

A simple gas chromatography method for the determination of ethylcarbamate in spirits

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Eine einfache gaschromatographische Methode zur Bestimmung von Ethylcarbamat in Spirituosen

Zusammenfassung. Ethylcarbamat ist ein cancerogener Inhaltsstoff von gewissen Spirituosen. Zu dessen Bestimmung wird die Probe auf $\frac{1}{5}$ ihres Volumens eingengt, mit Ethylacetat extrahiert und durch Capillar-GC mit FID nachgewiesen. Die Methode ist zeit- und materialsparend und hat sich bei einem Vergleich mit mehreren Laboratorien als zuverlässig erwiesen. Die Ethylcarbamatgehalte von 170 Spirituosenproben aus der Innerschweiz werden angegeben. Dabei liegen die Mittelwerte für Kirschwasser bei 1,0 mg/l, für Zwetschgen- und Pflümlwasser bei 1,5 mg/l und für Williamsbranntweine bei 0,4 mg/l. Die Ethylcarbamatgehalte von Spirituosen können bei der Lagerung stark zunehmen, dies vor allem unter dem Einfluß von Tageslicht.

Summary. Ethylcarbamate is a toxic component of certain spirits. For its determination, the spirit is concentrated to one-fifth of its volume, extracted with ethylacetate and analysed by capillary gas chromatography (GC) with flame ionization detection (FID). The method saves time and material and has proved to be a reliable test in different laboratories. The ethylcarbamate concentrations in 170 samples of Swiss origin are presented. The mean values were 1.0 mg/l in cherry brandies, 1.5 mg/l in plum brandies and 0.4 mg/l in Williams pear brandies. Ethylcarbamate concentrations may increase during storage, especially under the influence of daylight.

Introduction

Ethylcarbamate (urethan) has been known to be a powerful carcinogen since 1943 [1]. It was later found in beverages treated with diethyl pyrocarbonate [2] and different fermented foodstuffs such as wine, beer,

bread, yoghurt, soy sauce and sake have been found to contain ethylcarbamate at $\mu\text{g}/\text{kg}$ levels [3]. In 1985 Canadian chemists found considerable concentrations of ethylcarbamate in fruit spirits.

Taking into account the toxicological properties of ethylcarbamate and following the example of the Canadian food authorities, the German Federal health office established an upper limit of 0.4 mg/l for fruit spirits.

A method for the determination of ethylcarbamate at $\mu\text{g}/\text{l}$ levels in wine was published by Walker et al. [4]. The authors used chloroform to extract the substance followed by chromatography on a Florisil column. Detection was accomplished by packed column GC with a Coulson conductivity detector. Ough [3] improved the method, reaching a detection limit of 0.5 $\mu\text{g}/\text{l}$.

The method of Joe et al. [5] involved extraction with dichloromethane followed by chromatography on a mixture of aluminium oxide, silica gel and sodium sulphate (or Celite) and detection by packed-column GC with flame-ionization or nitrogen-phosphorus detection. More recently, Baumann and Zimmerli [6] presented a method based on chromatography using a mixture of Extrelut and sodium chloride followed by capillary GC with a nitrogen-phosphorus-detector.

The need for a method requiring less time and material led us to develop our own method. Before we developed the method described below, we tried to isolate the ethylcarbamate from the spirits by sorbent extraction, using different bonded silica phases and XAD resins. In no case was retention of the ethylcarbamate strong enough to satisfy our requirements. During these experiments, ethylcarbamate had to be monitored using GC, since no simple detection method such as colour reactions, UV-absorbance or thin-layer chromatography could be found for this purpose.

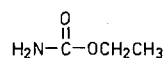


Fig. 1. Structural formula of ethylcarbamate

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Materials and methods

Principle

The spirit (adjusted to 40 vol-%) is concentrated under reduced pressure. The concentrate is extracted with ethylacetate and analysed by GC on a polar capillary column with FID.

