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Characterisation of whiskeys using solid-phase microextraction with gas chromatography–mass spectrometry

Gillian Fitzgerald[a], Kevin J. James[a], Kevin MacNamara[b], Mary A. Stack[a,*]

[a]*Ecotoxicology Research Unit, Chemistry Department, Cork Institute of Technology, Bishopstown, Cork, Ireland*
[b]*Irish Distillers Group Ltd., Bow Street Distillery, Smithfield, Dublin 7, Ireland*

Abstract

The application of solid-phase microextraction and gas chromatography–mass spectrometry to the detection of flavour volatiles present in Irish and Scottish whiskeys was investigated. A method was developed to characterise these volatiles which included the extraction, identification and quantification of 17 congeners which included fusel alcohols, acetates and esters. The method validation produced the optimum fibre [85 μm poly(acrylate)], extraction time (35 min), sample volume size (3 ml) and desorption time (5 min). The impact of salt on the absorption process was also studied. Characteristic profiles were determined for each whiskey and the flavour congeners were quantified using 4-methyl-2-pentanol as the internal standard. Calibration ranges were determined for each of the congeners with coefficients of linearity ranging from 0.993 (butan-1-ol) to 0.999 (ethyl laurate) and relative standard deviations ranging from 2.5% (2-methylbutan-1-ol) to 21% (furfural) at a concentration of 18.2 mg/l. Detection limits ranged from 0.1 mg/l (ethyl caprate) to 21 mg/l (butan-2-ol).

Keywords: Beverages; Food analysis; Solid-phase microextraction; Aroma compounds; Volatile organic compounds

1. Introduction

Distilled alcoholic spirits, such as whiskey, rum and gin, are characterised by the presence of congeners, which arise during fermentation, distillation and storage processes [1]. These components comprise mainly fusel alcohols [2], fatty acids and esters which are called organoleptic substances and can be present in relatively large amounts, up to 3 g/l [3,4]. These compounds may be determined by direct gas chromatography (GC) as pre-treatment is not essential. Fatty acids, such as capric acid and lauric acids, together with esters (phenylethyl acetate, etc.), are referred to as semi-volatile congeners and are present at much lower concentrations, ca. 0.01 g/l. Therefore, the determination of these constituents often requires the use of a pre-concentration step [1].

The study of congeners is of major importance in the detection of illicit spirits, as inconsistencies in the congener composition of the base spirit can be used to identify anomalies that are indicative of inconsistent manufacturing practices [5]. Simpkins established the typical concentrations for major congeners in authentic spirits with a view to developing rules of identification [5]. Alcohol congeners in Scotch whiskey, methanol, *n*-propanol, isobutanol and isoamyl alcohol, were studied by Aylott [6] for the purpose of identifying and authenticating the various brands available. In Europe, Scotch and Irish whiskeys are the most prevalent. Scotch whiskey is

*Corresponding author. Tel.: +353-21-4326-317; fax: +353-21-4345-191.

E-mail address: mstackenvdept@tinet.ie (M.A. Stack).

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mainly malt- or grain-based and most well known brands are blended from several individual whiskeys. However, data on the profiles of fusel oils in Scotch whiskeys are limited [7]. When malt is dried over an open peat fire additional flavour characteristics are imparted. Irish whiskey is made either from malted barley or a mixture of malted barley (not less than 25% of malted barley) with other cereals. This whiskey is produced after three separate distillation steps culminating in a final spirit of light and delicate character. The product (63% alcohol) is stored for 5 years in oak casks that were previously used to hold sherry and port. This storage contributes significantly to the whiskey's final colour and flavour [7]. Various analytical methods have been developed for the determination of the flavour constituents in distilled spirits. These methods include solvent extraction [8], simultaneous distillation/extraction [9], purge-and-trap [10], combined solvent extraction and purge-and-trap [11] and direct injection [12].

