

ETHYL CARBAMATE FORMATION IN GRAIN BASED SPIRITS

PART I: POST-DISTILLATION ETHYL CARBAMATE FORMATION IN MATURING GRAIN WHISKY

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Distilled spirits are subject to post-distillation ethyl carbamate formation in the presence of appropriate precursors. Freshly distilled grain whisky, produced by the continuous distillation Coffey still process, normally contains ethyl carbamate concentrations not exceeding 20 ppb (normalized to 43% v/v alcoholic strength). Further ethyl carbamate formation, dependent upon the presence of trace anionic precursors such as cyanate, cyanide, and copper cyanide complexes, may take place during normal maturation in oak casks. Related but different mechanisms may induce ethyl carbamate formation under normal daylight and artificial light in the laboratory. Ethyl carbamate precursors convert into ethyl carbamate during the initial three months of maturation and are not detectable in the final bottled product. Thus the final ethyl carbamate concentration in spirit is dependent upon the initial ethyl carbamate level measured after distillation plus ethyl carbamate formed from precursors. It is important during process control to monitor not only the ethyl carbamate level in freshly distilled spirit but also the spirit's potential to form ethyl carbamate from precursors with the objective of minimizing these components in the freshly distilled and maturing spirit. A scheme for predicting final ethyl carbamate concentrations from precursor concentrations in freshly distilled spirit is presented.

Key Words: *Ethyl carbamate, ethyl carbamate precursors, distilled spirits, Scotch whisky, post-distillation formation, maturation.*

INTRODUCTION

Ethyl carbamate ($C_2H_5OCONH_2$), otherwise known as urethane, is a naturally occurring compound present in most fermented foods and beverages²⁰. However, it is a chemical carcinogen¹³ and this has led to concern in recent years about its presence at trace levels in alcoholic beverages.

Canada introduced regulatory limits for various types of alcoholic beverage in 1985⁸ and the United States agreed voluntary limits for whiskey and wines in 1987 which were applicable to new production from 1st January 1989¹¹. These actions have resulted in considerable attention being given to the development of methods for ethyl carbamate analysis and to investigations into mechanisms for its formation. This paper deals with aspects of its formation in Scotch whisky.

Ough suggested in 1976 that an obvious source of natural ethyl carbamate was the hydrolysis of yeast carbamyl phosphate with ethanol, yielding phosphoric acid and ethyl carbamate²⁰. Recent work has identified a range of N-carbamyl compounds including allantoin and citrulline and the closely related arginine which can react with ethanol to form ethyl carbamate^{9,21,22}. Such pathways are important in alcoholic beverages such as beer and wine, where the final product remains associated with the solution from which it was fermented, or distilled spirits where the ethyl carbamate formed during fermentation is subject to distillation into the spirit.

Workers studying ethyl carbamate formation in stone fruit spirits associated its formation with yeast produced carbamyl phosphate and stone fruit reaction products⁷ and reported enhanced formation in distillates under the influence of light^{5,18}. Whilst various N-carbamyl compounds are also reported as potential ethyl carbamate precursors in stone fruit spirits, hydrocyanic acid and cyanate together with vicinal dicarbonyl compounds such as diacetal, 2,3-pentanedione and methyl glyoxal were included as important reactants^{4,29}. Such reactions

were accelerated under artificial light at increased temperature and with chemical additions of substances such as ascorbic acid, hydrogen peroxide and methyl glyoxal⁶.

The only published work to date on ethyl carbamate formation in pot-still whisky has implicated reactions involving copper and described the relative involatility of ethyl carbamate in pot-still distillations^{24,25}. This latter observation, together with reports of hydrocyanic acid involvement in ethyl carbamate formation in stone fruit spirits^{7,18}, make it reasonable to suspect that volatile precursors are involved in ethyl carbamate formation in other distilled spirits.

Initial investigations in this laboratory following analytical method development showed that ethyl carbamate concentrations in blended Scotch whisky ranged between 20 and 75 ppb with an average 48 ppb in the 181 samples examined¹. Further results encompassing 933 samples extended the range for Scotch whisky from 15 to 115 ppb with an average of 43 ppb². Despite average concentrations being well below the 150 ppb Canadian limit (applicable to distilled spirits) and the 125 ppb voluntary US limit (applicable to whiskey), a programme of work was initiated into understanding the mechanisms of ethyl carbamate formation in Scotch whisky. This paper is one of a series associated with that work^{9,16,17,28} and reports studies concerning the post-distillation formation of ethyl carbamate with particular reference to Scotch grain whisky.

For background information, Scotch grain whisky is produced from the fermentation of various cereals (normally wheat or maize), together with a small amount of malted barley, followed by continuous distillation in a Coffey (or Patent) still^{3,14,23,26}. The Coffey stills referred to in this paper, consist of two rectangular copper columns. The first, known as the analyser, separates alcohols from the fermented wash and the second, known as the rectifier, concentrates the ethanol to approximately 94% V/V alcoholic strength at the spirit take-off point. The new-make spirit is then reduced with water to typically 64% V/V strength for maturation in oak casks for at least 3 years and often over 12 years.

EXPERIMENTAL

Ethyl carbamate analysis

Ethyl carbamate (EC) was determined in the freshly distilled spirit and cask samples (at alcoholic strengths greater than 60% V/V) in the presence of n-propyl carbamate as internal standard by gas chromatography-mass spectrometry with mass detection at *m/z* 62 according to the direct injection procedures described by Aylott *et al.*¹. Throughout this paper ethyl carbamate concentrations are normalized to ppb (ng/ml) at 43% V/V; this is the common alcoholic strength of bottled distilled spirits for many export markets.

