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Determination of ethyl carbamate in alcoholic beverages by capillary multi-dimensional gas chromatography with thermionic specific detection

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Abstract

A specific, sensitive capillary multi-dimensional gas chromatographic method with thermionic specific detection (TSD) combined with internal standard methodology to identify ethyl carbamate (EC), a well known carcinogen, in various fermented alcoholic beverages is described. The basic procedures for sample preparation were similar to a modification of the LCBO (Liquor Control Board of Ontario) procedure, except that isopropyl carbamate (i-PC) was used as an internal standard. In the multi-dimensional gas chromatographic process, EC and i-PC were co-eluted on a polar capillary precolumn of BP-20, and then switched together to a non-polar OV-1 analytical column by the heart-cutting technique to resolve them and finally detected by TSD. The linear range of the calibration graph was from 10 to 550 ppb with a correlation coefficient of 0.9996. For a liquor containing 124.6 ppb of EC, the relative standard deviation was 2.0% and the detection limit was 1 ppb.

1. Introduction

Ethyl carbamate (EC) occurs in most fermented foods and alcoholic beverages [1,2]. However, it was recognized as a chemical carcinogen [3], and the Canadian Federal Government has introduced regulatory limits to control the incidence of EC in alcoholic beverages [4]. A number of methods for detecting and determining EC using different techniques have been reported. All of these methods involved some form of extraction and concentration treatment, followed by chromatographic separation with either flame ionization detection (FID), selective detection or some kind of mass spectrometric detection technique (MS). Dennis et al. [5] evaluated three different detection methods: with a thermal energy analyser (TEA), with a Hall electrolytic conductivity detector (HECD) in the nitrogen mode and MS. They found that the detection limits were similar and concluded that TEA was consistently more reliable than HECD in terms of detection limits. Two-dimensional capillary gas chromatography (GC) with dual flame ionization detectors using the external standard technique was introduced by Van Ingen et al. [6]. Sponholz [7] evaluated various methods, they believed that the two-dimensional capillary gas chromatography is the best method in terms of its relatively simple instrument requirements.

Recently, in cooperative trial studies on the

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determination of EC in alcoholic beverages, thermionic specific detection (TSD) or alkali flame ionization detection (AFID) methods have been widely used [8]. Although the absolute response of TSD may vary from one manufacturer to another, the detection limits are affected mainly by different chemical procedures and chromatographic methods. About 20 years ago, Walker et al. [9] described an analysis by packed column GC using a variety of detection methods including TSD. They reported that this procedure was able to detect 100 μg/l of EC in wine. Drexler and Schmid [10] developed a rapid and simple procedure using TSD after solid–liquid extraction. The detection limit of their method was also 100 μg/l. Joe et al. [11] were able to improve on the original method by further concentrating the sample and adopting a more rigorous clean-up regime, and were able to detect 10 μg/l of EC. Baumann and Zimmerli [12] reported a method using capillary AFID after column extraction, with a detection limit of 20 μg/l. For distilled spirits, Aylott et al. [13] reported capillary chromatography with TSD and a sample preparation procedure similar to that of the LCBO (Liquor Control Board of Ontario). The detection limit of their method was 5 μg/l. It seems that all these TSD methods are not sensitive enough to ensure compliance with the Canadian guidelines. Further improvement is still needed, and one approach is to form ethyl dimethyl carbamate by methylation. Bailay et al. [14] reported that this procedure can enhance the AFID response at least tenfold to obtain a detection limit of 1 μg/l.

In this paper, we describe an improved multidimensional capillary GC method using TSD and a special internal standard, with which very reliable and accurate results were obtained.

2. Experimental

2.1. Reagents

Reagents were of analytical-reagent grade from Beijing Chemicals Factory, (Beijing, China). Methylene chloride was purified by distillation on a 2-m Vigreux column. Before using, the purity was checked by concentrating 200 ml of the distilled solvent to 0.5 ml with a rotary evaporator and measuring the gas chromatogram of the residue, which must be free of interfering peaks for determination of EC. Other reagents used were sodium sulfate (anhydrous granular), ethyl acetate, potassium chloride, benzene, absolute ethanol and sodium hydroxide. Pure water was obtained by passing tap water through an ion-exchange column and then distilling it twice with a quartz glass device.