Solid-phase microextraction (SPME); for a review see Ref. [13] was developed by Pawliszyn and co-workers and this technique has been applied to a wide range of environmental and food applications [14–19]. SPME is particularly useful as a pre-concentration step for the direct extraction of analytes from a variety of samples matrices into a liquid or solid stationary phase coated onto a silica fibre. It is simple to use, with a high sensitivity and reproducibility and is solvent free. SPME has also been applied successfully for the quantitative analysis of flavour and fragrance components in foods and beverages [14,20]. The remarkable impact of SPME, as an extraction and concentration technique, for the determination of microorganic contaminants has recently been described [21]. This paper focuses on the development and optimisation of SPME to acquire profiles of a selection of Irish and Scotch whiskeys and to quantitatively determine the concentration of the flavour volatiles in these samples.

2. Experimental

2.1. Chemicals and materials

A manual SPME device was purchased from Supelco (Dorset, UK) including fibres; 65 μm poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB), 75 μm Carboxen–poly(dimethylsiloxane) (CAR–PDMS), 85 μm poly(acrylate) (PA). The six whiskeys used in this study were purchased locally. HPLC-grade water was obtained from (Labscan, Dublin, Ireland). All standard congeners were purchased from Sigma–Aldrich (Dorset, UK) and are listed in three groups A, B, and C. Group A standards were methyl acetate, butan-1-ol, butan-2-ol, isoamyl acetate, ethyl lactate (2-hydroxy-ethylpropanoic acid) and furfural. Group B standards were ethyl acetate, propanol, isobutanol, 2-methylbutan-1-ol and 3-methylbutan-1-ol. Group C standards comprised ethyl caproate (hexanoic acid ethyl ester), ethyl caprate (decanoic acid ethyl ester), ethyl caprylate (octanoioc acid ethyl ester), ethyl laurate (dodecanoic acid ethyl ester), 2-phenylethanol and phenylethyl acetate. The internal standard was 4-methyl 2-pentanol.

2.2. GC–mass spectrometry (MS) conditions

Chromatographic analysis was performed using a Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with a 5971A mass spectrometer. The target analytes were separated using a capillary column (CP Wax 57 CB), 50 m×0.25 mm with 0.2 μm film thickness (Chrompack, London, UK). The desorption temperature was 220°C and a desorption time of 5 min was used. The column was maintained at 40°C for 5 min after desorption and then ramped at 5°C/min up to 180°C where it was held for 15 min. Helium was used as the carrier gas at a head pressure of 18 p.s.i. (1 p.s.i.=6894.76 Pa). A 0.75-mm liner was used and analysis performed in the splitless mode. All mass spectra were acquired in electron impact (EI) mode at 70 eV, using full scan with a scan range of 27–400 amu, at a rate of 2.5 scans/s.

2.3. Preparation of standards

Two composite solutions containing the seventeen target analytes were prepared from 10 mg/ml standards. Composite 1 was prepared by adding 5.00 ml of group (A) standards and 10.00 ml of group (B) standards to a 100-ml volumetric flask, which was brought to the mark with ethanol-water (96:4).

Composite 2 contained 5.00 ml of group (C) standards and was prepared as composite 1 above. Calibration standards in the range 0.16–90.9 mg/l were prepared by dilution of the composite solutions into 10-ml volumetric flasks with the addition of 2 ml of ethanol and made up to the mark with water. Internal standard, 4-methyl 2-pentanol (1 ml), was then added.

2.4. SPME extraction procedures

Three fibres (PA, CAR–PDMS and PMDS–DVB) were conditioned in the GC injector port at 300, 260 and 280°C, respectively, for 3 h prior to use. The prepared standard solution (3 ml) was transferred to 6-ml vials containing a 10-mm magnetic stirring bar and capped with PTFE-faced butyl rubber seals. Extractions in headspace mode were carried out at 22±1°C while magnetically stirring the solutions (480 rpm). An extraction time of 20 min was selected and the experiment was conducted on all three fibres. The analytes were thermally desorbed in the GC injector port and subsequent chromatographic analysis was performed. Subsequent to these experiments, the PA fibre was selected for further studies.

