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Note

Determination of ethyl carbamate in alcoholic beverages by methylation and gas chromatography with nitrogen–phosphorus thermionic detection

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Ethyl carbamate has recently become of concern because it has been found in certain alcoholic beverages at levels much higher than would be expected as a result of the fermentation process. This carcinogenic substance[1] has appeared in alcoholic beverages in the past as a result of the use of diethyl pyrocarbonate[2] (an antimicrobial agent which, in Canada, is no longer permitted). It is also possible that ethyl carbamate could form from the use of urea as a yeast food. Urea is known to react with ethanol to produce ethyl carbamate[3] and its use is presently being reviewed.

Gas chromatography (GC) has proven to be one of the most useful techniques for the determination of ethyl carbamate in alcoholic beverages[4–6]. Detection of the compound has been achieved by Coulson electrolytic conductivity[4,5], flame ionization[6], alkali flame ionization[6] or by mass spectrometry[6]. Ethyl carbamate has been treated with trifluoroacetic anhydride and determined as the N-trifluoroacetyl derivative by GC with electron-capture or alkali-flame ionization detection[4]. Of these methods, electrolytic conductivity (Coulson or, more recently, Hall) because of its selectivity appears to be the most useful for routine analysis of alcoholic beverages at ethyl carbamate concentrations in the low μg/kg range. However, few laboratories are equipped with this type of detector. It is the purpose of this work to demonstrate the use of the nitrogen–phosphorus (NP) thermionic detector for the determination of low levels of ethyl carbamate in alcoholic beverages. Thermionic detection is selective; however, it is not sensitive enough to be useful for direct determination of ethyl carbamate at low μg/kg levels in many alcoholic beverages. By forming the dimethyl derivative the detector response is increased considerably enabling improved detection limits.

EXPERIMENTAL

Reagents

All chemicals and solvents were reagent grade materials. Sodium hydride was obtained as a 50% oil mixture (J. T. Baker).

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Gas chromatography

A Hewlett-Packard 5830 gas chromatograph equipped with a DBWAX-30W (0.25 μm) (J&W Scientific) capillary column and a Hewlett-Packard NP (thermionic) detector was employed for the analyses. The conditions were as follows: carrier gas, helium at *ca.* 1 ml/min; injector, 250°C; detector, 280°C. The temperature program was: 60°C hold 3 min, 10°C/min to 240°C, hold 15 min. Retention times were 10.3 min for ethyl carbamate and 4.6 min for ethyl N,N-dimethylcarbamate.

Sample extraction

Sample preparation and cleanup were carried out essentially as described elsewhere[7]. Briefly, alcoholic beverages were diluted where necessary to yield about a 10% concentration of ethanol. A 50-g sample of diluted beverage was mixed with 30 g of potassium chloride and the mixture extracted with 3 × 100 ml of methylene chloride. A 3-ml volume of toluene was added to the combined methylene chloride extracts and the solution evaporated (rotary evaporator, 28°C) to *ca.* 2 ml and then diluted accurately with toluene to 5.0 ml for derivatization or direct analysis by GC. Normally, 2 μl of solution were injected.

Alkylation

A 1-ml aliquot of the toluene extract from above was transferred to a 15-ml graduated centrifuge tube with PTFE-lined screw cap. To this were added 0.5 ml of dimethyl sulfoxide and 0.5 ml of methyl iodide and the contents mixed gently. About 20–50 mg of hexane-washed sodium hydride were added to the tube which was then capped and shaken gently on a wrist-action shaker for 15 min. After this, 3 ml of hexane were added followed by the careful dropwise addition of water until the evolution of hydrogen ceased. (Sodium hydride reacts rapidly with water to evolve hydrogen.) Additional water, to a total of 10 ml, was then added and the mixture shaken vigorously for 1 min. After the phases separated, the organic layer was removed to a second centrifuge tube for GC analysis. If the layer was cloudy, a small quantity of anhydrous sodium sulfate was added to remove moisture.

RESULTS AND DISCUSSION

In a comparison of the Hewlett-Packard NP detector with the Hall electrolytic conductivity detector it was found that the former was less sensitive by a factor of at least 10 for ethyl carbamate (0.4 cm/ng and 5 cm/ng, respectively). However, upon the addition of two methyl substituents to the nitrogen atom of the molecule, the NP thermionic response became equal to that of the Hall for ethyl carbamate. The Hall response, however, decreased by a factor of about 10 for the derivative. These results are expected since the detection principle with thermionic detectors is known to involve a nitrogen–carbon species. Therefore the addition of two carbon atoms to the nitrogen of ethyl carbamate would be expected to increase the abundance of the species in the plasma. On the other hand, the principle of the Hall detector involves pyrolysis of nitrogen compounds to yield ammonia. Thus the additional carbons in the methylated derivative would be expected to hinder the formation of ammonia compared to the $-NH_2$ moiety of the underivatized ethyl carbamate.

