FOOD COMPOSITION AND ADDITIVES

Gas Chromatographic Determination of Volatile Congeners in Spirit Drinks: Interlaboratory Study

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An interlaboratory study of a gas chromatographic (GC) method for the determination of volatile congeners in spirit drinks was conducted; 31 laboratories from 8 countries took part in the study. The method uses GC with flame ionization detection and incorporates several quality control measures which permit the choice of chromatographic system and conditions to be selected by the user. Spirit drink samples were prepared and sent to participants as 10 blind duplicate or split-level test materials for the determination of 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl ethanoate (ethyl acetate), butan-1-ol (*n*-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (*n*-propanol), and ethanal (acetaldehyde). The precision of the method for 9 of the 10 analytes was well within the range predicted by the Horwitz equation. The precision of the most volatile analyte, ethanal, was just above statistically predicted levels. This method is recommended for official regulatory purposes.

Council Regulation (EEC) No. 1576/89 (1) defines the description and composition of spirit drinks. The European Commission are currently drawing up legislation (2) that will prescribe methods of analysis to be used to monitor compliance with 1576/89.

Congeners are volatile substances formed along with ethanol during fermentation and maturation of spirit drinks and can be used to provide both qualitative and quantitative information for labelling purposes. In addition proposed European legislation specifically defines the volatile congener component of volatile substances as comprising the sum of: ethanal (acetaldehyde) and the ethanal fraction contained in 1,1-diethoxyethane (acetal) expressed as ethanal, and the sum of propan-1-ol (*n*-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (*n*-butanol), butan-2-ol (sec-butanol), 2-methylbutan-1-ol (active amyl alcohol) and 3-methylbutan-1-ol (isoamyl alcohol). Regulation 1576/89 also prescribes limits for methanol in wine spirit, brandy, grape marc, and fruit spirit drinks. The objective of this work, sponsored by the European Commission, was to formally validate methodology that would be suitable for use in monitoring compliance with 1576/89.

Interlaboratory Study

Collaborators from 31 laboratories in France, Germany, Greece, The Netherlands, Portugal, Spain, Ireland, and the United Kingdom took part in the study.

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Table 1. Sample scheme used in the interlaboratory study

Sample code	Test material No.	Sample description	Experimental design
A	30 & 40	Brandy	Blind duplicates
B	38 & 42	Kirsch	Blind duplicates
C	32 & 44	Grappa	Blind duplicates
D1 & D2	36 & 33	Whisky	Split level
E1 & E2	34 & 37	Rum	Split level

Pre-Trial Studies

Substantial testing of the method was performed prior to the interlaboratory study. The method was first assessed in-house by the coordinating laboratory and tested externally by 2 peer laboratories (Faculté d'OEnologie, University of Bordeaux 2, France and Bundesinstitut fur Gesundheitlichen Verbraucherschutz und Veterinarmedizin, Berlin, Germany). The peer laboratories tested the method and checked the designated assigned value attributed to the test materials by the coordinating laboratory during homogeneity testing. Finally, the participants were allowed to familiarize themselves with the method in a "pre-trial study." For the latter study each participant was sent a detailed method protocol as well as 4 test materials (2 sets of blind duplicates) to analyze. The participants were advised that for the purposes of the study it was not necessary to measure the alcoholic strength of the samples, and should therefore report results in µg/g and not g/100 L absolute alcohol as described in the method. They were also asked to submit copies of their chromatograms.

The results and chromatograms obtained from participants were scrutinized by the coordinating laboratory to assess whether their analytical systems were appropriate and met the specified quality assurance criteria. This assessment was communicated to participants to enable improvements to be made in their analysis where necessary. Minor amendments to the written method were made at this stage and circulated to participants before commencement of the main trial.

Sample Scheme

For the main trial, participants were sent 10 test materials, a method protocol, an instruction sheet, a result sheet, and an acknowledgement sheet. They were asked to ensure that each test material was analyzed once only, but to report the mean of 2 gas chromatographic (GC) injections. They were instructed to store the samples at 4°C prior to analysis and to submit copies of their chromatograms in addition to their results. The results were to be reported in µg/g.

The 10 test materials comprised 3 sets of blind duplicates and 2 split level (Youden) pairs. The test materials consisted of rum, whisky, brandy, kirsch, and grappa spirit drinks, with and without fortified levels of volatile congeners. The sample scheme is given in Table 1.

Preparation of Samples

Samples were purchased at retail stores from various parts of Europe. Samples A to C were prepared and dispensed into 15 mL amber vials by an external contractor (Laboratory of the Government Chemist, Teddington, UK) and dispensed into 15 mL amber vials. Split level samples D and E were produced by the coordinating laboratory. Sample D1 was used as purchased. Sample D2 was fortified by adding 25 mL of a solution containing all analytes (at 2000–4800 µg/mL) to 2.5 L D1 in a volumetric flask. Both samples, D1 and D2, were stored at <5°C prior to being dispensed into 15 mL amber vials. Sample E1 was used as purchased. Sample E2 was fortified by adding 25 mL of a solution containing all analytes (at 840 to 4500 µg/mL) to 2.5 L E1 in a volumetric flask. Both samples, E1 and E2, were stored at <5°C prior to being dispensed into 15 mL amber vials. All prepared test materials were stored at <5°C pending dispatch to participants. All test materials were labelled with their assigned sample code numbers by the coordinating laboratory.