Immersion extractions were conducted using 2-ml samples containing a congener mixture of 45 μg/ml in a 4-ml vial. Sorption–time profiles were carried out in headspace mode with the extraction time that varied from 1 to 50 min at 22±1°C. The effect of salt on the sorption–time profile was determined by spiking samples with 0.15, 0.3, 0.6 and 0.75 g NaCl, following the above procedure. The effect of sample volume on the sorption process was studied while keeping the liquid phase:headspace ratio constant and varying sample volume from 1 to 6 ml.

3. Results and discussion

The development of a suitable SPME method for the analysis of whiskey congeners involved the selection and optimisation of a number of parameters that influence SPME operation. These included the selection of the most appropriate SPME fibre, the choice of headspace versus immersion mode, the extraction time required to achieve equilibration between the analytes and the fibre, the desorption time in the GC injection port, the effect of sample volume size and the influence of salt in the sample solution.

3.1. SPME fibre selection

Three fibre coatings, PDMS–DVB, PA and CAR–PDMS, were used to experimentally determine which coating was the most appropriate for the characterisation of flavour volatiles that are typically found in whiskeys. The data obtained from the comparison of these different fibres are presented in Table 1. Subsequent to this determination, use of the CAR–PDMS fibre was discontinued due to is its poor sorption ability for the target analytes. The PA fibre gave the highest response for the congeners 5, 7, 8, 9, 13, 16 and 17 and all of the congeners listed in Table 1 were detected for the selected concentration. Although the PDMS–DVB fibre gave the greatest response for congeners 2, 6, 10 and 12, not all analytes were detected at the selected concentration. The PA fibre was therefore chosen for the remaining optimisation studies.

3.2. Comparison of headspace and immersion SPME

A comparison of headspace and immersion extraction modes was conducted using the PA fibre as outlined in the Experimental section. The measured response using immersion was considerably higher for six of the congeners but this extraction mode failed to detect three of the constituents, butan-2-ol, propanol and isobutanol. Chromatograms in Figs. 1 and 2, from headspace and immersion extraction modes, respectively, clearly demonstrate this. The relative standard deviation (RSD) data obtained for immersion and headspace extraction modes are listed in Table 2. The reproducibility of the method using headspace extraction was significantly better than immersion. It has previously been shown that volatile components present in the headspace layer sorb faster onto the fibre coating while the static layer surrounding the fibre in immersion probably inhibits analyte penetration [22]. However, both SPME extraction techniques performed favourably for the detection of the congeners when compared with direct liquid injection as illustrated in Fig. 3.

Table 1
Comparison of means for congeners using PA and PDMS-DVB fibres used for SPME

No.	Congener	PDMS–DVB (peak area·10^6) (n=3)	PA (peak area·10^6) (n=3)	Difference of area[a] (PA and PDMS-DVB) (%)
1	Methyl acetate	3.6	4.4	+18
2	Ethyl acetate	24	15	−38
3	Butan-2-ol	n.d.	17	–
4	Propanol	n.d.	12	–
5	Isobutanol	8	39	+80
6	Isoamyl acetate	456	276	−40
7	Butan-1-ol	2	18	+90
8	2-Methylbutan-1-ol	18	77	+77
9	3-Methylbutan-1-ol	22	93	+76
10	Ethyl caproate	1840	550	−70
11	Ethyl lactate	n.d.	9	–
12	Ethyl caprylate	6400	1981	−70
13	Furfural	33	57	+42
14	Ethyl caprate	3704	3012	−19
15	Phenylethyl acetate	310	2513	−19
16	Ethyl laurate	1356	1821	+26
17	2-Phenylethanol	8	17	+53

[a] + Denotes % increase in mean area for PA. − Denotes % increase in mean area for PDMS–DVB.