Under the chromatography conditions used, the ethyl N,N-dimethylcarbamate

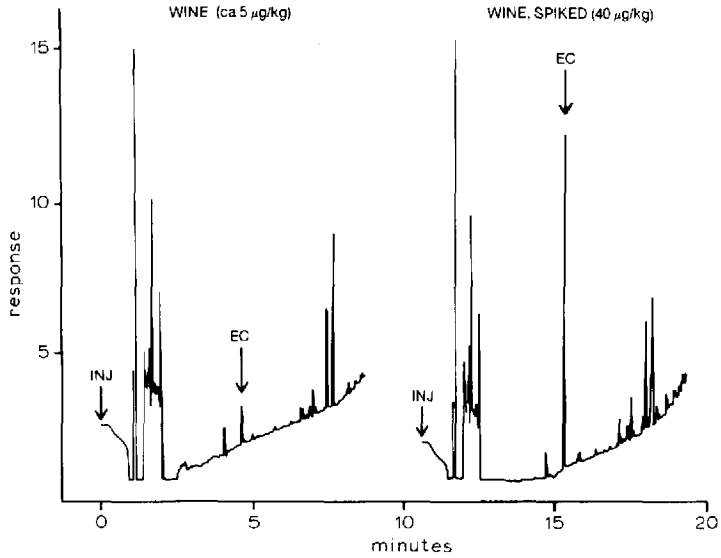

Fig. 1. Chromatograms of a wine sample containing *ca.* 5 μg/kg ethyl carbamate and the same sample spiked with 40 μg/kg ethyl carbamate. Chromatographic conditions are described in the text. EC = Ethyl carbamate.

was eluted at about 4.6 min compared to 10.3 min for the parent compound.

Fig. 1 shows typical results obtained for a wine sample containing *ca.* 5 μg/kg along with a similar sample spiked at 40 μg/kg. The ethyl carbamate derivative can be easily seen in both cases. Fig. 2 shows chromatograms of two samples (wine and

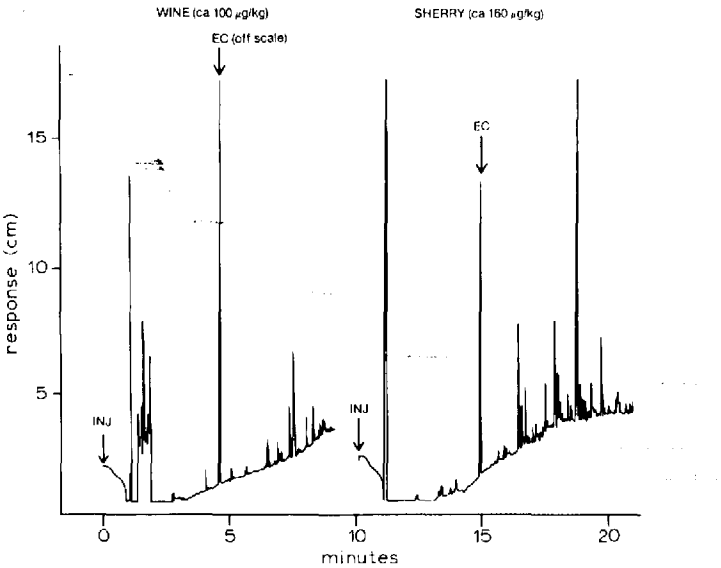

Fig. 2. Chromatograms of wine (*ca.* 100 μg/kg ethyl carbamate) using the DBWAX column and sherry (*ca.* 160 μg/kg ethyl carbamate) using a DB210 column. The sherry sample was diluted two fold before analysis. EC = Ethyl carbamate.

sherry) found to contain ethyl carbamate in excess of 100 μg/kg. The sherry sample was analyzed on a DB210 column under the same chromatographic conditions as described for the DBWAX column. Although the derivative was eluted in less than 5 min, it was necessary to continue temperature programming with both columns to 240°C and hold for about 15 min in order to remove other late eluting coextractives.

The recoveries of ethyl carbamate added to sherry at levels of 80 and 120 μg/kg were 91 and 95%, respectively, and agree well with values obtained by direct analysis with the Hall detector (92 and 87%, respectively). The repeatability of the derivatization reaction was tested by replicate derivatizations of 1-μg and 400-ng quantities of ethyl carbamate in toluene. The coefficients of variation were 3.3% and 2.5%, respectively. The reaction also was tested over a range of ethyl carbamate concentrations from 200 ng to 1.2 μg and found to yield linear results over the whole range. Solutions of the derivative were found to be stable for at least several weeks.

The identity of the alkylation product was determined by GC–mass spectrometry (VG 7070E at 4000 resolution) and found to have a molecular ion of 117 indicating the addition of two methyl groups to the nitrogen atom of ethyl carbamate.

The alkylation procedure described herein enables the use of the NP thermionic detector and thus provides an alternative to the Hall detector for the determination of ethyl carbamate in alcoholic beverages at levels in the low μg/kg range. This method has been successfully applied to a variety of beverages and can act as a confirmation of results obtained by direct analysis using the Hall conductivity detector.

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