

## Gas Chromatography/ Mass Spectrometry

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## Analysis of Ethyl Carbamate Using GC/MS

### Introduction

Ethyl carbamate is a naturally occurring ester formed in fermented foodstuffs such as bread and in beverages such as wine. Ethyl carbamate is formed from the reaction of urea with ethanol. The yeast and/or lactic acid bacteria added to catalyze the fermentation process generates urea by metabolism of amino acids such as arginine or citrulline<sup>1</sup>. The reaction is between urea that occurs naturally (as a yeast metabolite of arginine which is present in grape skins) and the ethanol generated during fermentation. An alternative source of urea is the citrulline amino acid which can be formed by lactic acid bacteria during wine production<sup>1</sup>. Animals injected with ethyl carbamate have been shown to develop cancer, and has resulted in the compound being classified as a Group 2A carcinogen<sup>2</sup>, "probably carcinogenic to humans." The U.S. Food and Drug Administration (FDA) and the European Food Safety Agency<sup>2</sup> (EFSA) have both reported on the presence of ethyl carbamate in various foodstuffs and have provided guidelines for maximum allowable limits, with the FDA advice going so far as to provide guidance on nitrogen fertilization to limit the naturally occurring ethyl carbamate. The EFSA report gave details on ethyl carbamate occurrence in a selection of alcoholic beverages including spirits made from "stone fruits" and "fruits other than stone fruits" and reported that in 95% of cases, ethyl carbamate was below the recommended 1 mg/L value for spirits made from fruits other than stone fruits and 100% below the recommended level for spirits from stone fruits.

The agencies also suggest steps to minimize formation of ethyl carbamate, such as minimizing exposure to heat and light and limiting storage time of finished product.

The method selected for this work is the AOAC International 944.07 method for Gas Chromatography and Mass Spectrometry detection (GC/MS). The method calls for calibration of ethyl carbamate with a propyl carbamate internal standard. As part of this experiment, butyl carbamate was investigated as an alternative internal standard.

## Experimental

A PerkinElmer Clarus® 580 GC was used for the separation of ethyl carbamate and its internal standards with a PerkinElmer Clarus SQ 8 S MS detector controlled through Waters® Empower® 3 software. A custom Mass Spectral library was created in the Waters® Empower® 3 software through the analysis of individual component standards. The MS experiment was conducted in SIFI (Selected Ion Full Ion) mode to obtain both scan and SIM (Selected Ion Monitoring) data simultaneously. The scan data provides library-searchable spectra for confirmation, and SIM data improves detection limits.

The GC method and MS method parameters used for the study are described in Tables 1 and 2. Figure 1 shows the MS method with the multiple channels created in Waters® Empower® 3 software and Figure 2 shows the subsequent channels associated with the method set. The generation of the Method Set enables multiple SIR channels to be created at the time of analysis.

Table 1. GC method parameters.

Gas Chromatograph	Clarus 580 GC	
Column	Elite-35 (30 m x 0.25 mm x 0.25 µm)	
Injector Type	Programmable Split Splitless	
Injector Temperature	180°C	
Injector Liner	2 mm i.d. glass (no glass wool)	
Carrier Gas	Helium @ 0.8 mL/min Pressure Pulse Injection 3 mL/min.	
Split Flow	No Split	
GC Oven Program	Temperature	
	<b>Rate</b>	<b>Set Point</b>
		<b>Hold Time</b>
	40°C	0.75
	20°C/min.	60°C
	3°C/min.	150°C
	45°C/min.	220°C
		8.7

Table 2. Mass Spectrometer method parameters.

Mass Spectrometer	Clarus SQ 8 S MS	
Inlet Line Temperature:	200°C	
Source Temperature	150°C	
Scan Conditions		
Mass Range	50 – 300 m/z	
SIR Channels m/z	Dwell Time (seconds)	Inter Channel Delay (seconds)
62	0.100	0.02
74	0.100	0.02
89	0.100	0.02

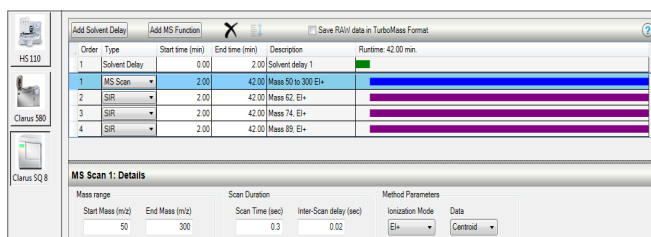


Figure 1. Mass spectrometer channels created in Waters® Empower® 3.

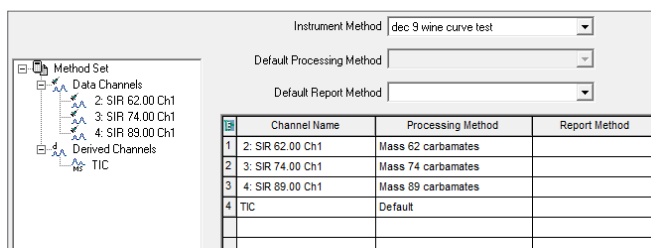


Figure 2. Waters® Empower® 3 Method set.

A calibration curve of ethyl carbamate at various concentrations (100, 200, 400 and 800 ng/mL) in methylene chloride containing 400 ng/ml butyl carbamate internal standard was set up to determine linearity and sensitivity of the technique.

## Results

Figure 3 shows the overlay of three SIM channels (m/z 62, 74, 89) monitored in 800 ppb standard of ethyl carbamate.