Table 2. Suggested columns and GC conditions

Column (s)	Column temp.	Injector temp., °C	Detector temp., °C
(1) Retention gap 1 m × 0.32 mm id connected to a CP-WAX 57 CB column 50 m × 0.32 mm id, 0.2 mm film thickness (stabilized polyethylene glycol) followed by a Carbowax 400 column 50 m × 0.32 mm id, 0.2 mm film thickness	35°C for 17 min, 35° to 70°C at 12°C/min, hold at 70°C for 25 min	150	250
(2) Retention gap 1 m × 0.32 mm id connected to a CP-WAX 57 CB column 50 m × 0.32 mm id, 0.2 mm film thickness (stabilized polyethylene glycol)	35°C for 10 min, 35° to 110°C at 5°C/min, 110° to 190°C at 30°C/min, hold at 190°C for 2 min	260	300
(3) Packed column (5% CW 20M, Carbopak B), 2 m × 2 mm id	65°C for 4 min, 65° to 140°C at 10°C/min, hold at 140°C for 5 min, 140° to 150°C at 5°C/min, hold at 150°C for 3 min	200	250

Table 3. Summary of calculated statistical parameters for test materials 30 and 40 (brandy), ethanal to propan-1-ol[a]

	Analyte						
Statistical parameter	Ethanal	Ethyl acetate	Acetal	Total ethanal	Methanol	Butan-2-ol	Propan-1-ol
Assigned value (homogeneity testing), µg/g	59.6	97.3	33.8	72.2	329.1	5.0	88.4
Mean, µg/g	63.4	96.8	35.04	76.5	319.8	5.88	86.4
n	31	31	31	31	31	31	31
*n*c	1	5	7	7	1	6	0
Outliers	2	2	4	1	4	4	2
n_1	28	24	20	23	26	21	29
r	9.3	6.2	1.6	9.8	12.3	1.1	8.3
s_r	3.3	2.2	0.58	3.5	4.4	0.40	3.0
RSD_r	5.2	2.3	1.7	4.6	1.4	6.8	3.4
Ho_r	0.9	0.4	0.3	0.8	0.3	0.8	0.6
R	33.5	17.9	11.8	35.2	35.2	2.5	14.8
s_R	12	6.4	4.2	13	13	0.89	5.3
RSD_R	18.9	6.6	12.1	16.4	3.9	15.2	6.1
Ho_R	2.2	0.8	1.3	2.0	0.6	1.2	0.8

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 4. Summary of calculated statistical parameters for test materials 30 and 40 (brandy), butan-1-ol to total higher alcohols[a]

	Analyte					
Statistical parameter	Butan-1-ol	2-Methylpropan-1-ol	2-Methylbutan-1-ol	3-Methylbutan-1-ol	Combined 2- and 3-methylbutan-1-ol	Total higher alcohols
Assigned value (homogeneity testing), µg/g	3.9	178.5	103.3	493.3	596.6	872.4
Mean, µg/g	3.79	174.2	113.0	459.4	571.3	842.0
n	31	31	28	28	31	31
*n*c	7	0	0	0	0	0
Outliers	4	3	3	5	4	4
n_1	20	28	25	23	27	27
r	1.1	6.4	6.0	13.9	16.8	26.9
s_r	0.43	2.3	2.1	5.0	6.0	9.6
RSD_r	11.2	1.3	1.9	1.1	1.1	1.1
Ho_r	1.3	0.3	0.4	0.3	0.3	0.3
R	1.7	24.9	20.8	83.4	93.2	117.6
s_R	0.59	8.9	7.4	29.8	33	42
RSD_R	15.7	5.1	6.6	6.5	5.8	5.0
Ho_R	1.2	0.7	0.8	1.0	1.0	0.9

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 5. Summary of calculated statistical parameters for test materials 38 and 42 (kirsch), ethanal to propan-1-ol[a]

	Analyte						
Statistical parameter	Ethanal	Ethyl acetate	Acetal	Total ethanal	Methanol	Butan-2-ol	Propan-1-ol
Assigned value (homogeneity testing), µg/g	62.2	974	33.2	74.6	2262	255	2680
Mean, µg/g	71.7	1046	36.46	85.3	2245	250.2	3541
n	31	31	31	31	31	31	31
*n*c	1	5	7	7	1	1	0
Outliers	4	2	3	5	3	3	4
n_1	26	24	21	19	27	27	27
r	5.3	40.7	2.4	3.5	74.4	6.1	68.5
s_r	1.9	15	0.84	1.3	27	2.2	24
RSD_r	2.6	1.4	2.3	1.5	1.2	0.9	0.7
Ho_r	0.5	0.4	0.4	0.3	0.4	0.2	0.2
R	38.9	221.9	12.2	41.8	278.3	35.5	407.2
s_R	14	79	4.4	15	99	13	150
RSD_R	19.4	7.6	12.0	17.5	4.4	5.1	4.1
Ho_R	2.3	1.4	1.3	2.1	0.9	0.7	0.9

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 6. Summary of calculated statistical parameters for test materials 38 and 42 (kirsch), butan-1-ol to total higher alcohols[a]

	Analyte					
Statistical parameter	Butan-1-ol	2-Methylpropan-1-ol	2-Methylbutan-1-ol	3-Methylbutan-1-ol	Combined 2- and 3-methylbutan-1-ol	Total higher alcohols
Assigned value (homogeneity testing), µg/g	5.99	111.9	43.2	266.3	309.5	3362
Mean, µg/g	5.57	111.7	48.3	242.7	291.7	4237
n	31	31	28	28	31	31
*n*c	5	0	0	0	0	0
Outliers	4	0	2	5	2	6
n_1	22	31	26	23	29	25
r	0.6	4.5	4.2	6.6	9.3	85.0
s_r	0.20	1.6	1.5	2.4	3.3	30
RSD_r	3.6	1.4	3.1	1.0	1.1	0.7
Ho_r	0.4	0.3	0.5	0.2	0.3	0.2
R	1.5	24.9	10.7	35.4	58.0	323.4
s_R	0.55	8.9	3.8	13	21	120
RSD_R	9.8	8.0	7.9	5.2	7.1	2.7
Ho_R	0.8	1.0	0.9	0.7	1.0	0.6

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 7. Summary of calculated statistical parameters for test materials 32 and 44 (grappa), ethanal to propan-1-ol[a]

