Determination of Ethyl Carbamate in Wine by Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry

R.S. Whiton[1] and B.W. Zoecklein[2]*

A method for the rapid determination of ethyl carbamate in wine by headspace solid-phase microextraction with detection by gas chromatography/mass spectrometry has been developed. The analysis parameters of fiber coating, extraction time, and sample temperature have been optimized. The optimized method of headspace sampling with a Carbowax/divinylbenzene fiber for 30 minutes has been found to be reproducible and linear from 10 to 80 µg/L, with a limit of detection of 9.6 µg/L. The method is simple, requires little operator effort, and can be automated.

Key words: Ethyl carbamate, solid-phase microextraction, SPME

Ethyl carbamate is a natural by-product of fermentation and is found in many products, including wines. In 1985, a significant concentration of this human carcinogen was reported in certain Canadian-produced baked sherries and other dessert wines [17]. The U.S. wine industry has established a voluntary target for ethyl carbamate of 15 µg/L or less in table wines and 60 µg/L or less in fortified wines. The U.S. Food and Drug Administration has published recommendations for minimization of ethyl carbamate in wine [3].

The formation of ethyl carbamate in wine is related to the urea and ethanol concentrations and time, and the rate of formation increases exponentially with increased wine storage temperature [25]. Urea is the principal precursor, and attempts to control the concentration of ethyl carbamate by controlling urea and the concentration of its precursor, arginine, have received significant attention [21,22,26,27]. Factors that influence the levels of urea, and hence ethyl carbamate, include yeast selection, nitrogen supplementation, and fortification. Arrested fermentation can result in increased urea levels due to production of urea during the early stages of fermentation. Due to the dependence of ethyl carbamate levels on time and temperature, investigations of processing techniques such as thermal vinification, as well as storage conditions, should be conducted with an awareness of the formation of ethyl carbamate. Efforts to monitor ethyl carbamate levels could be enhanced by development of a simple and rapid analysis method for wine.

The commonly used method for determination of ethyl carbamate involves methylene chloride extraction using a diatomaceous earth SPE column, followed by concentration of the extract by evaporation and analysis by gas chromatography (GC) [9]. This methodology has been used with gas chromatography/mass spectrometry (GC/MS) by Daudt et al. [5] to determine ethyl carbamate in fortified wines. Sen et al. [24] have used a similar extraction procedure and GC with thermal energy analyzer detection for the analysis of wine. Similar methodology has also been used to determine ethyl carbamate in soy sauce [19] and has been augmented by a pentane wash prior to methylene chloride extraction and GC/MS detection with chemical ionization [11]. Extraction of ethyl carbamate by methylene chloride using solid phase extraction columns followed by GC/MS with selected ion monitoring (SIM) has been adopted by the AOAC International (Association of Official Analytical Chemists) for analysis of alcoholic beverages [1]. Alternate extraction procedures that have been used include steam distillation followed by solvent extraction of the distillate [14], liquid-liquid extraction with ethyl ether [12], and continuous extraction with ethyl ether [10]. All of these methods are time-consuming and use large amounts of solvents.

Solid-phase microextraction (SPME) is a fast, simple, solvent-free analysis method that combines extraction, concentration, and chromatographic injection into one step, dramatically reducing labor, materials, and waste disposal costs. Since the introduction of SPME [2,30], several groups have studied the use of SPME-GC for analysis of wine volatiles [6,7,8,28,29]. Headspace SPME has been used to analyze wine for cork taint [13,4], sulfur compounds [20], methyl isothiocyanate [15], diacetyl [16], and oak lactones [23]. Immersion SPME has been proposed by Jagerdeo et al. [18] for determination of ethyl carbamate. The purpose of the work described here is to examine the possibility of using headspace SPME-GC/MS for the rapid determination of ethyl carbamate in wine.

Materials and Methods

Equipment. Solid-phase extraction fibers and manual fiber holders were purchased from Supelco (Bellefonte, PA, USA).

[1]Research Scientist and [2]Associate Professor, Enology and Grape Chemistry Group, Department of Food Science and Technology, Virginia Tech, Blacksburg, VA 24061.