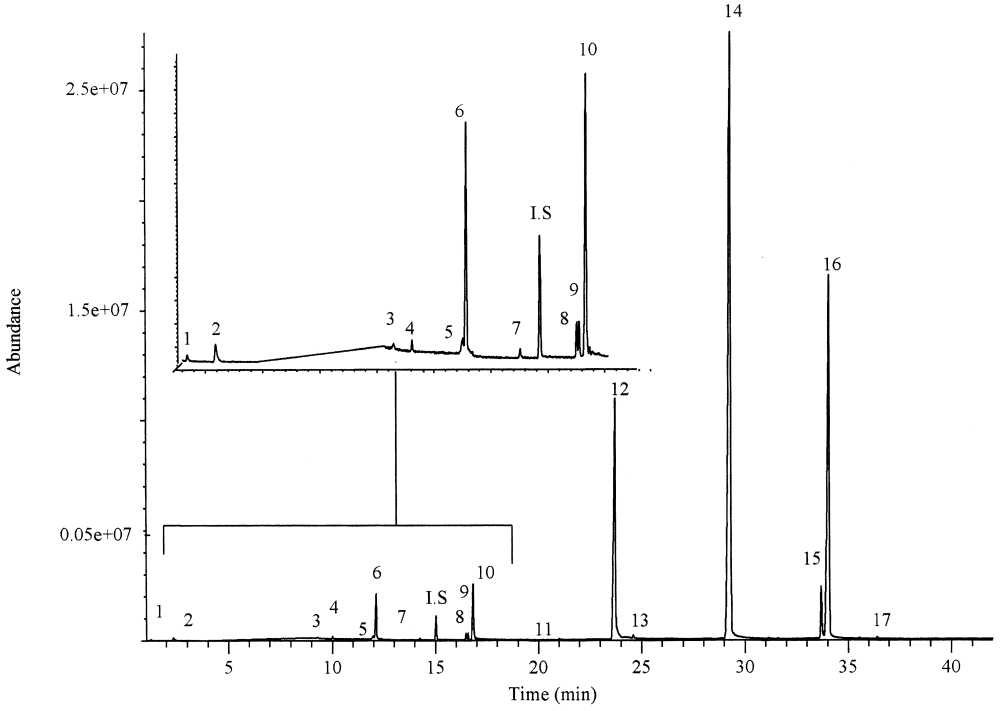

Fig. 1. Typical chromatogram of a standard mixture of congeners (45 mg/l) and 4-methylpentan-2-ol as the internal standard, extraction time of 35 min and using a poly(acrylate) fibre and headspace extraction. GC conditions were CP Wax 57 CB, wall-coated open tubular fused-silica (50 m × 0.25 mm column, 0.2 μm film thickness). Column temperature programme was: initial 40°C for 5 min, ramped at 5°C/min up to 180°C which was maintained for 15 min.