2.2. Primary standards

EC (chemical pure; Beijing Chemicals Factory) was purified by recrystallization from benzene. The purity was checked by GC with a OV-1 capillary column (15 m $\times$ 0.53 μm I.D., 1.2 μm film thickness). No impurities were found in the chromatogram.

Isopropyl carbamate (i-PC) (Tokyo Kasei Industry, Tokyo, Japan) was tested by the same GC method as for EC and no impurities were detected.

2.3. Standard solution preparation

A stock standard solution of EC in ethanol (1 mg/ml) was prepared, then a working standard solution of 100 μg/ml was prepared by diluting this solution tenfold with ethanol.

A stock standard solution of i-PC in ethanol (1 mg/ml) was prepared, then a working solution of 100 μg/ml was prepared by diluting this solution tenfold with ethanol.

2.4. Apparatus

All glass ware was obtained from the Beijing Glass Ware Factory. Before use, it was scrupulously cleaned with chromic acid, thoroughly washed with distilled water and dried at 120°C. An RE-51 rotary evaporator and an AC-11 micropipette were obtained from Yamato. The accuracy of the micropipette were calibrated by weighing with a precision balance. The calibrated volumes were used later in quantitative

calculations. A Sichromat-2 multi-dimensional gas chromatograph (Seimens) equipped with an SP3700 thermionic specific detector (Varian) was used throughout. The base and the heater of the detector were reconstructed to fit the Sichromat-2. A BP-20 (polyethylene glycol) column (25 m × 0.33 mm I.D. × 0.5 μm film thickness) (SGE) was installed in the first oven as a precolumn. An OV-1 fused-silica capillary column (25 m × 0.33 mm I.D. × 0.5 μm film thickness) (Chromatographic Laboratory, Beijing University) was installed in the second oven as an analytical column. FID and a TSD were used for detection of the chromatograms from the precolumn and analytical column, respectively. Nitrogen was used as the carrier gas with linear velocities of 22 cm/s for the precolumn and 27 cm/s for the analytical column. The initial temperature of the first oven was held at 55°C for 4 min, then programmed to 155°C at 4°C/min, held at 155°C for 8 min, then again programmed to 220°C at 15°C/min. The second oven was operated isothermally at 70°C. A Type 3056 multi-pen recorder (Yokagawa Kokuskin Electron) was used to recorded the chromatograms, and a Model 3394 integrator (Hewlett-Packard) was also connected to the amplifier output cable of the TSD instrument for processing the chromatographic trace. Splitless injection was used, 2 μl of extract were injected. Inlet purge was activated after 1 min and the injector temperature was 230°C.

2.5. Procedure

Sample preparation

First, the liquor samples were adjusted to an alcoholic strength near 20% (v/v) (10% for wine) with pure water, then 50 μl of 100 μg/ml i-PC internal standard solution were added to 50 g of each of these samples. These sample solutions were adjusted to pH 10 with 6 *M* NaOH solution and then saturated with KCl and extracted with 3 × 70 ml of methylene chloride. The extracts were dried with anhydrous sodium sulfate and concentrated under reduced pressure at 29°C with a rotary evaporator to a volume of about 5 ml. The residue was transferred to a concentrator tube and 0.50 ml of ethyl acetate was added as a keeper solvent. Under a gentle stream of nitrogen, the volume of the extracts were reduced to 0.5 ml.