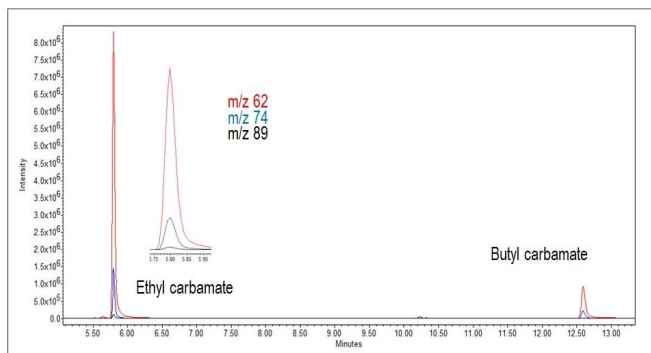


Figure 3. Representative 800 ppb standard of ethyl carbamate analyzed for three different ions.

We were able to easily detect 100 ppb ethyl carbamate at a S/N of 249:1. Figure 4 shows the calibration curve with excellent linearity ( $r^2 = 0.998$ ).

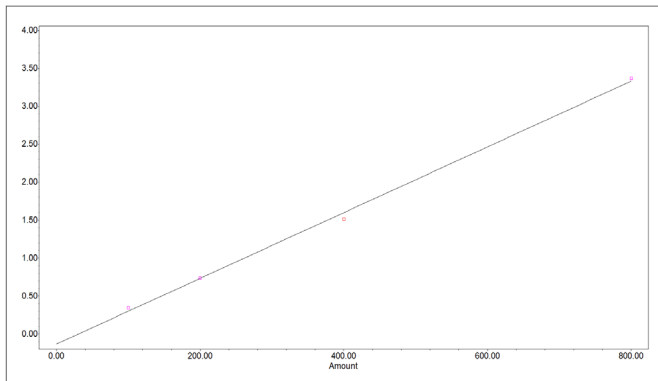


Figure 4. Calibration curve of ethyl carbamate ( $r^2$  0.998).

Figure 5 describes the match parameters used with the Retention Time Presearch filter selected as a presearch qualifier. The retention time window can be expanded or narrowed as required to improve search options and improve identification. The resulting spectral match has a probability based matching (PBM) value of 94% (Figure 6) indicating an excellent match to the library spectra.

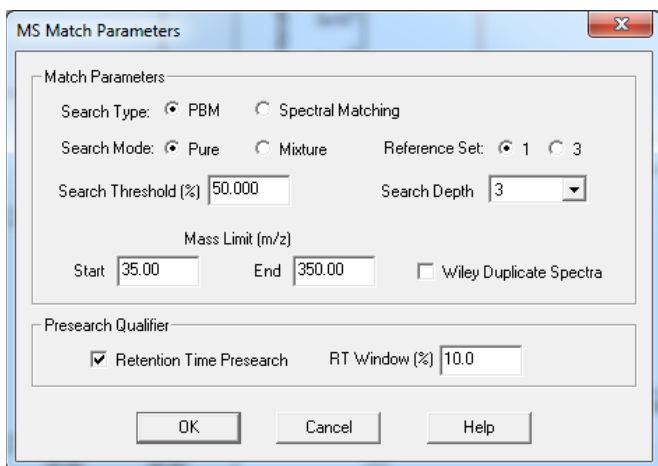


Figure 5. MS Match Parameters with Retention Time Presearch selected for additional result qualifying. The PBM threshold will further limit returns to only those that match greater than 50%.

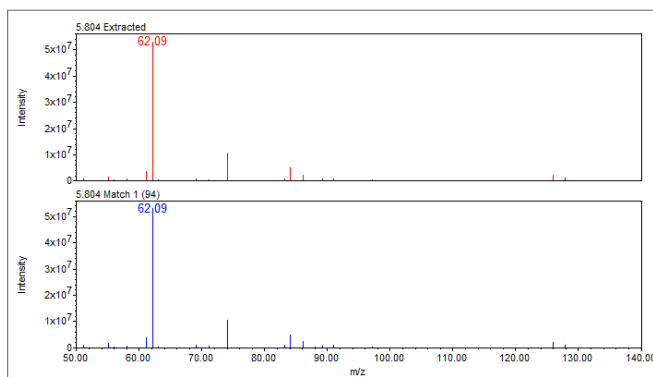


Figure 6. Library Match results showing 94% PBM fit value.

## Conclusion

The method described in the study for ethyl carbamate analysis was very sensitive with derived LOD and LOQ of 1.3 ng/L and 4 ng/L respectively, well below the recommended levels of 125 ppb mg/L for ethyl carbamate in wine. The Waters® Empower® 3 software easily enabled a custom library to be developed for this analysis, the library matching features a retention time prematch filter to limit search returns to the components that would be expected at that region of the chromatogram, and coupled with the Clarus SQ 8 MS, provides a complete solution for the determination of carbamates at the required sensitivity.

## References

- Christine E. Butzke, Linda F. Bisson, US FDA Ethyl Carbamate Preventative Action Manual, <http://www.fda.gov/Food/FoodborneIllnessContaminants/ChemicalContaminants/ucm078521.htm>.
- [http://www.efsa.europa.eu/sites/default/files/scientific\\_output/files/main\\_documents/578e.pdf](http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/578e.pdf).
- Alexander J, Auousson G, Benford D, Cockburn A, Cravedi J, Dogliotti E. 2007. Ethyl carbamate and hydrocyanic acid in food and beverages. Scientific opinion of the panel on contaminants. EFSA J 551:1–44.