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Note

Method for the analysis of ethyl carbamate in alcoholic beverages by capillary gas chromatography

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A number of methods have been described in the literature for the analysis of ethyl carbamate in alcoholic beverages. Walker *et al.*[1] described a suitable method of analysis for wine. After sample clean-up, packed column gas chromatography was used and a number of different detectors evaluated. Flame ionisation (FID), alkali flame ionisation (AFID) and electron capture were tried but only the Coulson electrolytic conductivity detector was considered suitable. With this technique it was possible to quantify ethyl carbamate in wine at a level of 100 μg/l. Mass spectrometry (MS) was used for identity confirmation but because of interferences it was necessary to analyse the ethyl carbamate as the trifluoroacetyl derivative. Ough[2] essentially followed Walker's procedure for his analyses of fermented beverages and foods. Joe *et al.*[3] were able to improve on Walker's original method. By further concentrating the sample and adopting a more rigorous clean-up regime they were able to use FID or AFID for levels of ethyl carbamate down to 10 μg/l although MS confirmation was still required.

A renewal of interest in this area of analysis, prompted by the Canadian Government decision to impose limits on the ethyl carbamate content of imported beverages[4], has led us to apply a number of new techniques to this problem. We have used solid-phase extraction and clean-up techniques to streamline the sample work-up procedures and employed capillary chromatography in place of the packed columns previously adopted. The performance of three different detectors has also been investigated in order to increase the sensitivity and selectivity of the analysis.

EXPERIMENTAL

Each sample of alcoholic beverage was analysed in duplicate. Recovery was

estimated by spiking a third aliquot of the sample with ethyl carbamate (5 μg in 50 μl ethanol) and taking this through the procedure.

Extraction and clean-up procedure

Samples of alcoholic beverage were taken and diluted to 50 ml so that the final alcohol content was below 5%. This solution was then absorbed on a CT 2050 Chemtube (Analytichem) (or Extrelut, Merck 42 g) and eluted with dichloromethane (3 × 50 ml). The eluent was passed through a short column containing anhydrous sodium sulphate (10 g) into a Kuderna-Danish concentrator. After rinsing the sodium sulphate with dichloromethane (5 ml) the combined extract was reduced to 4 ml on a waterbath at 55°C. The apparatus was then washed with dichloromethane (1 ml) and the concentrate applied to a Florisil Sep-Pak (Waters) (pre-rinsed with dichloromethane, 10 ml). A further 5 ml of dichloromethane was used to rinse the apparatus and applied to the Sep-Pak and the entire contents to this stage discarded. The fraction containing ethyl carbamate was eluted with 7% methanol in dichloromethane (5 ml) and the eluent was reduced in a micro Kuderna-Danish concentrator to about 0.7 ml. The apparatus was rinsed with dichloromethane (0.3 ml) and the final volume was measured with a syringe.

Gas chromatographic measurement

Three different detectors were evaluated under the following operating conditions:

(i) A thermal energy analyser Model 610 (Thermedics) was used in the nitrogen mode with an interface temperature of 200°C and a pyrolyser temperature of 800°C. The reaction chamber was operated at 2 mm mercury and no make-up gas was employed.

(ii) A Hall 700A electrolytic conductivity detector (Tracor) was used in the nitrogen mode without a scrubbing system. Furnace temperature was 840°C, the electrolyte was isopropanol–water (50:50) at 0.4 ml/min with manual venting and hydrogen fuel gas flow was 40 ml/min.

(iii) A VG 7070 H mass spectrometer was operated with electron impact ionisation (70 eV, 200 μA trap current, 200°C) and with the interface heater set to 200°C. Selected ion monitoring was employed, controlled by a VG 11/250 data system, at ion currents *m/z* 61, 62, 74.

Slightly different gas chromatographic conditions were employed for each detector to gain optimum performance from each instrument however the following, used for the thermal energy analyser (TEA) detector, was typical. A CP Wax 52 CB column (Chrompack), 25 × 0.31 mm I.D., 0.21 μm film thickness, was used with a temperature programme of 65°C (for 1 min) then 10°C/min to 100°C and 20°C/min to 200°C (for 10 min). On-column injection (2 μl) with secondary cooling was employed with helium carrier gas (0.6 bar).

RESULTS AND DISCUSSION

Extraction and clean-up procedure

The use of Chemtube or Extrelut materials greatly facilitates the dichloromethane extraction of the beverages since no emulsions are formed using this tech-

nique. It is not then necessary to clear the emulsions by centrifugation[3] and hence considerable savings in time are experienced. Similarly Florisil Sep-Paks can be used to clean-up the sample in about 1 min whereas the conventional column techniques previously used would require about 30 minutes. While this clean-up may not be essential where the use of the TEA detector is planned, we have found that it is necessary if a full mass spectrum is required for confirmatory purposes. It also leads to a significant extension of capillary column life.