Ethyl carbamate precursor analyses

Precursors, such as cyanide, cyanate, cyanohydrin, copper cyanide complexes and species described collectively as MC, were determined using the ion chromatographic methods developed by MacKenzie *et al.*¹⁷. All samples for both EC and MC analyses were stored in the dark prior to analysis unless otherwise stated.

Ammonium ion analysis

Ammonium ion was determined by ion chromatography with conductivity detection^{17,27}.

Lactonitrile analysis

Lactonitrile was determined by capillary column gas chromatography with nitrogen specific detection in the presence of 250 ppb ethyl cyanoacetate (Aldrich Chemical Co. Ltd.) as internal standard. The column oven temperature was programmed from 120°C to 220°C at 5°C/min after an initial fast programme from 50 to 120°C at 50°C/min. Splitless vapourizing injection at 230°C with splitting after 30 s was used. Samples and calibration standards (ranging from 0–500 ppb) were analysed on a Varian 6000 gas chromatograph (Varian Associates Ltd.) fitted with a thermionic specific detector for nitrogen and a 30 m × 0.32 mm i.d. fused silica capillary column with chemically bonded DB Wax as stationary phase (J. & W. Scientific Inc.).

Laboratory light box storage

Ethyl carbamate formation in spirit samples was induced in the laboratory by exposing samples in their clear glass bottles to intense fluorescent light for 72 hours at 43°C. The storage cabinet was constructed of Perspex and measured 100 cm × 100 cm × 25 cm. It was illuminated with three 25 Watt fluorescent lamps, heated using a hot water radiator device and thermally insulated.

Maturation studies

Whisky maturation studies were conducted in re-used 500 litre oak casks (butts) stored under normal warehouse conditions.

Grain distillation terminology

The term "Charge" refers to a filling of a spirit receiving vat, and "Period" to a single week of production. Vat volumes range from 8,000 to 70,000 litres depending on distillery.

RESULTS AND DISCUSSION

Initial Results

Preliminary research in 1986 showed that repeat ethyl carbamate analysis on freshly distilled grain scotch spirit samples conducted over a period of 3 weeks showed variable reproducibility, whereas repeat analysis of mature spirit samples showed good reproducibility similar to that reported during analytical method development¹. Successive analyses conducted on samples stored under normal laboratory light conditions between 1 day and 3 weeks after distillation gave either stable or increased ethyl carbamate concentrations but never decreased concentrations (Table I).

Scotch grain whisky, produced at 4 different distilleries during a typical production week, was sampled from cask 3 months into maturation. Samples from single casks, representing each of the Charges produced during that Period, were analysed for ethyl carbamate. Concentrations ranged from < 10 to 80 ppb with an average of 32 ppb for the 36 samples (Table II).

However, ethyl carbamate concentrations in samples of freshly distilled grain spirit, stored in the dark and analysed within 24 h of distillation, appeared to be generally lower than those levels observed in maturing samples (Table III). Concentrations ranged from "not detected" (< 5 ppb up to 20 ppb). Exposure to standardized conditions of intense fluorescent light and heat (43°C) for 3 days in the laboratory light box resulted in ethyl carbamate concentrations in some samples increasing by up to 160 ppb while others hardly changed (Table III). In addition, freshly distilled dark stored new-make spirit from Distillery B was analysed on two occasions within 30 min. of distillation and similarly low ethyl carbamate concentrations (< 20 ppb) were observed.

At this time MacKenzie, a co-author of this paper, during development of an ion chromatographic procedure for determining carbamyl phosphate, found that cyanic acid was the

TABLE I. Ethyl carbamate and MC concentrations in samples of new-make grain spirit stored in clear glass bottles under laboratory light at ambient temperature

Distillery	Sample No.	EC/MC	Concentration, ppb				
			< 1	3	7	14	21
A	2	EC	20	20	20	20	20
		MC	10	5	< 5	< 5	< 5
B	2	EC	45	55	60	NA	120
		MC	260	170	90	45	15
B	3	EC	15	NA	55	55	55
		MC	75	NA	< 5	NA	< 5
C	3	EC	25	25	30	NA	25
		MC	10	5	< 5	< 5	< 5
C	4	EC	35	35	50	NA	50
		MC	110	30	< 5	NA	< 5
D	4	EC	35	NA	45	65	85
		MC	210	NA	30	< 5	< 5

Notes: Concentration units— EC = ppb at 43% V/V alcoholic strength
MC = ppb

NA = Not analysed.

TABLE II. Ethyl carbamate concentrations in grain spirit sampled from cask 3 months into maturation and which was produced during a typical production week at 4 different distilleries

Charge Number	Ethyl carbamate, ppb at 43% v/v alcoholic strength			
	Distillery A	Distillery B	Distillery C	Distillery D
1	15	25	NA	15
2	NA	NA	25	20
3	NA	NA	NA	25
4	NA	20	NA	40
5	NA	30	40	40
6	< 10	35	NA	60
7	30	30	80	NA
8	10	20	20	NA
9	25	30	20	45
10	15	30	50	—
11	20	40	45	—
12	15	40	50	—
13	< 10	—	NA	—
14	—	—	70	—
15	—	—	70	—
Average	17	30	47	35
Range	< 10–30	20–40	20–80	15–60

Notes: NA denotes that a sample was not available. Distilleries A to D each produced 13, 12, 15 and 9 charges of spirit during their production weeks, respectively.