Quantification

To constructing a calibration graph, 50 g of a 20% (v/v) aqueous ethanol solution were added to each of six 200-ml separating funnels, then 5, 10, 20, 50, 100, 150, 200 and 250 μl of EC working solution were added to each of the six funnels. These correspond to standard concentrations of 10, 20, 40, 100, 200, 300, 400 and 500 μg/kg (ppb), respectively. Then 50 μl of the 100 μg/ml i-PC working standard solution were added to each separating funnel to obtain an internal standard concentration of 100 μg/kg. These standard solutions were extracted and concentrated in the same manner as in the sample preparation described above. Calibration graphs of peak-area ratio of EC to i-PC versus EC concentration were used to calculate unknown concentrations.

Gas chromatographic analysis

To determine the retention times of EC and i-PC, 0.2 μl of mixed standard solution (containing 10 ng each of EC and i-PC) was injected into the gas chromatograph. The retention times of EC and i-PC under the above conditions were 22 min 5 s. Then 2 μl of concentrated extract of standards or samples were injected into the gas chromatograph. With the heart-cutting technique, the fraction that eluted from the precolumn from 21 min 50 s to 22 min 20 s was switched to the analytical column, and was developed by a two-dimensional chromatographic process.

3. Results and discussion

3.1. Internal standard methodology in multi-dimensional gas chromatography

For quantitative analysis, choosing an appropriate internal standard to match the multi-dimensional gas chromatograph is always a troublesome problem. Obviously, with the heart-

cutting technique, the retention behaviour of the internal standard must be identical with that of the analyte on the pre-column, otherwise, the internal standard and the analyte will be detected by the monitoring detector and the main detector respectively. Hence there will be a standardization problem owing to the different responses of the two detectors. In addition, resolution on the pre-column will be difficult for complex samples. Therefore, the external standard method is always to be recommended in multi-dimensional GC.

Many compounds were screened and finally we found that i-PC fulfils the strict criteria for use as an internal standard. Fig. 1 shows the multi-dimensional gas chromatogram of a standard solution and indicates that EC and i-PC can be co-eluted on the BP-20 phase of the pre-column and with heart-cutting technique switched to the OV-1 phase of the analytical column and there be resolved. The co-elution of EC and i-PC on a Carbowax-20M phase was reported by Lau et al. [15] and they used a 30 m × 0.25 mm I.D. × 0.15 μm DB-Wax column to investigate the GC and MS properties of alkyl carbamates.

3.2. Detection limits

The chemical noise from the complex matrix was effectively minimized by the high resolving power of the multi-dimensional GC technique. Also, the instability of the thermionic specific detector that was always encountered with temperature programming was eliminated by operating the analytical column isothermally. Hence both the chemical and electrical noise were minimized. For these reasons, the detection limit was considerably improved, being as low as at least 1 ppb. Fig. 2 represents the multi-dimensional gas chromatograms of a wine sample that contained 22.1 ppb of EC.