Materials

Ethylacetate, ethanol, n-octanol and NaCl: p.a. of Merck, Darmstadt, FRG. Capillary gas chromatograph HP 5790A with FID and programmable attenuation factor. Vacuum rotary evaporator (Büchi, Flawil, Switzerland).

Procedure

The spirit to be analysed is adjusted to an alcohol content of 40 vol-% by the addition of water. In the case of spirits with an alcohol content of less than 40%, ethanol is added for this adjustment. The adjusted spirit (5 ml) is concentrated to a final volume of 0.8 to 1.0 ml (not less than 0.8 ml) in a conical flask by means of a rotary evaporator (bath temperature 30 °C, pressure 20 mbar) and boiling is avoided during this concentration step. The mass of the residue is adjusted to 1.0 g by the addition of water and 0.5 g NaCl is added followed by the addition of 0.5 ml of ethylacetate containing 100 mg/l n-octanol (internal standard). The contents are then shaken for 20 s and the ethylacetate phase analysed by GC.

Gas chromatography

Column: Chrompack CP Wax 57 CB fused silica capillary column i.d. 0.23 mm, length 16 m, film thickness 0.23 µm. – Carrier gas: H₂ 0.7 bar, Split: 5:1, injector temperature: 210 °C, detector temperature: 230 °C, injection volume: 1 µl. – Oven temperature program: initial isothermal 75 °C/0.1 min program rate 4.5 °C/min up to 105 °C, then 30 °/min up to 220 °C; final isothermal 220 °C/5 min. A typical gas chromatogram of a cherry brandy is shown in Fig. 2.

Calibration

Solution of 0.1 to 5 mg/l ethylacetate in 40% ethanol are prepared as described above and the peak heights of ethylcarbamate and of n-octanol are measured (by hand). It is necessary to change the attenuation factor between the two peaks by two orders of magnitude in order to obtain measurable peaks. The ratios of the peak heights of ethylcarbamate and n-octanol are calculated and plotted against the ethylcarbamate concentration in the initial solution. The calibration

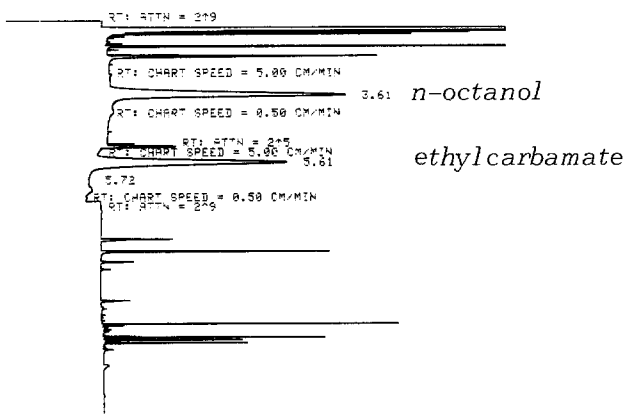


Fig. 2. Gas chromatogram of a cherry brandy containing 3.0 mg/l ethylcarbamate. As a control of peak purity the chart speed is increased to 5 cm/min for the n-octanol and ethylcarbamate peaks

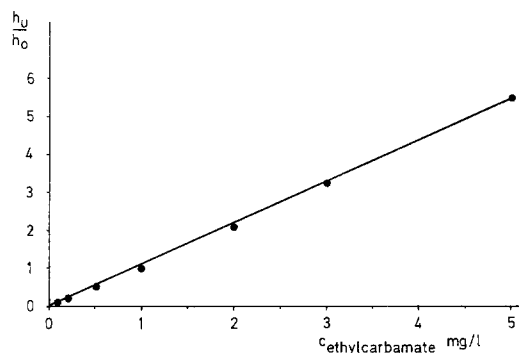


Fig. 3. Calibration curve: ethylcarbamate concentration in the sample versus peak height ratio of ethylcarbamate: n-octanol

curve is a straight line up to 5 mg/l (see Fig. 3). Spirits with higher ethylcarbamate concentrations have to be diluted to a concentration below this value. For spirits containing more than 40% alcohol, the results are referred to an alcohol content of 40%.