TABLE III. Ethyl carbamate and related species concentrations in freshly distilled new-make grain spirit produced at 4 different distilleries, and subsequently stored in the dark

Distillery	Sample No.	Concentration, ppb							
		Ethyl carbamate initial	Ethyl carbamate induced	MC	Cyanide	MC components Complex	Cyanohydrin	Lactonitrile	Cyanate
A	1	ND	ND	10	10	ND	ND	ND	ND
B	1	15	60	100	10	90	ND	ND	15
C	1	15	120	160	15	120	25	30	ND
C	2	< 10	20	60	10	50	ND	5	ND
D	1	20	180	250	15	200	35	55	ND
D	2	10	150	250	15	210	25	30	ND
D	3		45	65	15	40	10	15	ND

Notes: Concentration units— EC = ppb at 43% V/V alcoholic strength
MC = ppb

ND = Not detected. (The limit of detection for each species was approximately 5 ppb).

MC, MC components and cyanate were determined in the sample prior to induced formation in the laboratory light box. This data was reported, in part, in the corresponding analytical method development paper by MacKenzie *et al.*¹⁷.

active precursor in its conversion into ethyl carbamate. Fresh new-make grain spirit samples were found to contain only trace cyanate concentrations (< 5 ppb), whereas spirit stored for one week in the laboratory contained up to 40 ppb, thus inferring cyanate formation from a precursor. Furthermore, analysis of Coffey still plate deposits showed the presence of a range of copper salts incorporating cyanide, cyanate and thiocyanate¹⁷. MacKenzie's observations and the initial results described above contributed to the discovery that grain spirit could contain trace cyanide in both a free and a bound form, defined by the authors as MC (measurable cyanide). The MC analysis determined hydrogen cyanide, copper cyanide complexes and cyanohydrins as total cyanide.

When the newly developed MC analysis was applied to new-make grain spirit samples stored under normal laboratory light over a period of 3 weeks, low stable concentrations were detected in samples in which ethyl carbamate concentrations were stable and higher MC concentrations, which subsequently decreased, were detected in samples which exhibited increasing ethyl carbamate concentrations (Table I).

Thus these observations suggested that post-distillation ethyl carbamate formation could take place in the presence of

appropriate precursors. In particular, there appeared to be an inverse relationship between ethyl carbamate and MC concentrations during this period. Studies were then initiated on freshly distilled grain spirit in order that ethyl carbamate and its potential precursors could be monitored during maturation.

Ethyl carbamate formation during maturation

New-make grain spirit at 64% V/V alcoholic strength from Distillery C was sampled prior to filling into cask. The sample was analysed for ethyl carbamate, MC, cyanide, copper cyanide complexes, cyanohydrin, cyanate and thiocyanate. The corresponding maturing spirit was monitored in 5 casks for all these species over 6 weeks, and for MC and ethyl carbamate over 1 year.

The average and range of results for this experiment are reported in Table IV and indicate an initial ethyl carbamate concentration of 15 ppb (standardized to 43% V/V alcoholic strength) and an MC concentration of 70 ppb. The MC components comprised approximately 50 ppb cyanide, 15 ppb copper cyanide complexes and 5 ppb cyanohydrin (all of which were expressed as cyanide). During the period under study ethyl carbamate concentrations rapidly increased over the first three

TABLE IV. Average and ranges of concentrations of ethyl carbamate, its precursors and related compounds in 5 casks of new-make grain spirit during the first year of maturation.

	Concentration, ppb									
	Period of experiment									
	0 days	3 days	7 days	14 days	21 days	28 days	42 days	3 months	6 months	1 year
MC	70	41	40	29	25	26	26	20	15	15
Hydrogen cyanide	50	25	19	8	5	<5	<5	—	—	—
Cyanohydrin	5	16	19	21	20	23	24	—	—	—
Copper cyanide complexes	15	ND	ND	ND	ND	ND	ND	—	—	—
Cyanate (2 casks only)	2.4	8.7	10.2	2.6	3.0	3.5	8.0	—	—	—
Thiocyanate (3 casks only)	13	8	7	7	8	8	7	7	—	—
Ethyl carbamate	15	39	48	61	64	55	59	59	58	54

Notes: Range results are given in brackets.

ND = Not detected.

Concentration units— MC and other EC precursors = ppb
EC = ppb at 43% V/V alcoholic strength

weeks of maturation and plateaued at concentrations ranging from 45 to 65 ppb depending on cask.

MC concentrations rapidly decreased over a similar period and levelled at approximately 15 ppb. Hydrogen cyanide concentrations also rapidly decreased to a level below its limit of detection (<5 ppb). Copper cyanide complexes were not detectable when first sampled from cask after three days. Cyanohydrin concentrations increased to a maximum value of approximately 30 ppb. Cyanate concentrations (measured in two casks only) peaked at approximately 15 ppb during the first week of maturation. Thiocyanate concentrations (in 3 casks) initially were 13 ppb and thereafter were approximately 7 ppb. Data for a specific cask is shown graphically in Figure 1. All evidence to date suggests that ethyl carbamate, once formed, will maintain stable concentrations and that any variations observed reflected normal analytical precision¹.

From these results it was postulated that hydrogen cyanide and copper cyanide complexes were important ethyl carbamate precursors present in freshly distilled grain spirit. It was assumed that hydrogen cyanide entered the distillate by ascending the Coffey still rectifier column and copper cyanide complexes, being involatile, descended the rectifier column from areas at and above the spirit take-off point. Hydrogen cyanide exists in equilibrium with copper cyanide complexes and cyanohydrins, such as lactonitrile. Lactonitrile is present due to the slow reaction between cyanide and acetaldehyde and is not considered an ethyl carbamate precursor as the equilibrium is biased towards lactonitrile. Active MC components, such as hydrogen cyanide and copper cyanide complexes, were not detected in mature or bottled whiskies.

