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# Analysis of volatiles of malt whisky by solid-phase microextraction and stir bar sorptive extraction

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**Abstract**

Blended Scotch whisky was analysed by solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) to study the composition of the volatiles. For SPME analysis, three different fibres were compared, poly(dimethylsiloxane) (PDMS) (100 μm), poly(acrylate) (PA) (85 μm) and divinylbenzene–Carboxen on poly(dimethylsiloxane) (DVB–CAR–PDMS) (50/30 μm). It was found that the PDMS and DVB–CAR–PDMS fibres showed a higher enrichment capacity than PA, as well as a better reproducibility. The influence of sampling time, temperature and salt addition on the enrichment of volatiles as well as the difference between liquid and headspace SPME were studied. An optimum SPME method was developed. Finally a more recent sample preparation technique, namely SBSE was evaluated to extract whisky volatiles.


*Keywords:* Malt whisky; Stir bar sorptive extraction; Solid-phase microextraction; Volatiles

## 1. Introduction

The flavour of alcoholic beverages has been considered since early years as an image of their quality. With alcoholic beverages, a human being perceives an overall sensation, whereas, chemical analysis measures single aroma compounds qualitatively and quantitatively. Thus perceived sensations must be broken down into clearly defined elements, the chemical compounds. These components and their influence on the overall aroma are characterised.

In the past years, some extensive research has already been devoted to whisky flavour analysis by a Scottish group [1–3]. More than 1000 compounds have been identified in the aroma of whisky [4], including alcohols and fusel alcohols, fatty acids and esters, lactones and other carbonyl compounds, such as aldehydes, phenolic, sulfur and nitrogen compounds. Especially fatty acid esters form an essential group of aroma components in whisky. The esters generally have a pleasant, and some of them also a very intense odour and thus it may be assumed that they appear as important aroma components, even if they are present in relatively small amounts. The main ester component in alcoholic beverages is ethyl acetate, although ethyl, isobutyl and 3-methylbutyl esters of short-chain fatty acids, called ‘‘fruit esters’’ (due to their pleasant aromas), also appear. Quantita-

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tively significant components are ethyl esters of octanoic, decanoic and dodecanoic acids. Of the higher esters, ethyl *E*-11-hexadecenoate is interesting because significant amounts of this compound have been found mainly in Scotch whisky. The yeast used has a great influence on the production of esters in the fermentation process. The ester content of distilled beverages also depends on whether or not the yeast is present at the time of distillation. If distillation occurs in the presence of yeast, the ethyl ester concentrations of at least decanoic, dodecanoic and *E*-11-hexadecenoic acids increase [5].

Although the use of solid-phase microextraction (SPME) for the flavour analysis of orange juice [6], beer [7], fruit flavoured malt beverages [8], vodka [9] and especially wine [10,11] has been reported some years ago, the literature on SPME of whisky is much more recent and less extensive [12,13].

The aim of this work was to optimise SPME for the analysis of the composition of volatiles of blended Scotch whisky and to evaluate the novel sample preparation technique, namely stir bar sorptive extraction (SBSE) to extract whisky volatiles [14]. The principle of this method is the same as for SPME but it uses a magnetic stir bar covered with a poly(dimethylsiloxane) (PDMS) film instead of the fibre used in SPME. Whereas SPME is ideally suited for routine analysis, and for detection of compounds present at higher concentration, SBSE is more sensitive and can be used for trace and ultratrace analysis [15,16].

## 2. Experimental

### 2.1. Whisky

The whisky samples (65%, v/v, alcohol) were obtained from Bruggeman (Ghent, Belgium). All samples were adjusted to exactly 40% (v/v) alcohol by dilution with distilled water prior to extraction.

### 2.2. SPME

Aliquots of 17 ml of whisky for liquid SPME and 12 ml for headspace SPME were pipetted into 22-ml glass vials, which were closed with open-top phenolic closures with PTFE/silicone septa (Supelco, Bornem, Belgium). The fibres tested were 100 μm PDMS, 85 μm poly(acrylate) (PA) and 50/30 μm divinylbenzene–Carboxen on PDMS (DVB–CAR–PDMS) (Supelco, Bornem, Belgium). Different sampling times and different temperatures were used. Sodium chloride (NaCl) was added to some of the samples at a concentration of 0.15 g $ml^{-1}$ (i.e., until saturation), to test the influence of salt on SPME recovery. The samples were stirred (800 rpm) with PTFE magnetic stir bars (Supelco) during extraction.

### 2.3. SBSE

For the extraction of whisky by stir bar sorptive extraction [14], the stir bar (Twister, Gerstel, Mülheim a/d Ruhr, Germany) was placed into a 15-ml glass vial containing 10 ml of sample and the absorption was carried out for 30 or 60 min at 25 °C while stirring (800 rpm).

After the extraction, the bar was withdrawn and placed for a few seconds in warm distilled water (60 °C) in order to remove sugars from the stir bar which could interfere with the gas chromatography (GC) analysis, and it was dried with a lintfree tissue paper.

The bar was placed into a glass tube (Gerstel) (178 mm×6.0 mm O.D.) and then thermodesorption was carried out into the Gerstel Thermo Desorption System (TDS2, Gerstel).

Before each analysis, the stir bar was conditioned in an oven at 250 °C for 10 min by passing helium through the tube (30 ml $min^{-1}$).

