.

This technique should work well for short chain fatty acid analysis in solid samples, as well as other non-aqueous samples. Water tends prevent this esterification reaction from going to completion, therefore a water scavenger can be added to help remove water and improve the completion of the derivatization reaction. Addition of a salt may also help improve the detection limit by improving the partition coefficient of the analytes from the liquid phase to the gas phase.

Summary and Conclusions

In-vial derivatization using headspace is shown to be an effective way to analyze short chain fatty acids in non-aqueous samples by reducing chromatographic activity caused by the acid group of the analytes. Also, this technique allows direct headspace analysis by eliminating the need for multiple derivatization steps by performing the reaction in the headspace vial and sampling the analytes directly from the headspace. Multiple esterification alcohol options are available and n-butanol appears to produce the best results, but n-propanol and ethanol are viable options depending upon the other compounds of interest in the sample.

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