Ion chromatographic methods were normally used in these studies to determine cyanohydrins. However, it was found that there was reasonable correlation between these results and those for lactonitrile as determined by capillary column gas chromatography with nitrogen specific detection (Table III). As acetaldehyde is the principal aldehyde in Scotch whisky¹⁵, it was concluded that most of the cyanohydrin monitored was present as lactonitrile. A typical chromatogram from this analysis is shown in Figure 2 with well resolved peaks for lactonitrile and ethyl cyanoacetate (internal standard) eluting after 498.5 and 410 s, respectively. Glycolonitrile, the reaction product of hydrogen cyanide and formaldehyde, was not detected in new-make grain spirit by this technique.

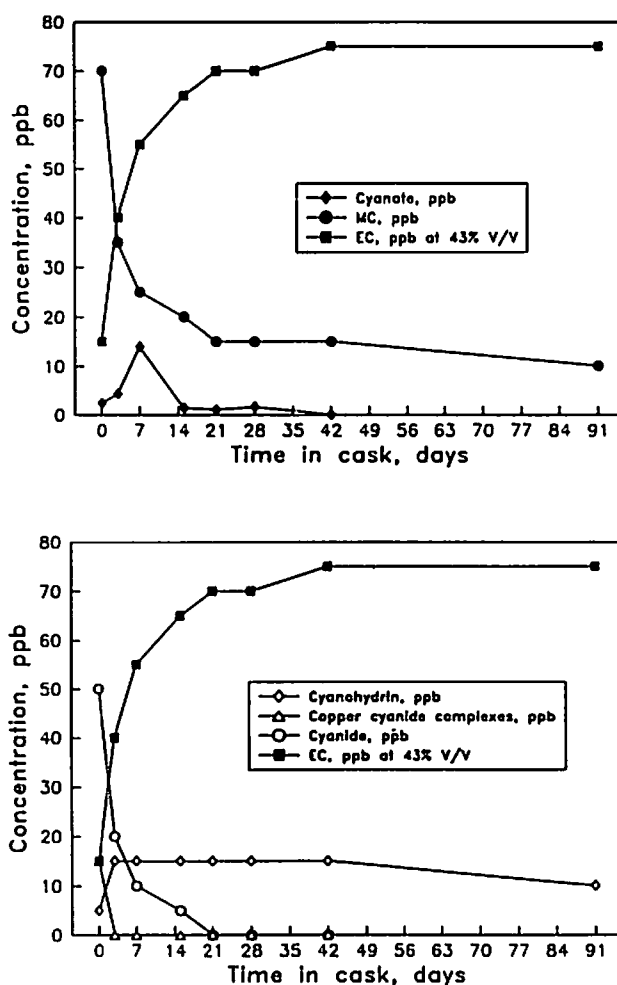


FIG. 1. Graphs showing post-distillation formation of ethyl carbamate and decrease of MC (both collectively and as its components) in grain spirit during the first 13 weeks' maturation. Ethyl carbamate concentrations are normalized to 43% V/V alcoholic strength. Other concentrations are quoted as received at the 64% maturation strength.

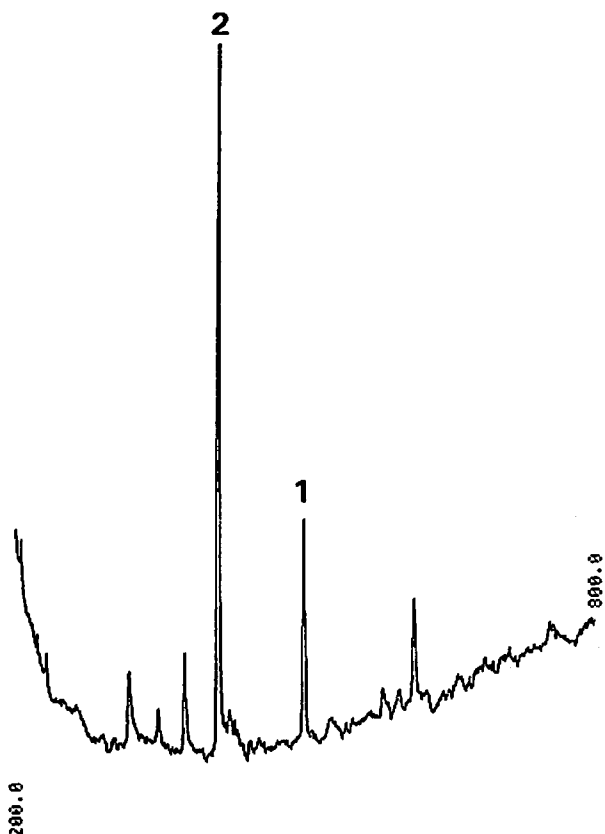


FIG. 2. Capillary column gas chromatogram with nitrogen specific detection of new-make grain spirit containing 85 ppb lactonitrile (1) and 250 ppb ethyl cyanoacetate (2) as internal standard.

Proposed mechanism for ethyl carbamate formation in Scotch grain whisky

Although ethyl carbamate formation may take place during both fermentation and distillation^{4,21,22,29}, its volatility (bp 182–184°C) suggests that ethyl carbamate formed during these processes should not be present in Coffey still distillate²⁸. Therefore, the trace levels of ethyl carbamate observed in freshly distilled grain spirit are thought principally to form in areas at or above the spirit take-off point in the rectifier column. It is further postulated that post-distillation ethyl carbamate formation is principally due to the reaction between ethanol and species yielding cyanide ion with cyanate ion as the reactive intermediate as summarized in Figure 3. The final amount of ethyl carbamate present in mature grain spirit therefore comprises the initial ethyl carbamate level measured immediately after distillation plus that formed from active precursors such as cyanate, hydrogen cyanide and copper cyanide complexes.