### 2.4. Gas chromatographic analytical conditions

Analyses were performed with an Agilent 6890 gas chromatograph coupled to a quadrupole mass spectrometer MSD 5973 (Agilent) and equipped with a HP-5MS capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness). The carrier gas was He at a flow-rate of 1.2 ml $min^{-1}$ (mean velocity 40 cm $s^{-1}$).

The analyses of the SPME extracts were performed with GC–MS under the following conditions: initial oven temperature 35 °C; 35–220 °C at 5 °C $min^{-1}$; 220–240 °C at 10 °C $min^{-1}$; final time 5 min. The SPME fibre was thermally desorbed in the programmed temperature vaporiser (PTV) injector (Gerstel) at 250 °C during 3 min and the injector was used in split mode (split ratio 1/10).

For the SBSE analyses the conditions were as follows: initial oven temperature 35 °C; 35–180 °C at 5 °C $min^{-1}$; 180–240 °C at 10 °C $min^{-1}$; final time 5 min. The stir bar was thermally desorbed and the volatiles were cryofocused in a cold trap and injected into the GC–MS system. The conditions for thermal desorption were: TDS2 oven (Gerstel) programmed from 10 to 250 °C at 60 °C $min^{-1}$; 10 min at 250 °C; cryo-focusing of the analytes in a CIS-4 PTV injector (Gerstel) at −50 °C; transfer of the analytes to the capillary column was done by fast heating the injector from −50 to +20 °C at 10 °C $min^{-1}$ and from 10 to 250 °C at 12 °C $min^{-1}$. The thermal desorption unit (TDS) was operated in split or splitless mode, whereas the injector (PTV) was operated in different split modes, namely 1/10, 1/20 and 1/50, and eventually three combinations were compared in order to select the best one, namely 1/10 (TDS)×1/10 (PTV), 1/20 (TDS)×1/20 (PTV) and splitless TDS×1/50 (PTV).

The mass spectrometer was operated in the electron impact (EI) mode (70 eV) and the ions were scanned over an $m/z$ range of 40–300 u (3–20 min) and 40–400 u (>20 min).

## 3. Results and discussion

### 3.1. List of target compounds

To compare the different sampling parameters for both SPME and SBSE, 21 target analytes were selected and divided into two groups for integration, namely volatile and semivolatile compounds. Integration was done by measuring the peak area after selection of a target ion (the main ion of the mass spectrum (relative abundance 100%) or a characteristic one) for each compound (Table 1).

### 3.2. Influence of the type of fibre on the enrichment of volatiles during SPME

In the first experiment, two different fibres were tested for SPME: PDMS (100 μm) and PA (85 μm), for which fibres the extraction is based on absorption. In this test, flavour compounds were extracted from the samples at two different temperatures (25 and 45 °C) during 30 min and also the influence of salt addition on the enrichment of the volatiles was investigated (Table 2). In the latter case, the whisky sample was saturated with NaCl prior to SPME. From the data displayed in Table 2 it is clear that the absorption of volatiles on the fibre is temperature dependent (tested for PA). Absorption at 25 °C is higher than at 45 °C. The most important parameter however is the type of fibre used and the amount of stationary phase on it. For both the volatile and the semivolatile flavour compounds enrichment by the PDMS fibre was much higher than with the PA fibre. It can also be noted that salt addition negatively affected the recovery during liquid SPME. This can be explained by the high alcohol content of the samples (40%) and the low solubility of salt in the whisky and hence the negative effect of salt addition

Table 1
List of target analytes for integration of whisky flavour compounds

| No. | Volatile compound | $t_R$[a] (min) | RI[b] | Target[c] ion | No. | Semivolatile compound | $t_R$ (min) | RI | Target ion |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Ethyl hexanoate | 8.35 | 1001 | 88 | 12 | Ethyl tetradecanoate | 28.96 | 1795 | 88 |
| 2 | Ethyl octanoate | 14.41 | 1199 | 88 | 13 | Isoamyl laurate | 29.94 | 1844 | 70 |
| 3 | Ethyl 9-decenoate | 19.56 | 1387 | 88 | 14 | 1-Hexadecanol | 30.69 | 1881 | 83 |
| 4 | Ethyl decanoate | 19.85 | 1398 | 88 | 15 | Ethyl pentadecanoate | 30.96 | 1894 | 88 |
| 5 | Isoamyl octanoate | 21.03 | 1446 | 70 | 16 | Ethyl *E*-11-hexadecenoate | 32.52 | 1974 | 55 |
| 6 | 2-Methylbutyl octanoate | 21.10 | 1449 | 70 | 17 | Ethyl hexadecanoate | 32.97 | 1997 | 88 |
| 7 | Isobutyl decanoate | 23.44 | 1545 | 56 | 18 | 2-Phenylethyl decanoate | 33.99 | 2053 | 104 |
| 8 | Ethyl dodecanoate | 24.68 | 1597 | 88 | 19 | Ethyl linoleate | 35.93 | 2162 | 67 |
| 9 | Isoamyl decanoate | 25.70 | 1644 | 70 | 20 | Ethyl oleate | 36.04 | 2168 | 88 |
| 10 | 2-Methylbutyl decanoate | 25.77 | 1647 | 70 | 21 | Ethyl octadecanoate | 36.52 | 2195 | 88 |
| 11 | 1-Tetradecanol | 26.43 | 1677 | 83 | | | | | |

[a] $t_R$=Retention time (for SPME analysis).
[b] RI=Kováts retention index.
[c] Target ion=Target ion for integration.