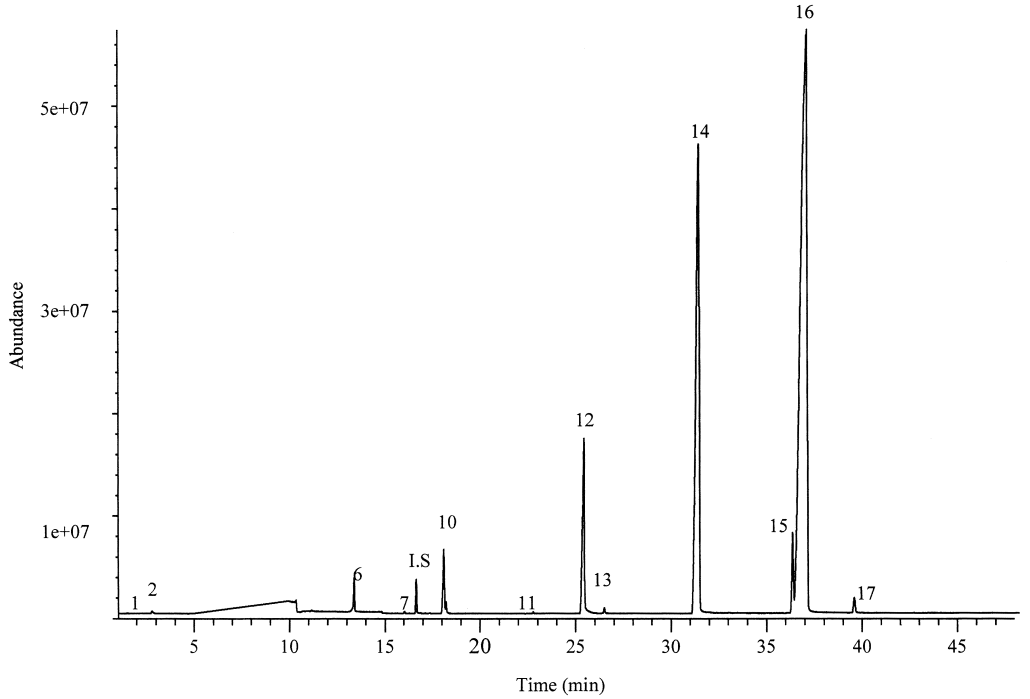

Fig. 2. Typical chromatogram for a standard mixture of congeners (45 mg/l) and 4-methyl pentan-2-ol as the internal standard. Extraction time was 35 min and a poly(acrylate) fibre was used and immersion extraction. GC conditions as in Fig. 1.

Table 2
Comparison of headspace versus immersion response data using SPME

Congener	Immersion mean peak area·10^7 (n=3)	RSD immersion (%, n=3)	Headspace mean peak area ·10^7 (n=3)	RSD headspace (%, n=3)	Difference between headspace and immersion response[a] (%)
1	0.4	23	0.04	5.0	−90
2	1.4	16	0.2	4.0	−86
3	n.d.	n.d.	0.3	2.0	–
4	n.d.	n.d.	0.1	29	–
5	n.d.	n.d.	2.3	21	–
6	16	19	15	3.0	−1.0
7	0.8	9.0	0.9	7.0	+12
8	3.4	2.0	4.0	0.3	+15
9	3.5	1.0	4.0	2.0	+13
10	25	23	24	0.1	−4.0
11	0.7	20	0.5	6.0	−29
12	103	14	113	3.0	+9.0
13	3.0	10	3.0	16	0
14	441	16	233	10	−47
15	37	10	16	7.0	−57
16	127	10	108	14	−15
17	9.0	4.0	1.0	6.0	−89

[a] + Denotes % increase in mean area for PA.