	Analyte						
Statistical parameter	Ethanal	Ethyl acetate	Acetal	Total ethanal	Methanol	Butan-2-ol	Propan-1-ol
Assigned value (homogeneity testing), µg/g	129.9	118.4	65.2	154.2	1334	26.6	162.9
Mean, µg/g	130.4	120.3	68.5	156.5	1326	27.57	159.1
n	31	31	31	31	31	31	31
*n*c	1	5	7	7	1	1	1
Outliers	3	1	2	2	3	1	3
n_1	27	25	22	22	27	29	27
r	19.1	7.2	4.4	18.3	62.5	2.5	10.0
s_r	6.8	2.6	1.6	6.5	22	0.87	3.6
RSD_r	5.2	2.1	2.3	4.2	1.7	3.2	2.3
Ho_r	1.0	0.4	0.4	0.8	0.5	0.5	0.5
R	62.4	22.9	25.0	67.4	169.1	8.9	18.2
s_R	22	8.2	8.9	24.1	60	3.2	6.5
RSD_R	17.1	6.8	13.0	15.4	4.6	11.5	4.1
Ho_R	2.2	0.9	1.5	2.1	0.8	1.2	0.6

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 8. Summary of calculated statistical parameters for test materials 32 and 44 (grappa), butan-1-ol to total highers alcohols[a]

	Analyte					
Statistical parameter	Butan-1-ol	2-Methylpropan-1-ol	2-Methylbutan-1-ol	3-Methylbutan-1-ol	Combined 2- and 3-methylbutan-1-ol	Total higher alcohols
Assigned value (homogeneity testing), µg/g	7.38	186.9	87.9	304.6	392.5	867
Mean, µg/g	7.54	185.0	91.6	288.4	380.6	757
n	31	31	28	28	31	31
*n*c	3	0	0	0	0	0
Outliers	6	1	3	4	1	3
n_1	22	30	25	24	30	28
r	1.2	6.9	4.7	9.6	16.4	34.9
s_r	0.43	2.5	1.7	3.4	5.8	13
RSD_r	5.6	1.3	1.8	1.2	1.5	1.7
Ho_r	0.7	0.3	0.3	0.3	0.4	0.4
R	2.3	27.2	18.4	58.8	68.5	105.9
s_R	0.82	9.7	6.6	21	24	38
RSD_R	10.8	5.2	7.2	7.3	6.4	5.0
Ho_R	0.9	0.7	0.9	1.1	1.0	0.9

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

Table 9. Summary of calculated statistical parameters for split level test materials 33 and 36 (whisky), ethanal to propan-1-ol[a]

	Analyte[b]					
Statistical parameter	Ethanal	Ethyl acetate	Acetal	Total ethanal	Methanol	Propan-1-ol
Assigned value (homogeneity testing), µg/g	45.4, <LOQ	122.6, 98	27.50, <LOQ	55.7, <LOQ	86.4, 73.0	272.8, 226.2
Mean, µg/g	38.4, 13.8	112.5, 91.8	20.36, 6.60	45.4, 15.8	83.0, 61.5	272.1, 229.3
n	31	31	31	31	31	31
*n*c	1	5	10	7	2	0
Outliers	3	2	4	3	1	2
n_1	27	24	17	21	28	29
r	11.6	5.8	2.3	12.2	4.3	6.4
s_r	4.1	2.1	0.82	4.4	1.5	2.3
RSD_r	15.8	2.0	6.1	14.2	2.1	0.9
Ho_r	2.4	0.4	0.9	2.3	0.4	0.2
R	19.1	17.5	4.0	20.3	12.5	25.2
s_R	6.8	6.2	1.4	7.3	4.5	9.0
RSD_R	26.2	6.1	10.7	23.7	6.2	3.6
Ho_R	2.7	0.8	1.0	2.5	0.7	0.5

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

[b] As butan-2-ol levels were at or below the limit of quantitation, no precision parameters were calculated for this analyte.

Homogeneity

Homogeneity was assessed with internationally agreed procedures (3). Five randomly selected containers were analyzed using the method, then injected in duplicate. Homogeneity was assessed using one-way analysis of variance (ANOVA; 3) and if necessary by a subsequent *F*-test. Only 3 out of the total 63 analyte/matrix combinations did not meet the criteria for homogeneity. This occurred for acetal in samples D2 (whisky), and E1 (rum), and ethanal in sample E2 (rum). However, as the matrixes were assessed to be homogenous for all the remaining analytes, these results were also assessed as satisfactory.

Stability

Samples were analyzed on 3 separate occasions during the lifetime of the study: (*1*) during homogeneity testing, (*2*) prior to the commencement of the trial proper, and (*3*) at the end of the trial proper. Each time a set of test materials was analyzed in duplicate using the candidate method. The levels obtained for most of the analytes/samples remained stable throughout the period of the trial. The main exceptions to this were the more volatile analytes (i.e., ethanal, ethyl acetate, and acetal) in some matrixes. This variability could be attributed to the greater imprecision of the method for these analytes. It would also appear that the level of propan-1-ol in kirsch had depleted by the time of the final stability testing. However, this was not deemed to be significant as all the trial results had been submitted by this time.

METHOD

Scope and Field of Application

The spirit drinks that can be analyzed using this method include whisky, brandy, rum, wine spirit, fruit spirit, and grape marc spirit. 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl ethanoate (ethyl acetate), butan-1-ol (*n*-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (*n*-propanol), and ethanal (acetaldehyde) in spirit drinks can be determined using GC. The concentrations of the analytes are expressed as g/100 L absolute alcohol (the alcoholic strength of the product must be determined prior to analysis).

Principle

Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, into a GC system. A suitable internal standard (for example pentan-3-ol) is added to the spirit drink prior to injection. The congeners are separated by temperature programming on a suitable column and are detected using a flame ionization detector (FID). The concentration of each congener is determined with respect to the internal standard from response fac-

Table 10. Summary of calculated statistical parameters for split level test materials 33 and 36 (whisky), 2-methylpropan-1-ol to total higher alcohols[a]

	Analyte[b]				
Statistical parameter	2-Methylpropan-1-ol	2-Methylbutan-1-ol	3-Methylbutan-1-ol	Combined 2- and 3-methylbutan-1-ol	Total higher alcohols
Assigned value (homogeneity testing), µg/g	292.7, 241.7	79.5, 55.9	148.7, 126.4	228.2, 182.3	793.7, 650.2
Mean, µg/g	291.0, 246.8	72.1, 45.2	142.2, 120.4	214.0, 165.4	777.4, 642.3
n	31	28	28	31	31
*n*c	0	0	0	0	0
Outliers	5	1	1	1	3
n_1	26	27	27	30	28
r	5.0	6.4	6.6	9.4	16.1
s_r	1.8	2.3	2.4	3.4	5.8
RSD_r	0.7	3.9	1.8	1.8	0.8
Ho_r	0.1	0.7	0.4	0.4	0.2
R	16.9	13.3	23.8	32.0	71.3
s_R	6.0	4.7	8.5	11	25.5
RSD_R	2.2	8.1	6.5	6.0	3.6
Ho_R	0.3	0.9	0.8	0.8	0.6

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

[b] As butan-1-ol levels were at or below the limit of quantitation, no precision parameters were calculated for this analyte.

tors, which are obtained during calibration using the prescribed chromatographic conditions.