Table 2
Influence of type of fibre and sampling temperature and effect of salt addition on the absorption efficiency of volatiles during liquid SPME sampling of whisky (30 min), expressed as relative peak area (100%=PDMS fibre, 25 °C, no salt addition)

| Compound | Fibre and temperature | | | |
|---|---|---|---|---|
| | PDMS, 25 °C (no salt) | PA, 25 °C (no salt)[a] | PA, 25 °C (salt) | PA, 45 °C (no salt) |
| Volatiles | 64.44 | 19.31±1.17 | 7.09 | 12.71 |
| Semivolatiles | 35.56 | 10.69±0.23 | 3.59 | 8.42 |
| Total | 100.0 | 30.00±1.40 | 10.68 | 21.13 |

PDMS=Poly(dimethylsiloxane), PA=poly(acrylate); salt=salt addition.
[a] Analysis performed in duplicate.

on the flavour release from the liquid into the headspace phase.

In the next experiment, one additional fibre was tested, namely DVB–CAR–PDMS (30/50 μm), where extraction is mainly based on adsorption. To compare this fibre with the other two (PDMS and PA—different batches of fibres were used than in the previous test), two different sampling times were used, namely 15 and 30 min. From the results (Table 3) it can be concluded that the best fibres were PDMS and DVB–CAR–PDMS (no significant difference could be found between the enrichment capacities of both fibres) and that equilibration was not reached after 15 min.

In all DVB–CAR–PDMS and PA extracts 2,4-diisocyanato-1-methylbenzene was found as a contaminant, which did not appear when a PDMS fibre was used. It has been suggested that these artefacts may result from insufficient conditioning of the fibres before use [17]. To avoid this phenomenon, new DVB–CAR–PDMS and PA fibres were conditioned at 270 and 300 °C, respectively, for at least 4 h prior to use, as recommended by the supplier. Using this treatment, bleeding of the fibres and artefact formation was largely reduced, but small traces of 2,4-diisocyanato-1-methylbenzene were still observed in the chromatograms.

In order to check the reproducibility of the extraction method, whisky samples were analysed after 30 min of liquid SPME sampling at 25 °C in triplicate using different fibres. The reproducibility was expressed as the relative standard deviation (RSD) on the peak areas obtained after triplicate extraction. For this test, a fresh whisky sample was taken for every extraction. The results obtained (Table 4) showed that the highest reproducibility (RSD<5% for the sum of all peak areas) was obtained when a PDMS or a DVB–CAR–PDMS fibre was used. For the PA fibre the obtained standard deviation values for each flavour compound were substantially higher, also resulting in an overall percentage of standard deviation of almost 20% for the sum of all peak areas indicating a poor reproducibility.

From these results it is clear that DVB–CAR–PDMS and PDMS are the most suitable fibres,

Table 3
Sorption efficiency (expressed as peak area) of different fibres and effect of sampling time during liquid SPME sampling of whisky at 25 °C (all tests performed in triplicate except 15 min with PDMS)

| Extraction time (min) | Fibre | | | | | |
|---|---|---|---|---|---|---|
| | DVB–CAR–PDMS | | PDMS | | PA | |
| | Peak area | RSD (%) | Peak area | RSD (%) | Peak area | RSD (%) |
| 15 | $3.94 \cdot 10^8$ | 15.82 | $4.47 \cdot 10^8$ | n.d. | $3.02 \cdot 10^8$ | 8.78 |
| 30 | $7.31 \cdot 10^8$ | 4.13 | $7.75 \cdot 10^8$ | 4.20 | $3.75 \cdot 10^8$ | 19.58 |

DVB–CAR–PDMS=Divinylbenzene–Carboxen–poly(dimethylsiloxane), PA=poly(acrylate), n.d.=not determined.

Table 4
Percentage of standard deviation values (% RSD) for the enrichment (measured as peak areas) of whisky flavour compounds by liquid SPME sampling using different fibres and by SBSE of whisky, using a split ratio of 1/20 (TDS)×1/20 (PTV) (all extractions performed in triplicate at 25 °C during 30 min)