Other species potentially involved in ethyl carbamate formation in grain whisky include thiocyanate and cyanogen (Figure 3). For example, laboratory experiments showed that up to 5 ppm ethyl carbamate was formed over 30 days in 40% V/V ethanol initially containing 9 ppm thiocyanate (as potassium thiocyanate) and 55 ppm diacetyl with exposure to normal laboratory light. No ethyl carbamate formation was observed in the absence of diacetyl and formation was enhanced as the proportion of diacetyl to thiocyanate increased. Cyanogen has been used for the synthesis of ethyl carbamate with ethanol in the presence of a dipolar, aprotic solvent and an acid catalyst¹². Another trace congener in Scotch whisky, methyl glyoxal, has recently been associated with the conversion of cyanide into ethyl carbamate⁶ and this reaction can be catalysed by copper¹⁰. One origin of methyl glyoxal in bourbon whiskey is the charred oak barrel used for maturation¹⁹.

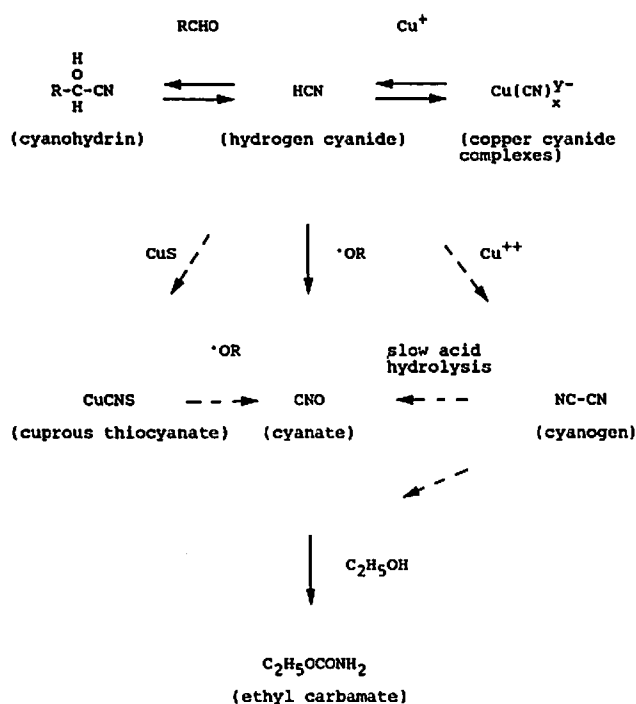


FIG. 3. Postulated ethyl carbamate formation mechanisms in the Coffey still and cask during maturation. Other potential reaction routes are shown with dotted arrows.

Laboratory induced ethyl carbamate formation

Freshly distilled grain spirit, as used in the maturation experiment described earlier, was subjected to storage under various laboratory conditions. Spirit was stored for 17 days under dark and normal laboratory light conditions at ambient temperature and under laboratory light box conditions at 43°C. In addition, a fourth sample was stored under dark conditions but in the presence of a small piece of American oak (with a surface area equivalent to 80 sq. cm. per 100 litres of spirit) simulating the area of contact between maturing spirit and an oak cask.

The results, shown in Table V, indicated that formation of ethyl carbamate and loss of MC occurred most rapidly in the laboratory light box (under hot, intense light conditions) with the reaction complete after three days' storage. Slower conversions occurred with spirit stored in the dark in the presence of wood and under laboratory light conditions. The sample stored in the dark with no additions underwent the slowest conversion.

In a second experiment, post-distillation ethyl carbamate formation was monitored in samples of fresh new-make grain spirit to which an ethanolic extract of oak was added. The extract was prepared by the Soxhlet extraction of 1 cm new oak cubes (20 g) into 150 ml ethanol over 20 h, followed by concentration from 75 ml to 10 ml under vacuum rotary evaporation. The extract was then added to new-make grain spirit at 1 and 5% V/V levels and the resulting solutions were analysed for ethyl carbamate at intervals over 7 weeks.

The results, shown in Figure 4, indicated that the oak extract accelerated post-distillation ethyl carbamate formation in new-make grain spirit, the 5% extract additions having a greater effect than the 1% additions, particularly in the presence of light. Most ethyl carbamate formation took place during the first 24 h and continued slowly thereafter with the reaction virtually complete after 7 weeks.

These and earlier results suggested that the conversion of MC into ethyl carbamate may be accelerated by light induction and also by the interaction with wood in the dark. The action of wood may be demonstrated both from the results generated in cask (Table IV), from the results generated in glass in the

TABLE V. Ethyl carbamate and MC concentrations in new-make grain spirit stored in glass bottles for 17 days under various storage conditions

Storage Condition		Concentration, ppb							
		Period of Storage, days							
Intense light, 43°C	EC	0	0.5	1	2	3	7	17	
	MC	15	40	65	70	80	75	80	
Dark, 20°C, American oak	EC	15	20	5	5	<5	<5	<5	
	MC	70	—	—	—	15	15	15	
Laboratory light 8 h/day, 43°C	EC	15	—	40	45	50	55	60	
	MC	70	—	20	20	20	15	10	
Dark, 20°C	EC	15	—	—	—	20	20	30	
	MC	70	—	—	—	50	50	45	

Notes: Concentration units EC=ppb at 43% V/V alcoholic strength
MC=ppb

This experiment used the same spirit as used in the maturation experiment (Table IV).