Results and discussion

For the same cherry brandy analysed in seven different laboratories, the results reported agreed well with each other (see Table 1).

It is very important to respect the lower limit of 0.8 ml in the concentration step, since below this limit uncontrolled losses of ethylcarbamate due to steam distillation may occur.

The overall loss of ethylcarbamate during the concentration and extraction steps is 26% and remains

Table 1. Results from seven different laboratories for the same cherry brandy

Laboratory	Method	Detector	Ethylcarbamate mg/l 40%
1	Present method	FID	2.1
2	Present method	FID	2.2
3	Present method	NPD	2.1
4	Present method	FID	2.0
5	[6]	NPD	1.9
6	[6]	FID	2.2
7	[6]	FID	1.8

Table 2. Ethylcarbamate concentrations found in spirit samples of central Swiss origin analysed in 1986 in Brunnen

Spirit	Ethylcarbamate mg/l (40%)			
	n	\bar{x}	x_{\max}	x_{\min}
Cherry	135	1.0	9.5	0.04
Plum	20	1.5	7.7	<0.2
Williams pear	8	0.4	1.9	<0.04
Apple	3	0.7	0.8	0.5
“Chrüter”	2	0.4	0.6	0.06
Peach liquor 20%	1	<0.05	–	–
Raspberry	1	0.1	–	–

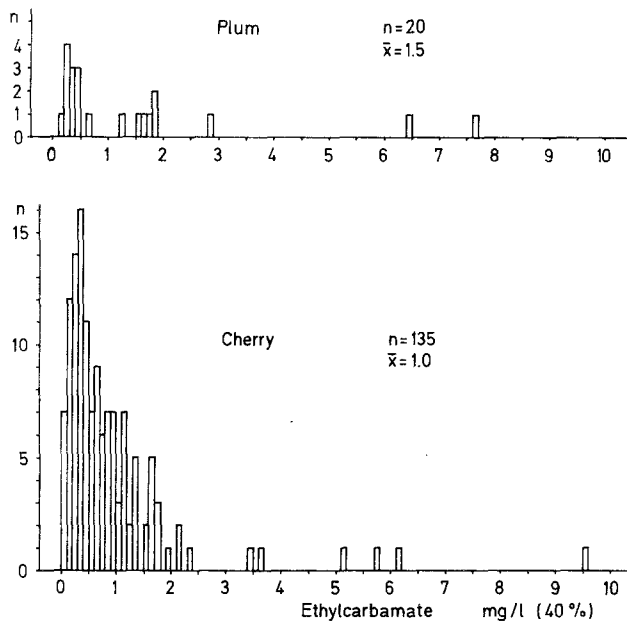


Fig. 4. Statistical distribution of ethylcarbamate concentrations in cherry and plum spirits

constant. The method gave the expected results when applied to a model mixture containing methanol, 1-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, ethylacetate and lactic acid ethyl ester in concentrations of 1500, 1039, 32, 49, 98, 68, 159, 6, 502, and 152 mg/100 ml absolute alcohol, respectively. Hence these spirit components have no adverse effect on the accuracy of the result even when they are present in large amounts.

With FID, a detection limit of 10–20 $\mu\text{g/l}$ 40% is obtained. Positive results can be confirmed using MS or thermionic detection (NPD).

Since January 1986, 170 samples of spirits have been analysed in our laboratory, the results of which are shown in Table 2 and Fig. 4.

The ethylcarbamate concentration in cherry brandies may increase very markedly during storage. This effect is particularly strong in samples in white glass bottles exposed to daylight. An example of this effect was shown in a freshly distilled cherry brandy where the concentration increased from 0.06 mg/l 40% to 0.6 mg/l 40% within 14 days. Hence, it is of crucial importance that samples to be analysed for ethylcarbamate be kept in the dark prior to analysis.

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