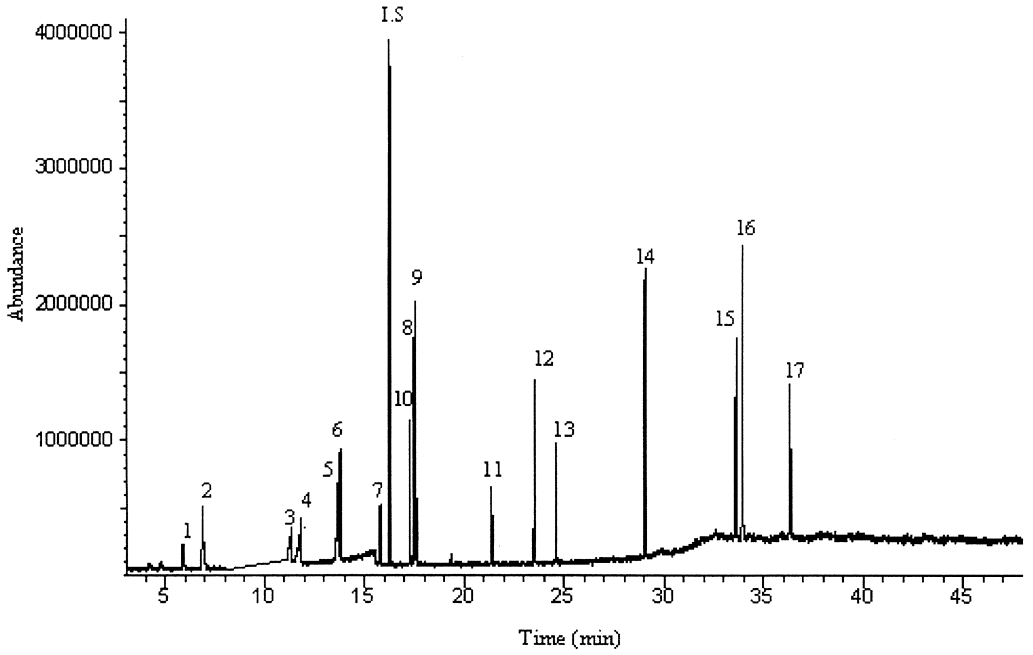

Fig. 3. Typical chromatogram for the direct injection of a 1 μl standard congener mixture (45 mg/l). GC conditions as in Fig. 1.

3.3. Sorption–time profiles

Fig. 4 shows the sorption–time profile achieved by measuring the peak area for selected analytes while increasing the extraction time. The time at which the peak area becomes constant was considered the equilibration time. There was little variation in peak area after 30 min for these congeners and, therefore, an extraction time of 35 min was selected for subsequent studies. A decrease in peak area was observed at 15 min for all of the congeners and was greatest for isoamyl acetate. This phenomenon was reproducible and a possible explanation for this decrease in peak area is that swelling of the fibre occurs which can lead to a temporary loss of analytes.

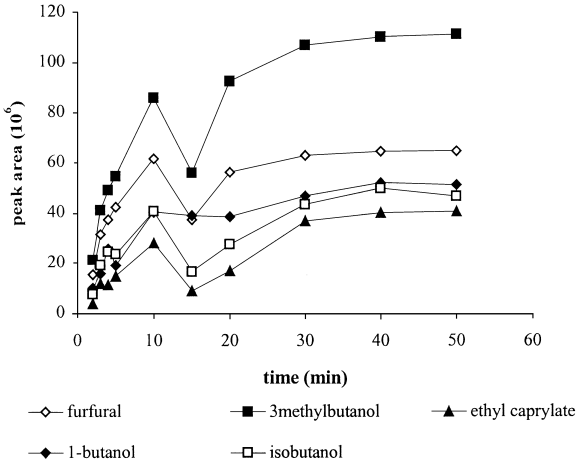

Fig. 4. Sorption–time profiles for five whiskey congeners (45 mg/l).

3.4. Effect of salt concentration on sorption efficiency

The addition of salt to the sample matrix decreases the solubility of the analytes in the sample matrix, allowing for more analytes to be sorbed onto the fibre and enhancing the extraction efficiency [20]. A standard flavour mixture (3 ml), prepared from the composite solutions 1 and 2, containing 45 μg/ml of each congener, in a 6-ml vial treated with NaCl to produce 5, 10, 20 and 25% (w/v) NaCl. The influence of salt on the sample matrix gave rise to three types of behaviour among the flavour com-

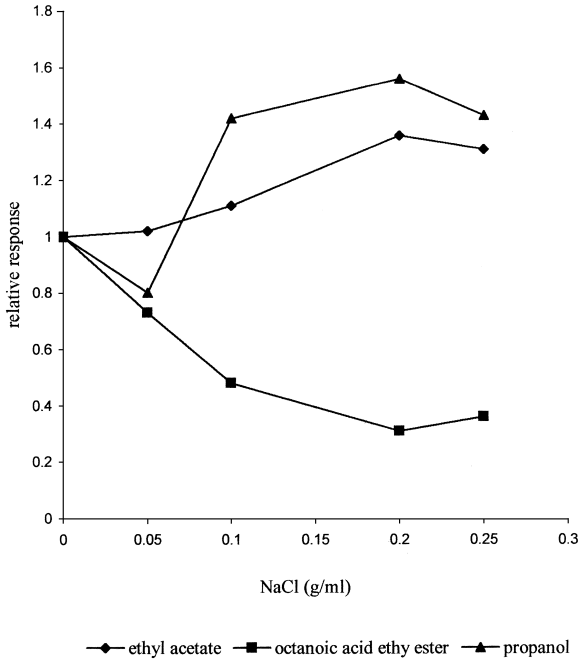

Fig. 5. Profile of the effect of salt addition on the sorption efficiency using a standard congener mixture (45 mg/l) using headspace SPME.