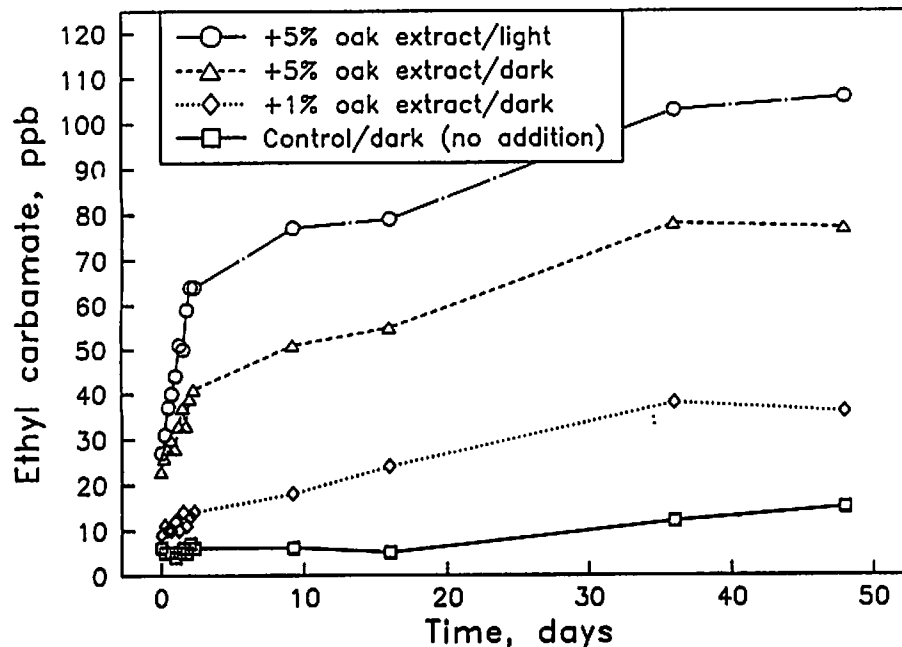


FIG. 4. Graph showing post-distillation ethyl carbamate formation in new-make grain spirit to which an ethanolic oak extract was added under various conditions. Ethyl carbamate concentrations are normalized to 43%V/V alcoholic strength.

presence of oak (Table V) and from the results generated in the presence of oak extract (Figure 4). There appeared to be two different mechanisms for the conversion of MC into ethyl carbamate, one based on light induction and another based on components extracted from oak. It was thought that both mechanisms involved the conversion of cyanide to cyanate by the action of peroxides⁵.

Most of the ethyl carbamate formation in cask appeared to be complete after 3 weeks. Formation in the laboratory light box at 43°C and in the dark in the presence of wood appeared to be complete after about 3 days. Conversion in glass stored samples under laboratory light conditions was still in progress after 17 days and under dark conditions it was found to be still in progress after 13 weeks.

Furthermore, a bottle of mature whisky was exposed to the aforementioned laboratory light box conditions for 15 months after which its ethyl carbamate concentration was found to remain unchanged at 55 ppb. It was therefore, concluded that ethyl carbamate concentrations in mature whisky (that is whisky at least 3 years old) will not increase.

The role of cyanide as an important ethyl carbamate precursor was also demonstrated in an experiment in which 10 ppm cyanide (as potassium cyanide) was added to mature

Scotch whisky at 40% V/V alcoholic strength and then stored for 8 weeks under dark and laboratory light box conditions. The results, shown in Table VI, indicated that the added cyanide was partially converted into ethyl carbamate and ammonium ion with the reaction proceeding further under the influence of heat and light. It is postulated that the cyanide first converted into cyanate which then reacted with water to form ammonium ion, and ethanol to form ethyl carbamate. A similar ethyl carbamate forming reaction has been reported following the addition of hydrogen cyanide to black rum²⁹.

Another worker has recently demonstrated the reaction of radio-labelled ¹⁴C cyanide with 94% V/V new-make grain spirit to give labelled ethyl carbamate with increased incorporation of the labelled ¹⁴C cyanide in the presence of cupric ion¹⁶.

Precursor monitoring as an aid to predicting ethyl carbamate formation

Clearly, it is undesirable for research workers and production quality control to have to wait until all post-distillation ethyl carbamate formation is complete before measuring its levels in maturing spirit. Investigations were therefore conducted in order to establish any relationship between initial MC concen-

TABLE VI. Cyanide ion, ammonium ion and ethyl carbamate concentrations 8 weeks after the addition of 10 ppm cyanide ion to blended Scotch whisky

Addition	Condition	Cyanide ion	Ammonium ion	Concentration, ppm		Percentage nitrogen recovery
				Ethyl carbamate	Total nitrogen	
None	dark	ND	0.7	0.065	—	—
10 ppm cyanide	dark	7.1(3.82)	2.2(1.09)	3.5(0.55)	5.46	101
None	light	ND	0.8	0.070	—	—
10 ppm cyanide	light	5.7(3.07)	2.8(1.51)	5.3(0.83)	5.41	101

Notes: 10 ppm cyanide is equivalent to 5.38 ppm nitrogen
 Results in brackets represent equivalent nitrogen concentrations
 ND = Not detected
 Limit of detection for cyanide = 5 ppb

trations in new-make spirit and ethyl carbamate concentrations following conversion in cask.

Freshly distilled spirit was sampled from 221 individual charges produced throughout the year at 4 grain distilleries and subjected to initial MC analysis and selected samples were subjected to initial ethyl carbamate analysis (as half-period composite samples for each distillery). After 3 months under normal maturation conditions, single casks representing each charge from each distillery were sampled and the spirit analysed for ethyl carbamate and selected samples were also analysed for MC. Sampling after 3 months' maturation was chosen as earlier experiments had shown that ethyl carbamate concentrations had clearly reached their maximum values by this time.