GC detector comparison

Two chromatograms of Scotch whisky samples obtained using the Hall and TEA detectors are shown in Figs. 1 and 2. The level of ethyl carbamate corresponds to 43 μg/l for the TEA analysis and 32 μg/l for the Hall analysis. The retention times for ethyl carbamate (peaks shadowed) varies for the different instruments because of minor differences in the columns and temperature programmes used. Both the TEA and Hall detectors show relatively few peaks indicating the selectivity of these instruments in the nitrogen mode. Some problems were initially experienced with the use of the Hall detector. Dichloromethane is not a good solvent to use for samples when using this instrument since it produces hydrochloric acid on reduction and this leads to a high background signal. This can be overcome by exchanging dichloromethane for a different solvent (*e.g.* ethyl acetate) during the sample work-up procedure. However, manual venting of the detector until just before the ethyl carbamate elutes is equally effective and more convenient. This procedure does however lead to a marked disturbance of the baseline as can be seen from the chromatogram. The Hall detector possesses a scrubbing system which is designed to remove traces of acidic gases from the GC eluent. In fact this system leads to significant band spreading of the chromatographic peaks and its removal considerably improved chromatographic performance without causing any increase in background noise.

The electron impact mass spectrum of ethyl carbamate (Fig. 3) was dominated by fragment ions of *m/z* 44, 45 and 62. The first two ions and the molecular ion (*m/z* 89) were either not sufficiently specific or not intense enough to be useful in

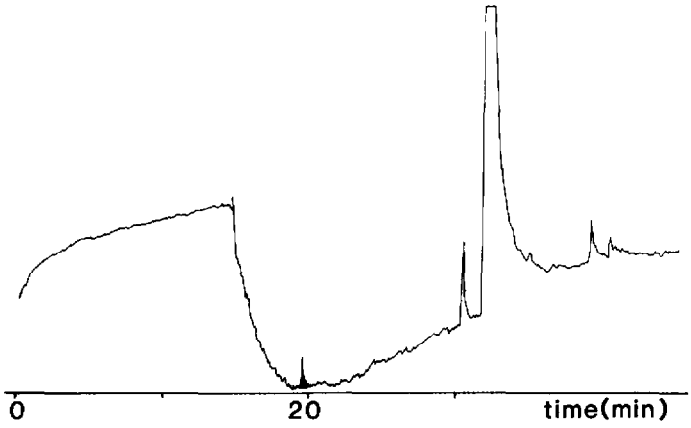

Fig. 1. Capillary GC analysis of whisky sample for ethyl carbamate (shadowed, 32 μg/l) using Hall 700A electrolytic conductivity detector.

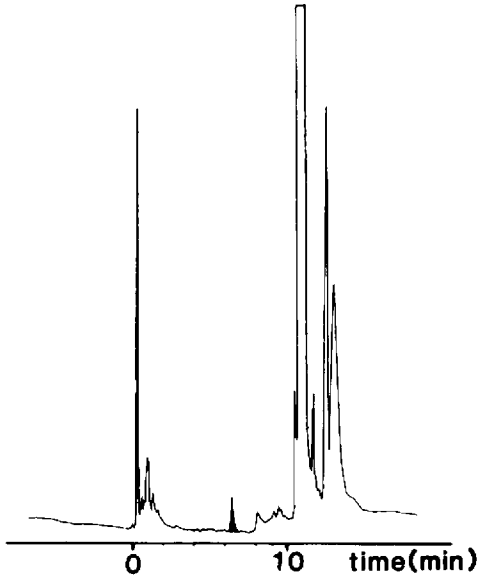

Fig. 2. Capillary GC analysis of whisky sample for ethyl carbamate (shadowed, 43 μg/l) using thermal energy analyser in the nitrogen mode.

trace analysis. However, as illustrated in Fig. 4, *m/z* 62 provided high selectivity and allowed a detection limit of 1 μg/l. The two additional ions chosen for selected ion monitoring (*m/z* 61 and *74*) were of low relative abundance and thus selectivity, but gave adequately resolved and measurable peaks for samples containing in excess of 30 μg/l. By ensuring that the ratios of all three ions were consistent with that of the ethyl carbamate standard, the identity of this component in the chromatogram could be ascertained with confidence.

No attempt was made to use the trifluoroacetylation recommended by Walker *et al.*[1]. The use of trimethylsilyl derivatives was examined but the EI spectra were dominated by non-specific fragments originating from the derivatising group. Chem-

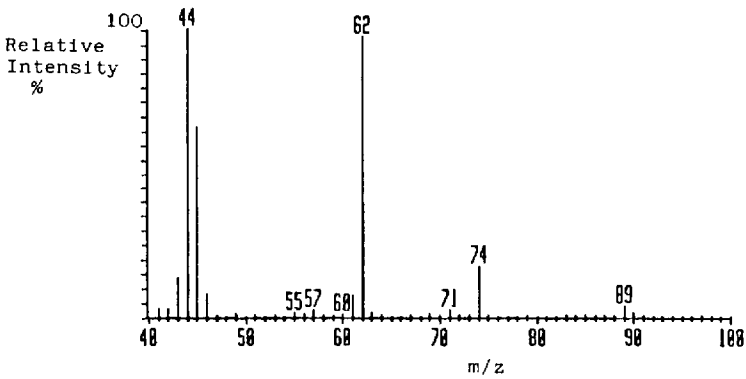

Fig. 3. Electron impact mass spectrum of ethyl carbamate.