Apparatus

(**a**) *Apparatus capable of measuring the density and alcoholic strength.*

(**b**) *Analytical balance.*—Capable of measuring to 4 decimal places.

(**c**) *Capillary GC.*—Temperature programmed, fitted with a FID and integrator or other data handling system capable of measuring peak areas or peak heights.

(**d**) *GC column(s).*—Capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is at least 1.3. The peak symmetry should ideally be between 0.5–1.5. Suitable GC columns and conditions are given in Table 2.

Reagents

Chemicals should be of a purity >99%, free from other congeners at test dilution (this may be confirmed by injection of individual congener standards at the test dilution) and water of at least grade 3 as defined in ISO 3696. The reagents should be replaced at 6 month intervals. Acetal and acetaldehyde must be stored in the dark at <5°C; all other reagents may be stored at room temperature.

(**a**) *Ethanol absolute*.

(**b**) *Methanol.*

(**c**) *Propan-1-ol.*

(**d**) *2-Methylpropan-1-ol.*

(**e**) *Pentan-3-ol*.—Other suitable internal standards are pentan-1-ol, 4-methylpentan-1-ol, and methyl nonanoate.

(**f**) *2-Methylbutan-1-ol.*

(**g**) *3-Methylbutan-1-ol.*

(**h**) *Ethyl acetate.*

(**i**) *Butan-1-ol.*

(**j**) *Butan-2-ol.*

(**k**) *Acetaldehyde.*

(**l**) *Acetal.*

(**m**) *Ethanol solution, 40 + 60.*—To 400 mL ethanol, add 600 mL distilled water and mix.

(**n**) *Standard solutions*.—Standard solutions must be stored at <5°C and must be prepared freshly on a monthly basis. Masses of components and solutions should be recorded to the nearest 0.1 mg.

(**o**) *Standard solution A.*—Pipette 3.0 mL of each analyte (methanol, propan-1-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, ethyl acetate, butan-1-ol, butan-2-ol, acetaldehyde, and acetal) into a 100 mL volumetric flask, containing ca 60 mL ethanol solution to minimize component evaporation, make up to volume with ethanol solution, and mix thoroughly. Record the weight

Table 11. Summary of calculated statistical parameters for split level test materials 34 and 37 (rum), ethanal to propan-1-ol[a]

Statistical parameter	Analyte						
	Ethanal	Ethyl acetate	Acetal	Total ethanal	Methanol	Butan-2-ol	Propan-1-ol
Assigned value (homogeneity testing), µg/g	31.4, 59.6	95.5, 113.2	13.5, 26.3	36.4, 69.4	15.2, 28.6	4.49, 13.82	176.6, 226.4
Mean, µg/g	28.6, 52.2	99.1, 117.0	15.1, 28.3	32.7, 61.8	18.6, 28.9	5.83, 14.12	177.1, 221.1
n	31	31	31	31	31	31	31
*n*c	1	5	7	7	2	6	0
Outliers	2	2	3	2	4	3	2
n_1	28	24	21	22	25	22	29
r	10.1	7.3	5.3	10.0	3.8	1.8	9.1
s_r	3.6	2.6	1.9	3.6	1.3	0.64	3.3
RSD_r	8.9	2.4	8.7	7.6	5.6	6.4	1.6
Ho_r	1.5	0.5	1.3	1.3	0.9	0.9	0.3
R	25.1	20.0	8.7	25.2	7.9	2.4	22.7
s_R	8.9	7.1	3.1	9.0	2.8	0.87	8.1
RSD_R	22.2	6.6	14.2	19.1	11.8	8.7	4.1
Ho_R	2.4	0.8	1.4	2.1	1.2	0.8	0.6

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

of the flask, each component added, and the total final weight of the contents. *Note*: It is preferable to add acetal and acetaldehyde last in order to minimize losses through evaporation.

(**p**) *Standard solution B.*—Pipette 3 mL pentan-3-ol, or other suitable internal standard, into a 100 mL volumetric flask containing ca 80 mL ethanol solution, make up to volume with ethanol solution, and mix thoroughly. Record the weight of the flask, the weight of internal standard added, and the total final weight of the contents.

(**q**) *Standard solution C.*—Pipette 1 mL solution A and 1 mL solution B into a 100 mL volumetric flask containing ca 80 mL ethanol solution, make up to volume with ethanol solution, and mix thoroughly. Record the weight of the flask, each component added, and the total final weight of the contents.

(**r**) *Standard solution D.*—In order to maintain analytical continuity, prepare a quality control standard using the previously prepared standard A. Pipet 1 mL solution A into a 100 mL volumetric flask containing ca 80 mL ethanol solution, make up to volume with ethanol solution, and mix thoroughly. Record the weight of the flask, each component added, and the total final weight of the contents.

(**s**) *Standard solution E.*—Pipet 10 mL solution B into a 100 mL volumetric flask containing ca 80 mL ethanol solution, make up to volume with ethanol solution, and mix thoroughly. Record the weight of the flask, each component added, and the total final weight of the contents.

(**t**) *Standard solutions used to check the linearity of response of FID.*—Into separate 100 mL volumetric flasks containing ca 80 mL ethanol solution, pipet 0, 0.1, 0.5, 1.0, and 2.0 mL solution A and 1 mL solution B, make up to volume with ethanol solution, and mix thoroughly. Record the weight of the flask, each component added, and the total final weight of the contents.