Initial MC and ethyl carbamate results 3 months into maturation are summarized in Table VII. Initial ethyl carbamate concentrations ranged from <5 to 20 ppb with an average of 11 ppb in the 32 samples analysed. This compared with an average concentration of 47 ppb ethyl carbamate in the 221 samples taken 3 months into maturation. MC concentrations measured in 12 cask samples after 3 months' maturation were all < 15 ppb compared with an average 63 ppb initially.

Linear relationships became apparent when initial MC concentrations were plotted against 3 month ethyl carbamate concentrations as shown in Figure 5. The regression line based on all 221 data points was:

$$\text{ethyl carbamate} = 0.5 \text{ MC} + 15$$

The slope of the line was taken to represent the conversion factor between MC and ethyl carbamate and the intercept on

the y-axis to represent the average ethyl carbamate concentration in new-make spirit. If all the MC converted into ethyl carbamate, the theoretical slope of the regression line would be 1.57. Therefore, the observed 0.5 conversion factor represents a 32% conversion of MC into ethyl carbamate during maturation. The remainder is thought to convert into ammonium ion and cyanohydrin. A line representing the 95% confidence interval for the upper band of ethyl carbamate concentrations for the various measurements of MC fell +25 ppb above the fitted regression line. This means that only 1 in 20 samples for a new-make grain spirit MC value would produce an ethyl carbamate concentration above this line. Thus this regression line provided these grain whisky distilleries with a "rule of thumb" for predicting ethyl carbamate concentrations in their mature spirit, namely 'EC = half MC plus 15'.

The individual regression lines for the 4 distilleries are shown in Table VII. Inspection of the graph (Figure 5) shows a number of outlying data points whose removal would aid the production of similar regression lines for each distillery. These outlying points may be associated with unknown production, sampling or analytical variables. In addition, a large proportion of the data points were biased toward low MC and ethyl carbamate concentrations. However, for the purpose of the "rule of thumb" it was considered important to incorporate all the available data in order to represent actual production conditions.

It was noteworthy that lower conversion factors have been observed in spirit where MC existed predominantly as unreactive cyanohydrin rather than as reactive cyanide or copper

TABLE VII. Correlation between initial MC concentrations in new-make grain spirit and ethyl carbamate concentrations 3 months into maturation

Distillery	Number of samples		Concentration, ppb		Regression line	Coefficient of correlation
			Average	Range		
All	221	MC	63	5-350	EC = 0.5MC + 15	0.92
		EC	47	5-180		
A	46	MC	29	5-190	EC = 0.37MC + 12	0.90
		EC	23	10-100		
B	73	MC	54	10-350	EC = 0.53MC + 19	0.92
		EC	48	15-180		
C	59	MC	39	5-125	EC = 0.47MC + 16	0.71
		EC	34	5-85		
D	43	MC	147	15-310	EC = 0.47MC + 20	0.90
		EC	88	15-160		

Notes: Concentration units— EC = ppb at 43% V/V alcoholic strength
 MC = ppb

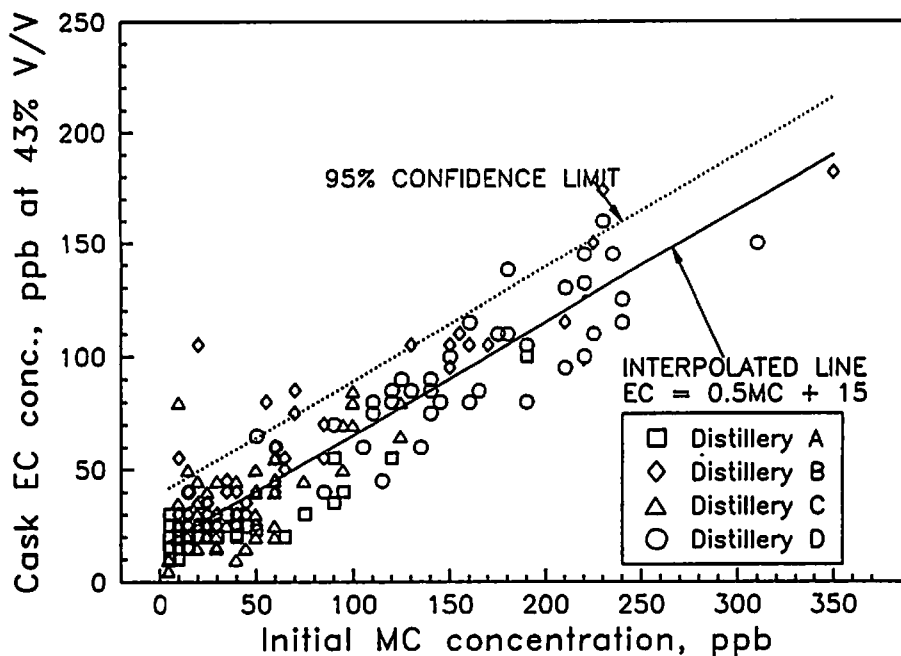


FIG. 5. Correlation between initial MC concentrations in 221 samples of new-make grain spirit produced at 4 different distilleries with ethyl carbamate concentrations in the same spirit sampled 3 months into maturation. Ethyl carbamate concentrations are normalized to 43% V/V alcoholic strength and MC concentrations are quoted as received at 94% V/V distillation strength. Regression analyses are reported in Table VI.

cyanide complexes. The copper surfaces within the Coffey still were also thought to be important as a catalyst and so different conversion factors may be encountered with stainless steel distillation systems (where copper cyanide complexes will be absent).