*Corresponding author [Fax: 540-231-9293; email: bzoeckle@vt.edu]

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The fiber coatings were 100 µm polydimethylsiloxane (PDMS), 65 µm PDMS/divinylbenzene (PDMS/DVB), 65 µm Carbowax/divinylbenzene (CW/DVB), 85 µm polyacrylate (PA), and 75 µm Carboxen/PDMS (Carb/PDMS). The samples were analyzed using a 5890 GC interfaced to a 5972 Mass Selective Detector (Hewlett Packard, Palo Alto, CA, USA). The GC was equipped with a 30 m x 0.25 mm DB-WAX fused silica open tubular column (J & W Scientific, Folsom, CA, USA) with a 0.25 µm coating. The carrier gas was ultra pure carrier grade helium at an average velocity of 36 cm/sec. The injector temperature was 250°C and the transfer line temperature was 240°C. The column was initially held at 40°C for 5 min, then programmed at 10°C/min to 60°C, then at 3°C/min to 140°C, then at 10°C/min to 230°C and held for 3.3 min, for a total run time of 46 min. The injection mode was splitless for 5 min. The MS was operated in selected ion monitoring (SIM) mode with electron impact ionization. The ions monitored were m/z 44, 62, and 74. The signal at m/z 62 was used for quantitation and the others for confirmation.

Chemicals and solutions. Ethyl carbamate and propyl carbamate were purchased from Aldrich (Milwaukee, WI, USA). Propyl carbamate was chosen as the internal standard because its extraction and chromatographic behavior should be very similar to ethyl carbamate, and it is used in existing methods [1]. The internal standard spiking solution was prepared at 2.00 mg/L in 15% (v/v) ethanol in distilled water. The calibration and test solutions were prepared in a synthetic wine base consisting of distilled water containing 12% (v/v) ethanol and 1 mM tartaric acid at a pH of 3.1. Calibration standards were prepared with ethyl carbamate concentrations of 10, 20, 40, 60, and 80 µg/L and propyl carbamate concentration of 40 µg/L. The solutions should be stored in a refrigerator. *Note:* We have observed a loss of propyl carbamate from the synthetic wine solutions after two months of refrigerated storage. These solutions should be monitored for decomposition if they are stored or should be prepared fresh as needed. Similar decomposition of solutions prepared in distilled water or ethanol/water has not been observed.

Extraction. A 7-mL sample was pipetted into a 16-mL sample vial and spiked with 140 µL of the 2.00 mg/L propyl carbamate internal standard solution. A small magnetic stirring bar and 2.0 g of sodium chloride were added, and the vial was capped with a PTFE/silicone septum. The vial was placed in an aluminum block thermostated at 22°C on a stirrer and allowed to equilibrate, with stirring, for 15 min. A CW/DVB SPME fiber was inserted into the headspace for 30 min, then the fiber was removed from the sample vial and inserted into the injection port of the GC for 15 min. Before use, the fiber was conditioned at 250°C for 30 min in the injection port with the split vent open.

Results and Discussion

The first step in evaluating SPME as a sampling method for ethyl carbamate was to test the response for the target analyte and internal standard using different extraction fibers. For this experiment, a solution of 600 µg/L of ethyl carbamate and 120 µg/L of propyl carbamate in a synthetic wine base (12% ethanol adjusted to pH 3.1 with tartaric acid) was used. The headspace over 5 mL of the solution was sampled for 20 min at room temperature. The results, shown in Figure 1, indicate a wide range of responses with different fibers. The strongest responses for ethyl carbamate were obtained with the CW/DVB and PA fibers. Although the responses were similar with these two fibers, the CW/DVB results were more reproducible and that fiber was used for all subsequent experiments.

Because headspace SPME is a technique based on equilibrium of the analytes between phases [30], it is important to sample for a period of time sufficient to allow the system to reach equilibrium. The responses of ethyl and propyl carbamate as functions of sampling time were determined and are shown in Figure 2. The response increased up to 30 min of fiber exposure, then leveled off, indicating equilibrium. The response ratio of ethyl carbamate to propyl carbamate also varied at sampling times less than 30 min, but was constant from 30 min on. For this reason, 30 min was chosen as the sampling time.

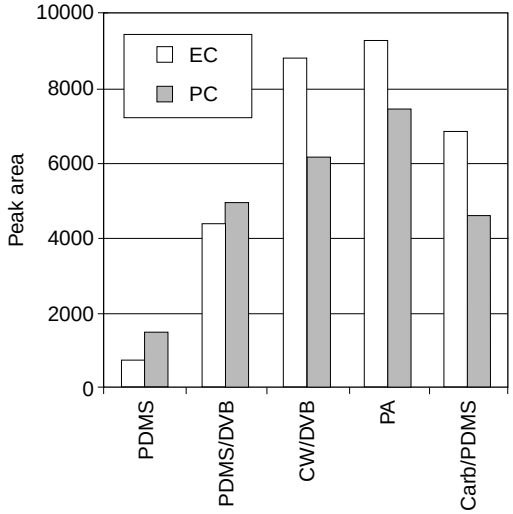

Figure 1 Comparison of various SPME fibers for extraction of ethyl carbamate and propyl carbamate from a model solution. Headspace was sampled for 20 min at room temperature. (EC = ethyl carbamate. PC = propyl carbamate. PDMS = polydimethylsilicone. PDMS/DVB = PDMS/divinylbenzene. CW/DVB = Carbowax/divinylbenzene. PA = polyacrylate. Carb/PDMS = Carboxen/PDMS.)