(**u**) *QC standard solution.*—Pipet 9 mL standard solution D and 1 mL standard solution E into a weighing vessel and mix thoroughly. Record the weight of the flask, each component added, and the total final weight of the contents.

Procedure

(**a**) *Alcoholic strength.*—On receipt, the apparent alcoholic strength of each sample is measured. When not in use, samples should be stored at <5ºC.

(**b**) *Samples.*—Weigh an appropriate sealed weighing vessel and record the weight. Pipet 9 mL sample into the vessel and record the weight. Add 1 mL standard solution E and record the weight. Shake the sample vigorously (at least 20 inversions). Samples must be stored at <5°C prior to analysis in order to minimize any volatile losses.

(**c**) *Blank.*—Weigh an appropriate sealed weighing vessel and record the weight. Pipette 9 mL 400 mL/L ethanol solution into the vessel and record the weight. Add 1 mL standard solution E and record the weight. Shake the test material vigorously (at least 20 inversions). Samples must be stored at <5°C prior to analysis in order to minimize any volatile losses.

(**d**) *Preliminary test.*—Inject standard solution C to ensure that all the analytes are separated with a minimum resolution of 1.3 (except 2-methylbutan-1-ol and 3-methylbutan-1-ol)

Table 12. Summary of calculated statistical parameters for split level test materials 34 and 37 (rum), 2-methylpropan-1-ol to total higher alcohols[a]

	Analyte[b]				
Statistical parameter	2-Methylpropan-1-ol	2-Methylbutan-1-ol	3-Methylbutan-1-ol	Combined 2- and 3-methylbutan-1-ol	Total higher alcohols
Assigned value (homogeneity testing), µg/g	112.9, 133.3	39.1, 57.4	218.2, 258.2	257.3, 315.6	551, 691
Mean, µg/g	116.0, 133.9	39.5, 61.5	212.3, 245.6	251.4, 304.7	549, 674
n	31	27	27	31	31
*n*c	0	0	0	0	0
Outliers	6	2	6	0	0
n_1	25	25	21	31	31
r	2.1	6.3	9.1	18.4	36.7
s_r	0.74	2.3	3.2	6.6	13
RSD_r	0.6	4.5	1.4	2.4	2.1
Ho_r	0.1	0.8	0.3	0.5	0.5
R	17.4	12.5	18.7	51.4	100.1
s_R	6.2	4.5	6.7	18.4	36
RSD_R	5.0	8.8	2.9	6.6	5.9
Ho_R	0.6	1.0	0.4	1.0	1.0

[a] *n* = total number of sets of data submitted; *n*c = number of results excluded from statistical analysis due to noncompliance; outliers = number of results excluded from statistical analysis due to determination as outliers by either Cochran's or Grubbs' tests; n_1 = number of results used in statistical analysis.

[b] As butan-1-ol levels were at or below the limit of quantitation, no precision parameters were calculated for this analyte.

and that all the analytes have a peak symmetry factor between 0.5 and 1.5.

(**e**) *Calibration.*—Ensure that the response is linear by successively analyzing in triplicate each of the linearity standard solutions containing internal standard. From the peak areas or peak heights for each injection, calculate the ratio R for each congener and plot a graph of R vs the concentration ratio of congener to internal standard, C. A linear plot should be obtained, with a correlation coefficient of at least 0.99.

$$R = \frac{\text{Peak area or height of congener}}{\text{peak area or height of IS}}$$

$$C = \frac{\text{Concn of congener (µg / g)}}{\text{concn of IS (µg / g)}}$$

(**f**) *Determination.*—Inject standard solution C and 2 QC standard solutions. Follow with unknown samples inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C after every 5 samples.

Calculation

Measure either peak areas or peak heights for congener and internal standard peaks. From the chromatogram of the injection of standard solution C, calculate response factors for each congener with the following equation:

$$\text{Response factor} = \frac{\text{peak area or height IS}}{\text{peak area or height congener}} \times \frac{\text{concn congener (µg / g)}}{\text{concn IS (µg / g)}} \quad (1)$$

where concn congener is the concentration of congener in solution C and concn IS is the concentration of internal standard in solution C. The concentration of each congener in the samples is calculated with the following equation:

$$\text{Congener concentrations, µg/g} = \frac{\text{peak area or height congener}}{\text{peak area or height IS}} \times \frac{M_{IS}\,(g)}{M_{SAMPLE}\,(g)} \times \text{concn IS (µg / g)} \times \text{RF} \quad (2)$$

where M_{SAMPLE} is the mass of sample; M_{IS} is the mass of internal standard; concn IS is the concentration of internal standard in solution E; and RF is the response factor calculated using the equation above.

Table 13. Comparison of columns used by participants in pre-trial and trial proper