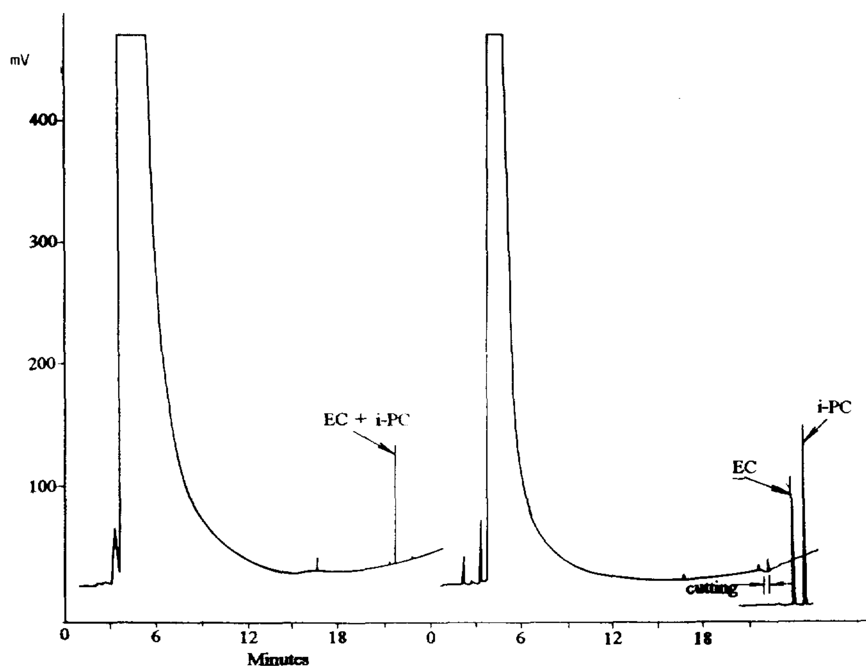


Fig. 1. Multi-dimensional gas chromatograms of a standard solution (containing 100 ng each of EC and i-PC). Left: precolumn chromatogram obtained on a BP-20 column (25 m × 0.33 mm I.D. × 0.25 μm) using FID without heart-cutting. Right: precolumn chromatogram (top) and an analytical column chromatogram (bottom) obtained on an OV-1 column (25 m × 0.33 mm I.D. × 0.25 μm) using FID with heart-cutting.