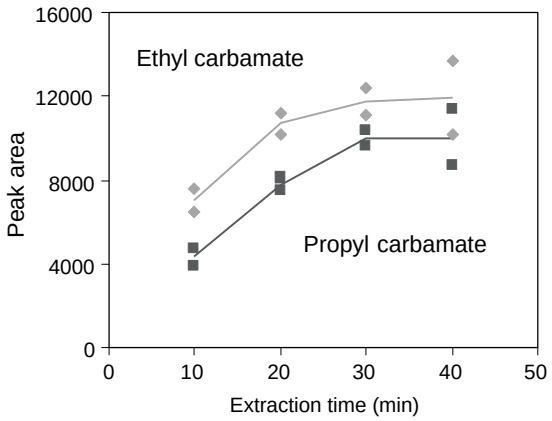

Figure 2 Extraction of ethyl carbamate and propyl carbamate from model solutions as a function of sampling time, using a CW/DVB fiber at room temperature.

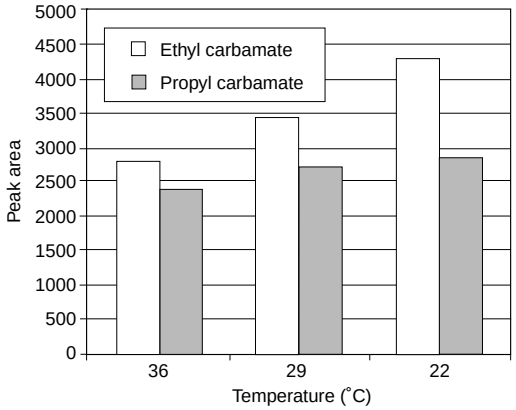

Figure 3 Effect of temperature on extraction of ethyl carbamate and propyl carbamate from spiked wine using a CW/DVB fiber and 30 min sampling time.

Extraction of volatile compounds from wine by headspace SPME has been shown to be highly dependent on sample temperature [29]. The dependence of ethyl carbamate response on sampling temperature was determined by varying the temperature from 22 to 36°C, using an aluminum block through which water from a controlled temperature bath circulated. This experiment was conducted using a spiked wine sample to control for matrix effects that might be temperature dependent. Results are shown in Figure 3. The response decreased as temperature increased over the range tested. No tests were conducted at temperatures below 22°C due to difficulties in maintaining subambient temperatures.

As a result of these tests, the optimum sampling conditions were determined to be 30 min headspace sampling at 22°C, using a CW/DVB fiber. Immersion sampling was also tested using CW/DVB and Carb/PDMS fibers. As the results were no better than with headspace sampling (and worse for CW/DVB), headspace sampling was used to prevent fiber exposure to nonvolatile materials and minimize the transfer of water into the GC/MS. The effects of sample size and headspace/sample volume ratio were also investigated and found to have little effect.

Calibration with five levels of standards in synthetic wine base produced a linear response from 10 to 80 µg/L (slope = 0.009165, intercept = -0.043927, r^2 = 0.9990). The limit of detection was determined to be 9.6 µg/L (three times the standard deviation of seven replicate analyses of a wine sample containing 23 µg/L ethyl carbamate). Analysis of spiked wine samples gave recoveries of 85% at 20 µg/L (n = 7, RSD = 15%) and 121% at 40 µg/L (n = 3, RSD = 4%).

Conclusions

Headspace solid-phase microextraction has been shown to have potential as an alternative to solvent extraction for determination of ethyl carbamate in wine. The method is rapid and simple and does not involve use and disposal of hazardous, toxic, and expensive solvents. The technique has been optimized through selection of extraction fiber coating, sampling time, and sample temperature. The resulting method has been shown to be linear from 10 to 80 µg/L, and to produce reproducible results.

Although the limit of detection is somewhat higher than is achieved by methylene chloride extraction, the SPME method eliminates the need for solvents and reduces technician effort substantially. The method can also be automated with an SPME autosampler, virtually eliminating labor. For these reasons, this method may be attractive for research laboratories monitoring ethyl carbamate formation during processing experiments.

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