Pre-trial			Trial proper		
Lab	Column type	Phase	Lab	Column type	Phase
2	Capillary	CP WAX 58CB (PEG)	2	Capillary	CPWAX 57CB
3	Capillary	CP-WAX 57CB	3	Capillary	CPWAX 57CB - Carbowax 400
6	Capillary	CP-WAX 57CB	6	Packed	Carbowax 20M
8	Capillary	CP-WAX 57CB	8	Capillary	CPWAX 57CB
9	Packed	5% CW 20M Carbopak B	9	Packed	5% Carbowax 20M
10	Capillary	Carbowax 20M - CP-WAX 57CB	10	Capillary	CPWAX 57CB
11	Capillary	CP-WAX 57CB	11	Capillary	CPWAX 57CB
12	Capillary	CP-WAX 57CB - Carbowax 400	12	Capillary	CPWAX 57CB - Carbowax 400
13	Capillary	CP-WAX 57CB	13	Capillary	CPWAX 57CB
14	Capillary	CP-WAX 57CB	14	Capillary	CPWAX 57CB
15	Capillary	CP-WAX 57CB	15	Capillary	CPWAX 57CB
17	Capillary	CP-WAX 57CB	17	Capillary	CPWAX 57CB
18	Capillary	CP-WAX 57CB	18	Capillary	CPWAX 57CB
19	Capillary	CP-WAX 57CB - Carbowax 400	19	Capillary	CPWAX 57CB - Carbowax 400
21	Capillary	CP-WAX 57CB	21	Capillary	CPWAX 57CB
22	Capillary	CP-WAX 52CB	22	Capillary	CPWAX 57CB
24	Capillary	CP-WAX 57CB	24	Capillary	CPWAX 57CB
25	Packed	0.5% Carbowax 1500	25	Packed	0.5% Carbowax 1500
26	Capillary	DB-WAX (PEG)	26	Capillary	DB-WAX
27	Capillary	HP 19091X-116 (HP-WAX bonded PEG)	27	Capillary	HP-WAX
29	Capillary	DB-WAX (PEG)	29-1	Capillary	DB-1
			29-2	Capillary	DB-WAX
30-1	Packed	Carbowax 20M	30	Packed	60/80 Carbopak B/5% Carbowax 20M
30-2	Capillary	SE54			
31	Packed	80/120 Carbopak B/5% Carbowax 20M	31	Packed	80/120 Carbopak B/5% Carbowax 20M
32	Packed	Carbowax 20M on Carbopak BAW	32	Packed	Carbowax 20M on Carbopak B-AW
33	Capillary	CP-WAX 57CB	33a	Packed	80/120 Carbopak B/5% Carbowax 20M
			33b	Capillary	CPWAX 57CB
34	Capillary	CP-WAX 57CB	34	Capillary	CPWAX 57CB
35	Packed	5% Carbowax 20M on 80-120 mesh Carbopak BAW	35	Packed	80/120 Carbopak B/5% Carbowax 20M
36	Capillary	CP-WAX 57CB - Carbowax 400	36	Capillary	CPWAX 57CB
37	Capillary	CP-WAX 57CB	37	Capillary	CPWAX 57CB
38	Packed	80/100 Carbopak B/5% Carbowax 20M	38	Packed	80/120 Carbopak B/5% Carbowax 20M
39	Capillary	Permabond CW 20 M - DF 0.5	39	Capillary	Permabond CW 20M - DF 0.5

Recovery of the target value for each congener in the quality control standards is calculated as follows:

$$\text{Recovery of QC sample, \%} = \frac{\text{concn analyte in QC standard}}{\text{concn analyte in solution D}} \times 100 \qquad (3)$$

The concentration of the analyte in the QC standard is calculated using equations 1 and 2.

Results are converted from µg/g to g/100 L absolute alcohol for samples using the following equation:

$$\text{Concn, g/100 L absolute alcohol} = \frac{\text{concn } (\mu g/g) \times SG \times 10}{\%\,\text{strength } (v/v)} \qquad (4)$$

where SG is the specific gravity of the sample (density at 20°C/0.99715). Results are calculated to 3 significant figures and a maximum of one decimal place, e.g., 11.4 g/100 L absolute alcohol.

Using equation 2 above, calculate the concentration of each congener in the quality control standard solutions. Using equation 3, calculate the percentage recovery of the target value. If the analyzed results are within ± 5% of their theoretical values for each congener, analysis may proceed. If not, an investigation should be made to find the cause of the inaccuracy and remedial action taken as appropriate.

Results

Statistical Analysis of Results

The trial results were examined for evidence of individual systematic error ($p < 0.025$) using Cochran's and Grubbs' tests progressively, by procedures described in the internationally agreed *Protocol for the Design, Conduct, and Interpretation of Method-Performance Studies* (4).

Repeatability and Reproducibility

Calculations for repeatability (r) and reproducibility (R) as defined by that protocol (4) were performed on those results remaining after removal of outliers (Tables 3–12).

Horwitz-Predicted Precision Parameters

When assessing a new method, there is often no validated reference or statutory method with which to compare precision criteria, hence it is useful to compare the precision data obtained from a collaborative trial with "predicted" levels of precision. These "predicted" levels are calculated from the Horwitz equation. Comparison of the trial results and the predicted levels give an indication as to whether the method is sufficiently precise for the level of analyte being measured (5).

The Horwitz predicted value is calculated from the Horwitz equation (5):