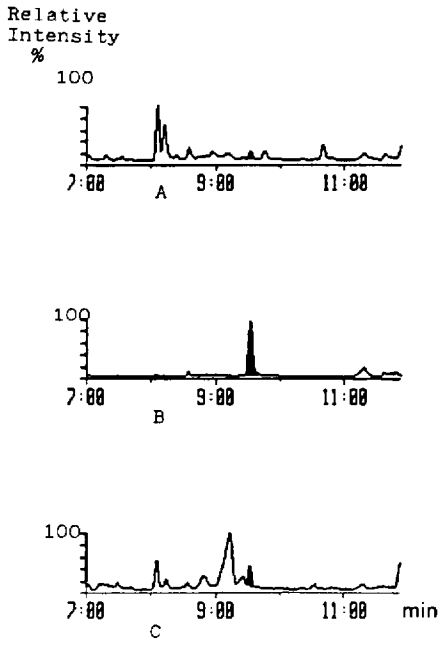

Fig. 4. Capillary GC analysis of North American whisky sample for ethyl carbamate (shadowed, 124 μg/l) using selected ion monitoring at *m/z* 61 (A), 62 (B) and 74 (C).

ical ionisation did not offer any advantage in terms of sensitivity compared with the monitoring of *m/z* 62.

Each of the detectors studied had similar detection limits for wine (defined as 3 × noise level) with the TEA (1 μg/l) and Hall (2–5 μg/l) detectors having comparable sensitivity to the mass spectrometer (1 μg/l). The results obtained from the three detectors (Table I) showed remarkable consistency between the different instruments

TABLE I

COMPARISON OF RESULTS FOR ETHYL CARBAMATE (μg/l) OBTAINED FROM DIFFERENT DETECTORS

Sample	*Mass spectrometer (m/z 62)*	*TEA detector*	*Hall detector*
Bourbon whisky a*	216**	204	176
b	212**	208	184
Scotch whisky a	75	80	72
b	77	99	84
Red wine a	22	16	13
b	Not analysed	13	10

* Each sample was extracted and concentrated in duplicate (a and b). Each concentrate was then analysed by the different detectors.

** Confirmed from *m/z* 61, 74.

given that some weeks elapsed between individual analyses. This serves further to confirm the identity of the contaminant measured as ethyl carbamate and indicates that any of these detectors might reasonably be used for monitoring the levels of ethyl carbamate in alcoholic beverages. In practice the TEA detector is used on a routine basis for this analysis since it proved consistently reliable and also permitted better detection limits than the Hall detector.

Analytical precision

On a day-to-day basis the sample clean-up and GC–TEA technique works very reliably. Blanks were consistently below the detection limit of the method and an average recovery of 84% (minimum 75%) was obtained for 12 whisky and wine samples. Duplicate analyses also proved very reproducible with the range normally within ±5% of the mean. The repeatability shown by replicate analyses on three separate days proved consistent with a mean of 303 μg/l (coefficient of variation 6.34%) obtained for six analyses of a bourbon whisky.

In a limited study of the application of the methodology, ethyl carbamate levels in various alcoholic beverages showed a wide range of concentrations. Wines averaged 5 μg/l with a range of < 1 to 18 μg/l (13 samples), whilst the mean for 6 samples of sherry was 28 μg/l, range < 1 to 60 μg/l. The level in gin (2 samples) and vodka (2 samples) was below the 1 μg/l detection limit. In the case of whiskies concentrations in the range 20–90 μg/l were detected in 11 samples of Scotch whisky and in the range < 1 up to 230 μg/l for six imported whiskies.

CONCLUSION

The procedures described in this study are suitable for routine analysis of ethyl carbamate in alcoholic beverages at levels of down to 1 μg/l. The use of the Chemtube or Extrelut materials improves the sample analysis time by avoiding the use of liquid-liquid extraction and time consuming centrifugation of resultant emulsions. Additionally the Florisil clean-up stage provides a sample extract that is sufficiently clean that little deterioration in performance of the gas chromatographic analysis was noticeable even after several hundred injections. Studies are currently in progress to identify the reasons for the presence of ethyl carbamate in some alcoholic beverages. These findings will be reported elsewhere as will an investigation into the possible presence of ethyl carbamate in other fermented foodstuff.

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