| No. | Flavour compound | SPME fibre: Mean $t_R$ (min) | PDMS, RSD (%) | DVB–CAR, RSD (%) | PA, RSD (%) | SBSE | |
|---|---|---|---|---|---|---|---|
| | | | | | | $t_R$ (min) | RSD (%) |
| 1 | Ethyl hexanoate | 8.35 | 11.46 | 21.53 | 38.60 | 8.98 | 21.14 |
| 2 | Ethyl octanoate | 14.41 | 7.32 | 17.03 | 37.52 | 14.61 | 4.28 |
| 3 | Ethyl 9-decenoate | 19.56 | 3.39 | 5.46 | 32.47 | 19.69 | 7.03 |
| 4 | Ethyl decanoate | 19.85 | 3.46 | 5.64 | 32.47 | 19.94 | 3.64 |
| 5 | Isoamyl octanoate | 21.03 | 4.09 | 8.79 | 42.21 | 21.15 | 2.09 |
| 6 | 2-Methylbutyl octanoate | 21.10 | 3.83 | 6.66 | 29.18 | 21.23 | 11.88 |
| 7 | Isobutyl decanoate | 23.44 | 5.97 | 13.04 | 22.72 | 23.54 | 3.31 |
| 8 | Ethyl dodecanoate | 24.68 | 4.55 | 5.58 | 23.61 | 24.72 | 1.83 |
| 9 | Isoamyl decanoate | 25.70 | 9.50 | 5.18 | 21.55 | 25.80 | 3.22 |
| 10 | 2-Methylbutyl decanoate | 25.77 | 9.45 | 6.78 | 21.47 | 25.87 | 7.32 |
| 11 | 1-Tetradecanol | 26.43 | 20.30 | 55.16 | 38.64 | 26.66 | 68.52 |
| 12 | Ethyl tetradecanoate | 28.96 | 9.23 | 6.92 | 13.17 | 29.02 | 5.57 |
| 13 | Isoamyl laurate | 29.94 | 16.51 | 7.81 | 7.24 | 29.96 | 5.89 |
| 14 | 1-Hexadecanol | 30.69 | 8.64 | 35.99 | 36.97 | 30.69 | 34.20 |
| 15 | Ethyl pentadecanoate | 30.96 | 16.79 | 11.19 | 7.32 | 30.79 | 2.90 |
| 16 | Ethyl *E*-11-hexadecenoate | 32.52 | 9.29 | 8.04 | 7.77 | 31.93 | 3.39 |
| 17 | Ethyl hexadecanoate | 32.97 | 15.10 | 11.50 | 3.23 | 32.21 | 5.25 |
| 18 | 2-Phenylethyl decanoate | 33.99 | 5.31 | 4.10 | 12.32 | 32.96 | 7.97 |
| 19 | Ethyl linoleate | 35.93 | 15.33 | 7.32 | 6.08 | 34.14 | 5.84 |
| 20 | Ethyl oleate | 36.04 | 21.71 | 9.92 | 9.20 | 34.20 | 5.59 |
| 21 | Ethyl octadecanoate | 36.52 | 35.18 | 15.27 | 23.14 | 34.46 | 5.88 |
| | RSD (%) on sum of volatiles | | 2.80 | 4.47 | 28.80 | | 3.00 |
| | RSD (%) on sum of semivolatiles | | 11.28 | 6.99 | 6.61 | | 4.68 |
| | RSD (%) on sum of all peak areas | | 4.20 | 4.13 | 19.58 | | 3.33 |

(Mean) $t_R$=(Mean) retention time (min). DVB–CAR=Divinylbenzene–Carboxen on PDMS.

compared to PA, for the analysis of whisky volatiles, and that an extraction time of 30 min results in the highest enrichment.

When there is only a limited amount of sample available for analysis it can be interesting to do multiple SPMEs of the same sample. To test this, the effect of successive extractions of whisky samples on the response of the SPME analysis was studied. Therefore, one and the same sample was extracted three times with the same fibre and the repeatability (RSD) of the three extractions was determined; this was done for every fibre, every time using a fresh whisky sample and the results obtained with the three different fibres were compared (Table 5). RSD values were calculated for every selected whisky flavour compound, for the sum of the peak areas of the volatiles and the semivolatiles and for the sum of the peak areas of all flavour compounds.

From the data shown in Table 5 it is clear that a very good repeatability could be obtained with a PDMS fibre (RSD value <1% for the sum of all flavour compounds), which means that the SPME of one whisky sample by PDMS did not result in changes in the relative composition of the esters in the sample. When the peak areas obtained after three successive extractions were compared for every ethyl ester, a slight decrease in the enrichment of the semivolatile esters (ethyl hexadecanoate to ethyl octadecanoate) could be observed, demonstrating a small effect of depletion during liquid SPME (Table 6). This effect was different for the DVB–CAR–PDMS fibre, where a small decrease in the enrichment of volatile esters was observed after each extraction, and a progressive increase in the sorption of the semivolatile compounds, which was even more pronounced for the PA fibre (Table 6). This

Table 5
Percentage of standard deviation values (% RSD) for the enrichment (measured as peak areas) of whisky flavour compounds for three successive extractions of one whisky sample by liquid SPME sampling at 25 °C—tested with three different fibres (for each new fibre, a fresh whisky sample was used)

| No. | Ester | Fibre | | |
|---|---|---|---|---|
| | | PDMS | DVB–CAR–PDMS | PA |
| 1 | Ethyl hexanoate | 0.87 | 9.57 | 6.06 |
| 2 | Ethyl octanoate | 2.50 | 14.17 | 4.96 |
| 3 | Ethyl 9-decenoate | 4.26 | 12.06 | 12.99 |
| 4 | Ethyl decanoate | 2.33 | 9.98 | 6.86 |
| 5 | Isoamyl octanoate | 2.30 | 12.62 | 11.09 |
| 6 | 2-Methylbutyl octanoate | 3.63 | 9.89 | 19.44 |
| 7 | Isobutyl decanoate | 2.19 | 13.83 | 16.67 |
| 8 | Ethyl dodecanoate | 2.39 | 8.40 | 11.58 |
| 9 | Isoamyl decanoate | 1.61 | 12.15 | 20.44 |
| 10 | 2-Methylbutyl decanoate | 2.32 | 11.63 | 21.77 |
| 11 | 1-Tetradecanol | 55.69 | 10.30 | 18.32 |
| 12 | Ethyl tetradecanoate | 0.58 | 14.60 | 23.47 |
| 13 | Isoamyl laurate | 3.29 | 25.08 | 34.29 |
| 14 | 1-Hexadecanol | 12.50 | 9.06 | 30.73 |
| 15 | Ethyl pentadecanoate | 1.98 | 27.72 | 32.54 |
| 16 | Ethyl *E*-11-hexadecenoate | 2.41 | 17.70 | 30.29 |
| 17 | Ethyl hexadecanoate | 4.47 | 28.72 | 31.25 |
| 18 | 2-Phenylethyl decanoate | 6.28 | 10.88 | 21.59 |
| 19 | Ethyl linoleate | 2.04 | 30.00 | 33.78 |
| 20 | Ethyl oleate 1 | 3.75 | 27.98 | 37.65 |
| 21 | Ethyl octadecanoate | 8.85 | 51.32 | 49.58 |
| | RSD (%) on sum of volatiles | 1.77 | 8.13 | 10.56 |
| | RSD (%) on sum of semivolatiles | 1.24 | 23.25 | 29.30 |
| | RSD (%) on sum of all peak areas | 0.89 | 10.83 | 20.79 |