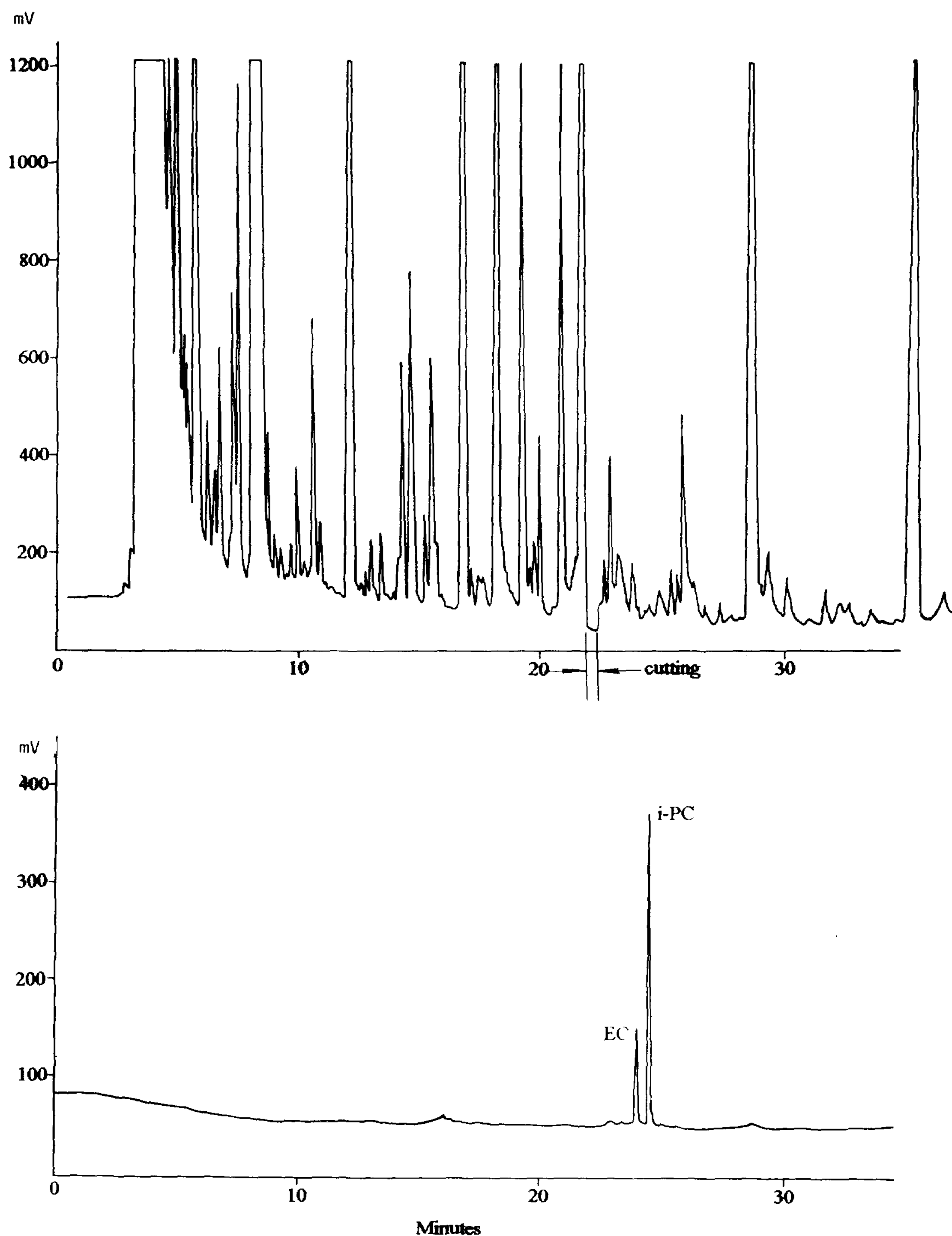
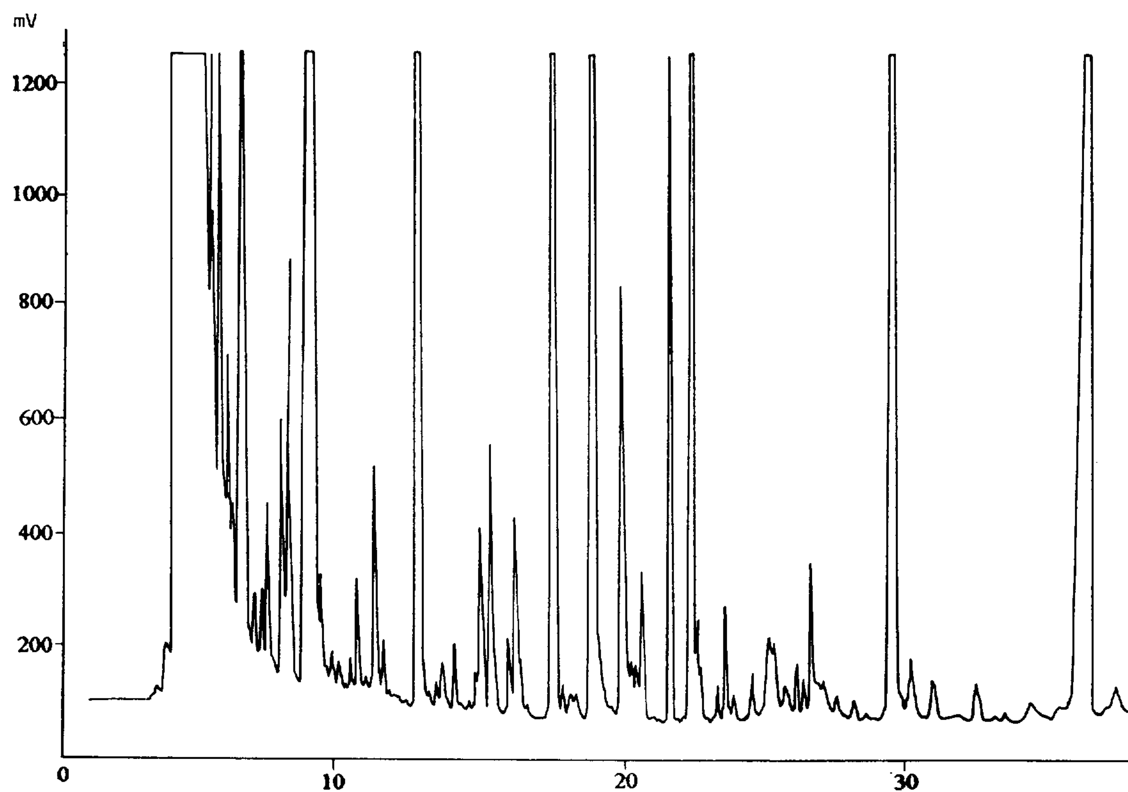


Fig. 2. Multi-dimensional gas chromatograms of a wine containing 22.1 ppb of EC. Left: capillary precolumn chromatogram obtained by FID without heart-cutting. Right: capillary precolumn chromatogram obtained by FID (top) and an analytical column chromatogram obtained by TSD (bottom) with heart-cutting.