ponents studied and this is illustrated in Fig. 5. Firstly, a decrease in absorption for compounds, ethyl caprylate, phenylethyl acetate, phenylthanol, ethyl laurate, ethyl caprate, butan-2-ol, 3-methylbutan-1-ol, 2-methylbutan-1-ol, ethyl caprate, was observed with an increase in salt concentration, levelling off at a concentration of 20% (w/v). Secondly, an initial decrease in sorption was observed for methyl acetate, propanol, ethyl lactate and furfural, followed by an increase at a concentration of 5% (w/v). Thirdly, 1-butanol and isoamyl acetate displayed an initial increase in sorption at concentrations up to 20% (w/v). Overall, a decrease in sorption was obtained as salt solution increased. Since no real advantage was gained by salt addition, it was not used in the remainder of this study.

3.5. Desorption–time study

The desorption–time trials determined the minimum time required to remove all of the analytes from the PA fibre at an injection port temperature of 220°C. Again, a congener mixture (3 ml), containing 45 μg/ml of each analyte was used with an SPME time of 35 min, and the selected desorption times ranged from 1 to 5 min. These experiments showed that all the congeners were desorbed from the fibre after 4 min.

3.6. Influence of sample volume on SPME efficiency

The quantity of analyte sorbed by the SPME fibre is dependent on the initial concentration present in the sample and not on sample volume, unless a very small sample volume is used and rapid exhaustion of the analytes occurs [20]. In this study, the optimum volume was found to be 3 ml. The volume ratio of liquid phase to headspace was 1:1 and this ratio was kept constant when the sample volume was increased from 1 to 6 ml. Fig. 6 shows that the response increased initially for all congeners and remained relatively constant at a volume of 2 ml for ethyl laurate and at 3 ml for the other congeners.

3.7. Calibration, limits of detection and precision data

After the preliminary investigations were completed and the extraction parameters optimised, the feasibility of using this SPME method for the determination of congeners in whiskey was investigated. The assessment of the method included

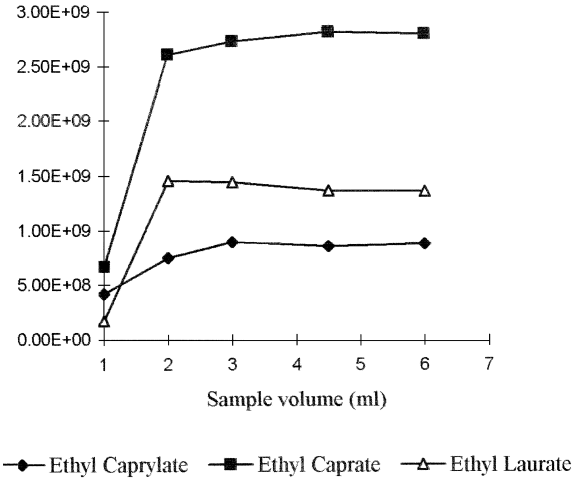

Fig. 6. Effects of sample volume on headspace SPME efficiency.