$$RSD_R = 2^{(1 - 0.5 \log C)}$$

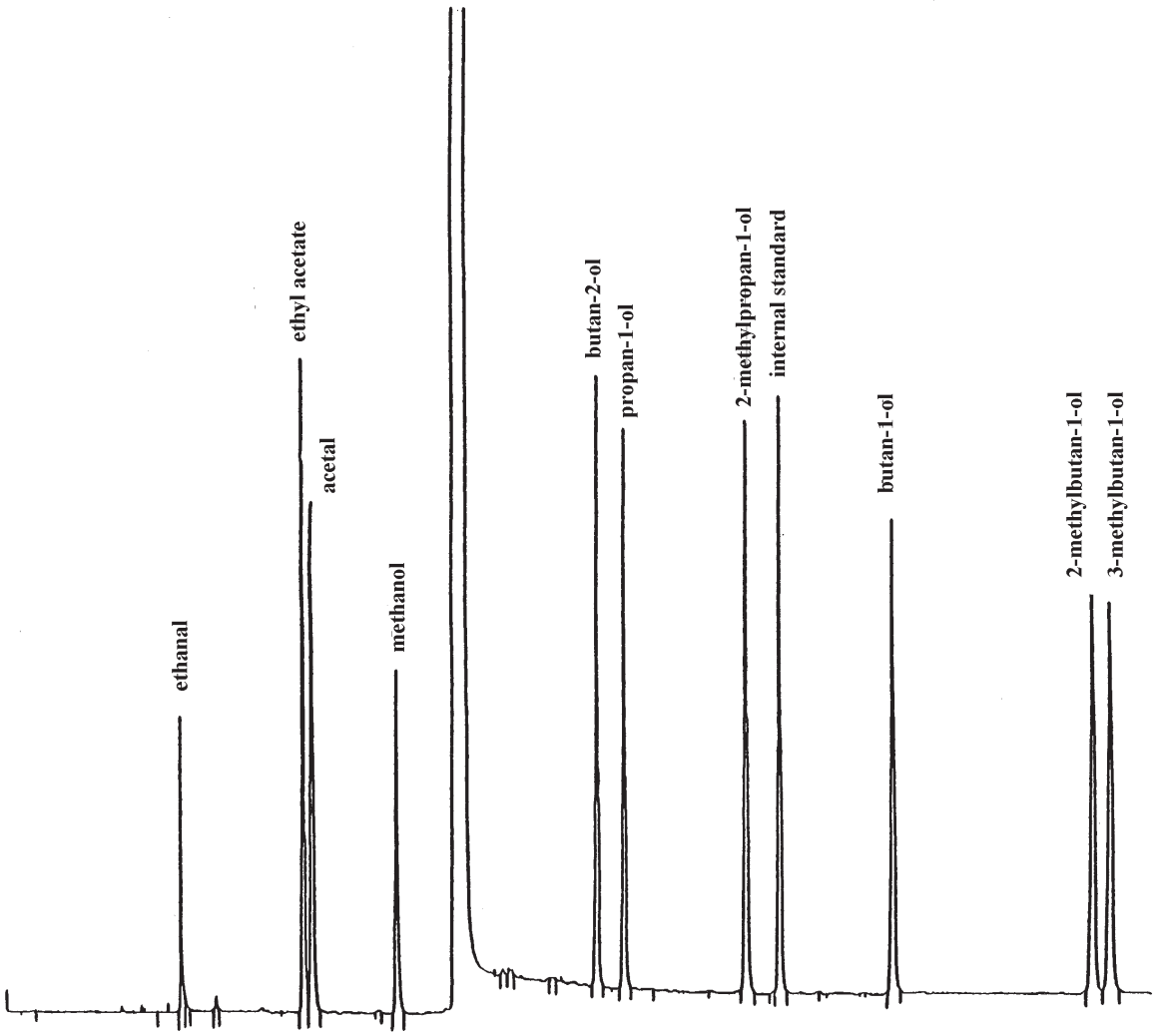

Figure 1. Chromatogram obtained using CP-WAX 57 CBCarbowax 400 columns.

where C = measured concentration of analyte expressed as a decimal (e.g., 1 g/100 g = 0.01) and RSD_R = reproducibility standard deviation.

Horrat Value (Ho)

The Horrat (6) value gives a comparison of the actual precision measured with the precision predicted by the Horwitz equation for a method measuring at that particular level of analyte. It is calculated as follows:

$$Ho_R = \frac{RSD_R \text{ (measured)}}{RSD_R \text{ (Horwitz)}}$$

A Ho_R value of 1 usually indicates satisfactory interlaboratory precision, whereas a value of >2 usually indicates unsatisfactory precision, i.e., one that is too variable for most analytical purposes or where the variation obtained is greater than that expected for the type of method used. Ho_r is also calculated and used to assess intralaboratory precision, using the approximation $RSD_r(\text{Horwitz}) = 0.66\ RSD_R(\text{Horwitz})$. This assumes the approximation r = 0.66 R. The Horwitz values calculated from the results of this trial are given in Tables 3–12.

Discussion

A range of columns and conditions were used by participants. The method criteria stipulated that satisfactory chromatographic separation (minimum resolution of 1.3) of the analytes involved should be achieved, therefore the statistical data provided was derived only from those laboratories that satisfied this criterion. However as the pre-trial results had suggested that there was little difference to the total congener concentration whether 2-methylbutan-1-ol and 3-methylbutan-1-ol are quantitated individually or as one peak, this criterion was withdrawn for these 2 analytes for the main interlaboratory study. For the remaining analytes, a resolution of 1.3 (baseline resolution) was prescribed. Participants who had obtained poor resolution and/or peak shape in the pre-trial were contacted and advised on how to improve their chromatographic performance prior to the main study.

The proposed legislation requires that ethanal (acetaldehyde) be quantitated as "total ethanal" (i.e., together with the ethanal fraction of acetal). The calculation of "total ethanal" was performed for the participants by the coordinating laboratory from their ethanal and acetal data.

Although many laboratories used similar columns, chromatographic performance varied considerably. A list of the columns used is given in Table 13. The method produced for this study contained quality assurance parameters which could be easily checked in order to ascertain that the method used by participants was indeed appropriate, i.e., resolution >1.3 and peak symmetry ideally between 0.5 and 1.5. Assessment by the coordinating laboratory of chromatograms submitted by participants identified some laboratories who had not optimized their chromatography. Their results for some analytes were deemed as not being valid ("noncompliant") and were therefore not included in the subsequent statistical analysis. Those laboratories using polar capillary columns obtained best separations when using programs similar to those recommended in the method, i.e., low starting oven temperature (35°C), long isothermal temperature (>10 min), and a relatively slow ramp. Packed columns generally gave satisfactory results with good separation, although with broader peaks than capillary columns. Only 5 laboratories achieved complete separation of all the analytes. Three laboratories (3, 12, and 19) used the coupled system described in the method, a fourth laboratory (17) used a narrow bore CP-WAX 57CB, while a fifth laboratory (25) used a packed column. Example chromatograms are given in Figures 1–3. Some participants had modified their conditions in the light of their pre-trial performance.

The precision obtained for the vast majority of analyte/matrix combinations was good, with Horrat values of the order of ≤1 in many cases. There were only 9 (out of 65 of the analyte/matrix combinations) where Horrat values were >2, i.e., above the precision predicted by the Horwitz equation. These were for ethanal and total ethanal. Ethanal can be a problematical analyte to determine due to its greater volatility