Table 6
Relative enrichment (expressed as % normalised peak area relative to peak areas obtained after first extraction, i.e., 100%) of ethyl esters after three successive SPMEs of one whisky sample at 25 °C

| Ethyl ester | Fibre | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PDMS | | | DVB–CAR–PDMS | | | PA | | |
| | Extr. 1 | Extr. 2 | Extr. 3 | Extr. 1 | Extr. 2 | Extr. 3 | Extr. 1 | Extr. 2 | Extr. 3 |
| C6 | 100 | 124 | 121 | 100 | 95 | 83 | 100 | 111 | 100 |
| C8 | 100 | 116 | 107 | 100 | 92 | 75 | 100 | 110 | 103 |
| C10 | 100 | 106 | 100 | 100 | 97 | 83 | 100 | 114 | 104 |
| C12 | 100 | 95 | 91 | 100 | 113 | 97 | 100 | 125 | 108 |
| C14 | 100 | 86 | 85 | 100 | 134 | 125 | 100 | 160 | 124 |
| C16:1 | 100 | 86 | 85 | 100 | 166 | 180 | 100 | 193 | 167 |
| C16 | 100 | 76 | 80 | 100 | 169 | 186 | 100 | 205 | 164 |
| C18:2 | 100 | 77 | 78 | 100 | 152 | 179 | 100 | 225 | 196 |
| C18:1 | 100 | 67 | 73 | 100 | 131 | 161 | 100 | 222 | 192 |
| C18 | 100 | 52 | 63 | 100 | 265 | 331 | 100 | 302 | 300 |

Test performed with three fibres.
Extr.=extraction No.

Table 7
Influence of extraction condition (headspace vs. liquid) and sampling temperature on the enrichment of whisky flavours during SPME (PDMS, 30 min), expressed as relative peak area (% relative to standard method, i.e., 100%=30 min liquid SPME at 25 °C without salt addition)

| Extraction condition | Sampling temperature | | | | |
|---|---|---|---|---|---|
| | 25 °C | Room temperature | 30 °C | 45 °C | 55 °C |
| Headspace | n.d. | 40 | 29 | 23 | 21 |
| Liquid | 100 | 99 | 82 | 63 | 50 |

n.d.=Not determined.

behaviour could be due to the existence of a competitive effect between the volatile and the semivolatile compounds for the adsorption place on the fibre. This effect resulted in high standard deviation values within the successive extractions and therefore, to a poor repeatability when a DVB–CAR–PDMS or PA fibre was used (Table 5).

Thus it is only possible to do multiple SPME analyses from the same whisky sample with good repeatability (RSD<1%) if a PDMS fibre is used, and when an extraction time of 30 min is used. With the other two fibres a higher repeatability is obtained for the extraction of the more volatile flavours than for the semivolatile ones.

### *3.3. Effect of sampling conditions on the enrichment of the volatiles during SPME*

The influence of sampling conditions (temperature, liquid vs. headspace extraction, sampling time) on the enrichment of the whisky volatiles was measured using the PDMS fibre (Tables 7 and 8).

Table 8
Influence of extraction condition (headspace vs. liquid) and sampling time on the enrichment of whisky flavours during SPME (PDMS, room temperature), expressed as relative peak area (% relative to standard method, i.e., 100%=30 min liquid SPME at 25 °C without salt addition)

| Extraction condition | Sampling time (min) | | |
|---|---|---|---|
| | 15 | 30 | 60 |
| Headspace | 22 | 40 | 44 |
| Liquid | 69 | 99 | 123 |

From these results, it can be deduced that temperature is a very important parameter during SPME sampling. Different temperatures from room temperature to 55 °C were tested for both headspace and liquid SPME. The results (Table 7) indicated that a higher sampling temperature resulted in a lower enrichment of flavour volatiles on the fibre. However, since the difference between room temperature (22–23 °C) and standard operating temperature (25 °C) is negligible, and since SPME is a method that should be standardised thoroughly, 25 °C was chosen as optimal working temperature rather than room temperature.

Since SPME is based on an equilibrium and a partition between three phases in a sample, i.e., the liquid phase, the gas phase and the fibre itself, the best results will only be achieved after complete equilibrium has been reached. Depending on the type of analytes this time can vary between a few minutes and 1 h or more. From the results depicted in Table 8, it could be concluded that equilibrium was not fully reached after 30 min and that a slightly higher recovery was achieved after 60 min. However in many cases, equilibrium must not be reached to perform a good SPME, as long as a standardised method is applied and all samples are extracted during exactly the same time. Therefore, a sampling time of 30 min was selected as optimum time.