Further demonstration that low initial MC concentrations in freshly distilled grain spirit lead to low ethyl carbamate concentrations in mature spirit were obtained in a separate monitoring of 14 charges from one period of Distillery B production. Initial MC and ethyl carbamate concentrations ranged from <5 to 10 ppb and 5 to 10 ppb, respectively. After 5 months matur-

ation in cask, ethyl carbamate concentrations ranged from 5 to 15 ppb.

In addition, the relationships between MC, ethyl carbamate and copper concentrations were examined. A typical set of results, representing production during a single Period at Distillery B, is shown in Figure 6. Initial ethyl carbamate concentrations in the half-period new-make spirit composites were <10 and 15 ppb and MC concentrations after 3 months in cask ranged between 10 and 15 ppb. The graph showed approximate relationships between initial MC and copper concentrations and ethyl carbamate concentrations measured

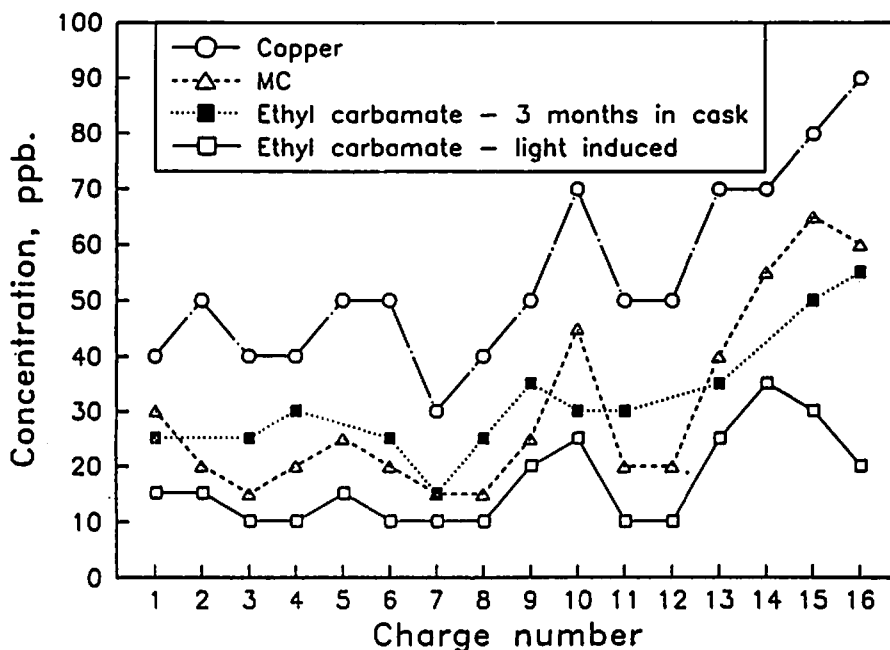


FIG. 6. Typical relationships between ethyl carbamate, MC and copper concentrations in grain spirit produced at Distillery B during a typical production period. Ethyl carbamate concentrations are normalized to 43% V/V alcoholic strength and MC and copper concentrations are quoted as received at 94% V/V distillation strength.

after light box induction at 43°C and after 3 months in cask.

A tendency for MC, copper and subsequent ethyl carbamate concentrations to increase during the production period was found to be an operating characteristic of individual distilleries. The resting of a Coffey still during the mid-week shutdown between the distillation of long and short fermentations was found to give reduced concentrations of all these species when the still was restarted. These congeneric effects are discussed further in the related engineering paper dealing with the grain distillery process by Whitby *et al.*²⁸

The relationship between MC and copper concentrations was associated with MC existing as copper cyanide complexes¹⁷. Light box induced ethyl carbamate concentrations were slightly lower than those observed in cask. However, the reverse relationship has been observed in samples with higher MC concentrations (>150 ppb), and therefore caution is required in using light box ethyl carbamate induction for predicting final ethyl carbamate concentrations in mature whisky.

In summary, the levels of MC in freshly distilled spirit may be variable and appear dependent upon distillery conditions, production period and charge number. This has been demonstrated by monitoring the same distilleries at other times when different ranges of MC and 3 month ethyl carbamate concentrations were observed. However, the general relationship between MC and ethyl carbamate was maintained. Thus it was concluded that the monitoring of initial MC concentrations in freshly distilled grain spirit is a useful guide for predicting final ethyl carbamate concentrations in mature spirit. The accuracy of the "rule of thumb" estimate may then be checked by monitoring ethyl carbamate levels in cask samples 3 months into maturation.

Post-distillation ethyl carbamate formation in Scotch malt whisky and bourbon whiskey

Preliminary results indicated the potential for post-distillation formation of ethyl carbamate from MC in both Scotch malt and American bourbon whiskeys. It appeared that higher ethyl carbamate concentrations may exist in freshly distilled malt spirit and new-distillate bourbon and that MC to ethyl carbamate conversion may be more rapid compared to that observed in Scotch grain whisky.

A sample of freshly distilled Highland malt spirit was found to contain 70 ppb MC and 45 ppb ethyl carbamate. The spirit was filled into 3 casks and monitored at 1, 3, 7, 14 days, 3 months and finally after 6 months in cask. Since day 1 of the monitoring programme ethyl carbamate concentrations averaged 70 ppb for each cask and MC was not detected (<5 ppb). This suggested that the conversion of MC into ethyl carbamate was essentially complete after 24 hours in cask.

A fresh sample of high wines from a Kentucky bourbon whiskey distillery was found to contain 40 ppb MC and 30 ppb ethyl carbamate. Following maturation in a new 50 US gallon oak barrel for one month the ethyl carbamate concentration had risen to 50 ppb. Initial results from subsequent studies have indicated that MC conversion into ethyl carbamate also takes place within the first 24 hours in cask.

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