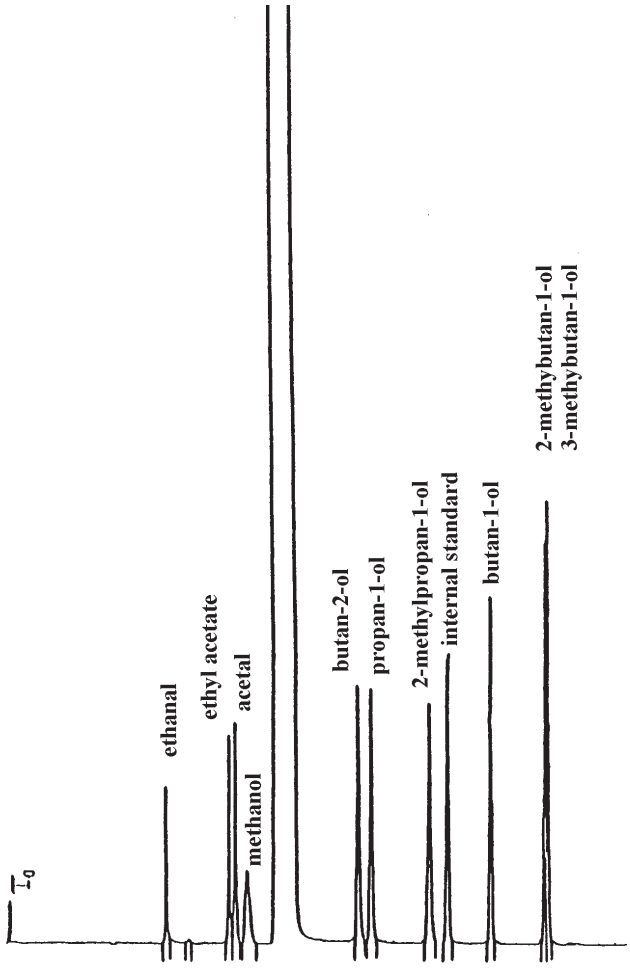

Figure 2. Chromatogram obtained using CP-WAX 57 CB column.

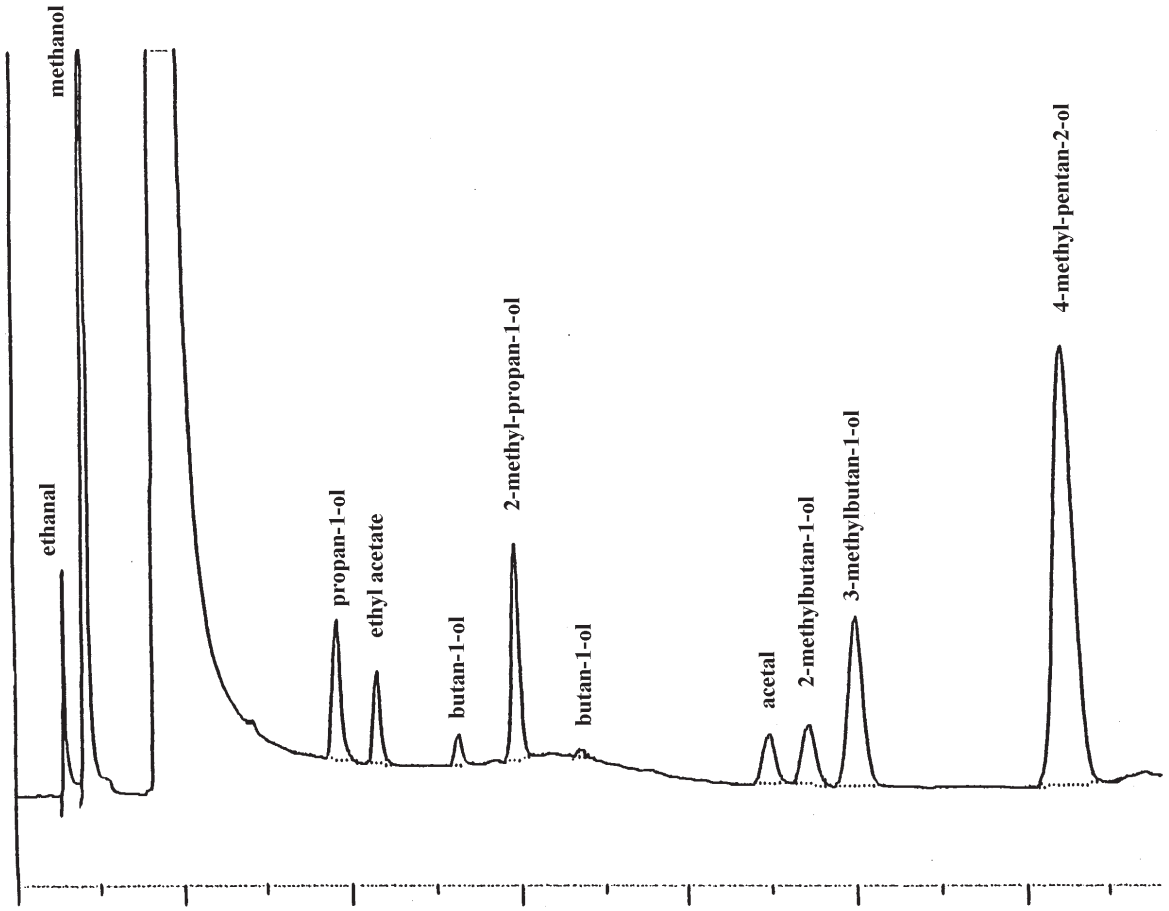

Figure 3. Chromatogram obtained from a participant using a 0.5% Carbowax 1500 column.

and its interconversion with acetal. The slightly worse precision for ethanal is therefore not unexpected.

As the levels of butan-2-ol and butan-1-ol in samples D1 and D2 (whisky) were below the limit of quantitation (resulting in many participants reporting zero values), no statistical analyses were performed on these analytes in these matrixes. Similarly, no statistical analyses were performed on butan-1-ol in samples E1 and E2 (rum).

Although there were several comments on the method at the pretrial stage on the large amount of quality assurance and weighing required in the method, in general participants were more content with the method during the trial proper. Participants were very positive about the method at a subsequent meeting of participants held to discuss the results of the study.

Conclusions

The results of this study have demonstrated the successful validation of this GC method for a wide variety of spirit drinks. The results were satisfactory, with only a small minority of data for ethanal and total ethanal being greater than theoretically predicted levels, possibly due to the high volatility and interconversion that is associated with these 2 compounds.

Incorporation of quality assurance procedures in the method permits the choice of chromatographic system and conditions to be selected by the user. The method is recommended for use in monitoring compliance with Council Regulation (EEC) No. 1576/89 and for official purposes in general.

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