A comparison between headspace and liquid SPME was also made (Tables 7 and 8). Liquid SPME resulted in higher enrichment of the volatiles than headspace SPME, since semivolatile esters such as ethyl oleate or ethyl linoleate were in many cases not extracted by headspace SPME, while both volatile and semivolatile compounds were well extracted by liquid SPME.

From these results, it can be concluded that the best condition for whisky sampling was liquid SPME of a stirred solution at 25 °C during 30 min without salt addition.

Chromatograms obtained after SPME of whisky with the three different fibres are depicted in Fig. 1. From Fig. 1c it can be observed that some organic acids, namely *n*-decanoic and dodecanoic acid were also extracted by the more polar PA fibre, resulting in small but broad peaks. In the chromatograms obtained after extraction of the whisky with the DVB–CAR–PDMS and PA fibres (Fig. 1b and c,

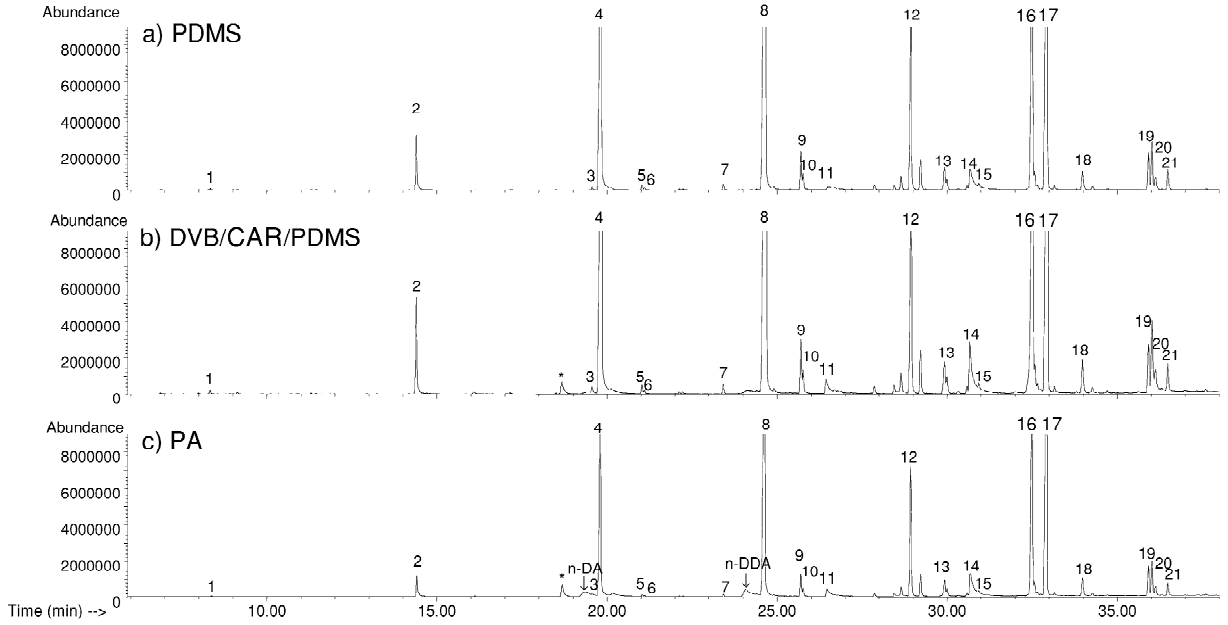


Fig. 1. Chromatograms obtained after liquid SPME of whisky samples using different fibres (30 min, 25 °C): (a) PDMS; (b) DVB–CAR–PDMS; (c) PA. Peak numbers refer to compounds listed in Table 4. *=2,4-Diisocyanato-1-methylbenzene; n-DA=*n*-decanoic acid; n-DDA=*n*-dodecanoic acid.

respectively) a small peak corresponding with the artefact 2,4-diisocyanato-1-methylbenzene was also noticed (marked with an asterisk).

### *3.4. Semi-quantitative analysis of the composition of whisky by SPME using three fibres*

Finally the relative composition of the flavour volatiles of the whisky was analysed by SPME using the three fibres in triplicate and applying the optimised method, i.e., liquid SPME at 25 °C during 30 min. For every fibre the relative composition (% contribution) of every flavour compound was calculated (Table 9) as the percent ratio of the respective peak area relative to the total peak area. In this table, the 21 target analytes as well as other minor flavour volatiles including some unidentified esters and alcohols are included to verify if the type of fibre had an influence on the determination of the relative composition of the volatiles.

From the results it can be concluded that the main flavour volatiles of the whisky are ethyl esters and that the type of fibre only had a minor influence on the calculation of the relative contribution of the flavour volatiles. Whereas the results obtained with the PDMS and DVB–CAR–PDMS fibres were very similar, the profile of the extracts obtained after SPME with the PA fibre differed slightly, which was in agreement with the patterns of the chromatograms (Fig. 1).

### *3.5. Stir bar sorptive extraction of whisky samples*

Whisky samples were finally analysed by means of the relatively new sampling technique SBSE [14] and the results were compared with those obtained with SPME.