Table 3
Main analytical parameters for the determination of 17 congeners using headspace SPME–GC–MS

No.	Congener	LOD[a] (mg/l)	Coefficient of linearity, r^2	RSD[b] (%, $n=7$)
1	Methyl acetate	12	0.970	8.7
2	Ethyl acetate	10	0.998	13
3	Butan-2-ol	21	–	–
4	Propanol	4.4	0.999	22
5	Isobutanol	6.0	0.995	16
6	Isoamyl acetate	0.58	0.999	15
7	Butan-1-ol	6.9	0.993	9.2
8	2-Methylbutan-1-ol	3.4	0.993	2.5
9	3-Methylbutan-1-ol	3.2	0.995	5.4
10	Ethyl caproate	0.26	0.994	4.8
11	Ethyl lactate	9.5	0.998	15
12	Ethyl caprylate	0.08	0.995	12
13	Furfural	4.1	0.994	21
14	Ethyl caprate	0.06	0.993	5.7
15	Phenylethyl acetate	0.53	0.998	5.7
16	Ethyl laurate	0.08	0.999	11
17	2-Phenylethanol	9.1	0.997	16

[a] LOD=Limit of detection (3×baseline noise).
[b] RSD=Relative standard deviation.

investigation of its reproducibility and calculation of the limits of detection (LODs). The precision was estimated by performing seven replicate extractions on a standard congener mixture, containing 18.2 μg/ml of each of the 17 congeners, and calculating the RSDs. The RSDs for the PA fibre revealed values

Table 4
Quantitative data for congeners in five Irish and two Scotch whiskey samples

No.	Congener	Concentration (mg/l)						
		Irish					Scotch	
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2
1	Methyl acetate	<12	<12	<12	<12	<12	<12	<12
2	Ethyl acetate	<10	67	73	51	130	81	110
3	Butan-2-ol	<21	<21	<21	<21	<21	<21	<21
4	Propanol	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4	<4.4
5	Isobutanol	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0	<6.0
6	Isoamyl acetate	9	9	13	13	31	21	28
7	Butan-1-ol	<6.9	<6.9	<6.9	<6.9	<6.9	<6.9	<6.9
8	2-Methylbutan-1-ol	59	81	126	79	76	47	82
9	3-Methylbutan-1-ol	168	247	314	256	217	135	225
10	Ethyl caproate	1.1	<0.26	1.2	0.9	1.4	0.7	0.9
11	Ethyl lactate	<10	<10	<10	<10	<10	20	<10
12	Ethyl caprylate	0.2	0.2	0.4	0.2	0.4	0.2	0.8
13	Furfural	6.2	<4.0	7.3	5.7	8.8	5.0	<4.0
14	Ethyl caprate	0.6	0.5	0.8	0.4	0.2	<0.1	1.0
15	Phenylethyl acetate	<0.5	<0.5	<0.5	<0.5	1.4	<0.5	<0.5
16	Ethyl laurate	0.3	0.3	0.2	<0.1	<0.1	<0.1	0.4
17	2-Phenylethanol	<9.1	<9.1	<9.1	<9.1	<9.1	<9.1	<9.1

ranging from 2.5% for 2-methylbutanol to 22% for propanol. Seven of the compounds had values <10% and the remaining congeners had RSD values<15% (Table 3). SPME calibration graphs were prepared for all of the congeners. The coefficients of linearity data can be seen in Table 3 and they varied from 0.993 to 0.999. The LODs, based on three-times the baseline noise, ranged from 0.1 mg/l for ethyl caprate to 21 mg/l for butan-2-ol.

After the method has been optimised, the congeners in the seven whiskeys were quantified. These concentrations are shown in Table 4. Among the congeners listed this table, seven to nine congeners were detected in all whiskey samples. Methyl acetate was not detected in any sample and 3-methylbutan-1-ol was the congener that was found in the highest concentration in all of the whiskey samples.

4. Conclusion

An SPME–GC method has been developed and optimised for the determination of congeners in distilled spirits and can be considered complementary to the commonly used method, direct injection GC. SPME provides many advantages over other methods that have been used including, purge-and-trap, solvent extraction and distillation/extraction. It requires only a few minutes to complete the SPME step and no solvent is used. An improved sensitivity for the determination of esters congeners was also demonstrated.

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