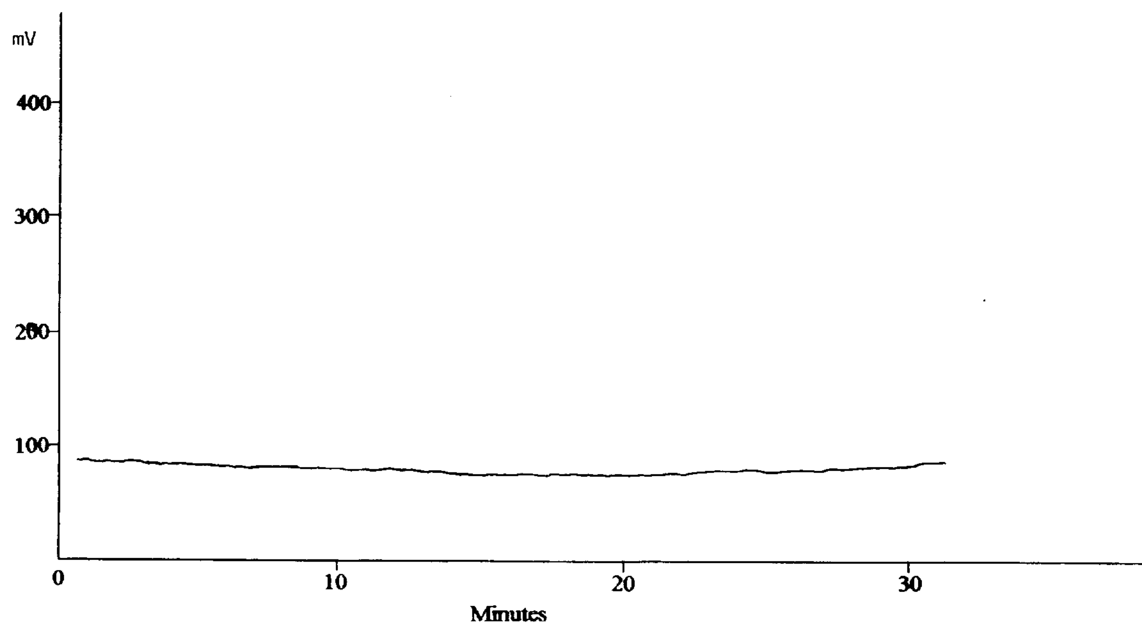


Fig. 2 (*continued*).

Table 1
Measurement precision

Sample	Concentration measured (ppb)	Mean value (ppb)	R.S.D. (%)
Liquor	123.3, 123.7, 124.3, 122.2, 130.8, 122.2, 125.7	124.6	2.0
Rice wine	72.6, 70.0, 68.2, 70.7, 67.3, 66.4, 67.0	68.9	3.0

3.3. Calibration and calculation

From 10 to 500 ppb of EC, the measured relationship between peak-intensity ratios (y) and concentration of EC (x) for the calibration mixtures can be expressed by the regression equation $y = 0.0007 + 0.0161x$, with a correlation coefficient of 0.9994. This shows that the linearity is very satisfactory, and the straight line almost passes through the origin.

3.4. Precision and accuracy

The precision of the method was calculated by analysing seven replicates each for two typical Chinese wines, one a distilled liquor and the other a rice wine. The results obtained are given in Table 1.

The assay accuracy of the method was determined by analysing a wine sample that contained 22.1 ppb of EC as the mean value of three determinations. To this wine sample, 50 ppb of EC were added prior to extraction and the mean recovery determined was 96%.

4. Conclusion

Using a nitrogen-specific detector and a special internal standard technique, a multi-dimensional gas chromatographic method was developed that offers a routine method for detecting ethyl carbamate in alcholic beverages.

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