The qualitative profile obtained after SBSE was the same as with SPME showing the same peaks corresponding mainly to the ethyl esters (Fig. 2). On the other hand, the enrichment of volatiles with SBSE was higher than with the SPME fibres, even when a higher split ratio was applied for SBSE than for SPME (e.g., 1/100 or 1/50, Fig. 3) because of the higher amount of polymer that covers the bar, proving the higher sorption capacity of SBSE. Although the higher sensitivity of SBSE can be important for trace and ultratrace analysis, it is not a

Table 9
Relative composition of flavour volatiles (% contribution) of the whisky determined by SPME–GC–MS analysis using three fibres, namely PDMS, DVB–CAR–PDMS and PA

| Mean $t_R$ | Flavour compound | Fibre | | |
|---|---|---|---|---|
| | | PDMS | DVB–CAR | PA |
| 4.35 | Isoamyl acetate | 0.07 | 0.11 | 0.19 |
| 4.65 | Styrene | n.d. | 0.11 | 0.02 |
| 8.36 | Ethyl hexanoate | 0.07 | 0.10 | 0.09 |
| 14.41 | Ethyl octanoate | 2.28 | 2.54 | 1.61 |
| 16.09 | 2-Phenylethyl acetate | 0.02 | 0.13 | 0.13 |
| 19.31 | *n*-Decanoic acid | n.d. | 0.15 | 1.40 |
| 19.58 | Ethyl 9-decenoate | 0.14 | 0.19 | 0.25 |
| 19.85 | Ethyl decanoate | 19.09 | 19.57 | 12.64 |
| 20.15 | Unidentified ester | 0.07 | 0.17 | 1.10 |
| 21.05 | Isoamyl octanoate | 0.24 | 0.20 | 0.09 |
| 21.12 | 2-Methylbutyl octanoate | 0.09 | 0.08 | 0.03 |
| 22.14 | Unidentified ester | 0.04 | 0.03 | 0.02 |
| 22.25 | Ethyl undecanoate | 0.05 | 0.04 | 0.02 |
| 23.44 | Isobutyl decanoate | 0.26 | 0.19 | 0.13 |
| 24.07 | *n*-Dodecanoic acid | 0.01 | 0.18 | 1.43 |
| 24.68 | Ethyl dodecanoate | 26.59 | 26.24 | 20.52 |
| 24.93 | Unidentified alcohol | 0.14 | 0.14 | 0.07 |
| 25.69 | Isoamyl decanoate | 1.59 | 1.38 | 1.03 |
| 25.74 | 2-Methylbutyl decanoate | 0.68 | 0.60 | 0.42 |
| 26.31 | 1-Tetradecanol | 0.87 | 0.97 | 1.45 |
| 26.53 | Propyl dodecanoate | 0.07 | 0.06 | 0.03 |
| 26.61 | Ethyl tridecanoate | 0.04 | 0.03 | 0.02 |
| 27.43 | Unidentified compound | 0.23 | 0.19 | 0.16 |
| 27.85 | Unidentified compound | 0.23 | 0.26 | 0.28 |
| 27.99 | Unidentified ester | 0.71 | 0.73 | 0.71 |
| 28.18 | Ethyl tetradecanoate | 6.64 | 6.59 | 6.65 |
| 28.38 | Unidentified alcohol | 1.24 | 1.19 | 1.12 |
| 28.48 | Hexadecanal | 0.04 | 0.03 | n.d. |
| 28.84 | Isoamyl laurate | 1.03 | 0.96 | 1.20 |
| 28.89 | 2-Methylbutyl laurate | 0.43 | 0.49 | 0.47 |
| 29.11 | Unidentified ester | 0.09 | 0.08 | 0.09 |
| 29.26 | Unidentified ester | 0.18 | 0.12 | 0.06 |
| 29.32 | 1-Hexadecanol | 3.62 | 3.43 | 3.54 |
| 29.47 | Ethyl pentadecanoate | 0.19 | 0.18 | 0.18 |
| 30.34 | Hexadecanoic acid | n.d. | 0.06 | 0.11 |
| 30.42 | Ethyl *E*-11-hexadecenoate | 12.89 | 13.21 | 15.51 |
| 30.66 | Ethyl hexadecanoate | 14.25 | 14.03 | 19.62 |
| 30.78 | Unidentified hydrocarbon | 0.21 | 0.18 | 0.26 |
| 31.29 | 2-Phenylethyl decanoate | 1.01 | 1.08 | 1.15 |
| 31.43 | Unidentified ester | 0.19 | 0.13 | 0.20 |
| 32.44 | Ethyl linoleate | 1.43 | 1.22 | 1.77 |
| 32.50 | Ethyl oleate | 2.28 | 1.97 | 3.06 |
| 32.79 | Ethyl octadecanoate | 0.63 | 0.51 | 1.09 |
| 33.57 | 2-Phenylethyl ester | 0.07 | 0.13 | 0.09 |
| | Sum | 100 | 100 | 100 |

Measurements are mean values of triplicate analyses using optimised SPME conditions (30 min liquid SPME at 25 °C). The relative composition of every flavour compound was calculated as the percent ratio of the respective peak area relative to the total peak area. Mean $t_R$ = mean retention time (mean for the three SPME fibres), n.d. = not detected, DVB–CAR = divinylbenzene–Carboxen on PDMS.

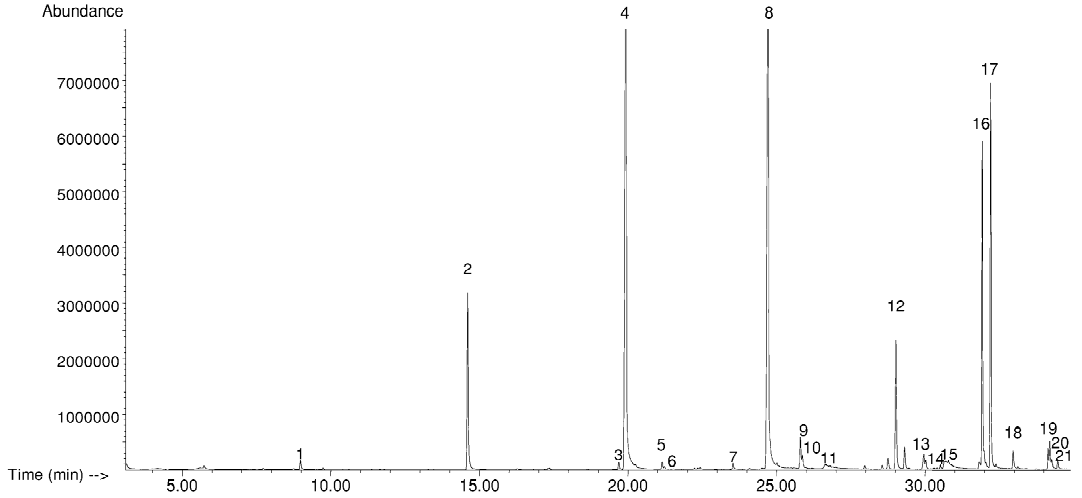


Fig. 2. Chromatogram obtained after SBSE of whisky using a split ratio of 1/20 (TDS)×1/20 (PTV injector) (30 min, 25 °C). Peak numbers refer to compounds listed in Table 4.

determinant condition to select SBSE as a better technique than SPME for whisky analysis since in many cases, the higher recovery of compounds just leads to overloaded chromatograms with broad and distorted peaks (Fig. 3a). Using splitless thermal desorption combined with split injection (split ratio PTV 1/50) however resulted in chromatograms with higher resolution and sharper peaks (Fig. 3b). Also a combination of two higher split ratios (1/20 (TDS)× 1/20 (PTV injector)) resulted in chromatograms with sharper but smaller peaks (Fig. 2).

The reproducibility of SBSE (triplicate) was also

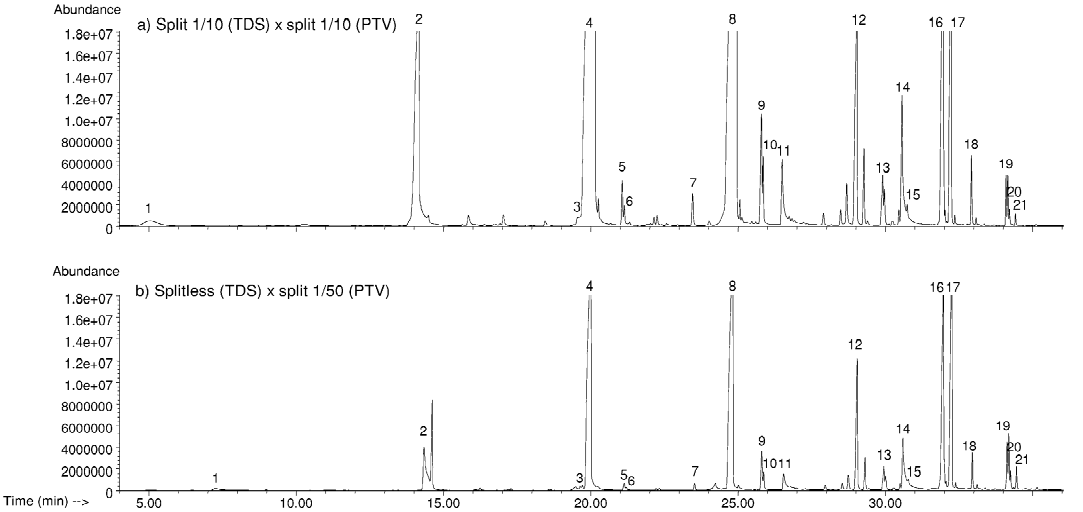


Fig. 3. Chromatograms obtained after SBSE (30 min, 25 °C) of whisky using two split modes: (a) ratio of 1/10 (TDS)×1/10 (PTV injector); (b) splitless (TDS)×1/50 (PTV). Peak numbers refer to compounds listed in Table 4.

tested and compared with SPME (for the PDMS fibre), showing comparable standard deviation values for the enrichment of the volatiles and lower RSD values for the extraction of the semivolatiles (Table 4). The overall reproducibility was slightly higher for SBSE than for SPME (RSD on the sum of all peak areas after triplicate analysis 3.3%, compared to 4.2% for SPME).

The effect of the split mode applied (split/splitless desorption and split ratio of injector) and sampling time (measured for the 1/20×1/20 split mode) on the relative enrichment of the whisky flavour volatiles was also investigated and compared (Fig. 4). From these results it can be observed that a higher sampling time (60 vs. 30 min) only resulted in a significant increase of the enrichment of the semivolatiles (53% higher recovery) but not in a drastic increase of the enrichment of the volatiles (only 30% higher recovery). Therefore it can be concluded that 30 min results in good enrichment of the flavour volatiles with good reproducibility (RSDs 3.0 and 4.7% for the volatiles and the semivolatiles, respectively). When the effect of the split condition was investigated, it could be concluded that the best results were obtained using a splitless thermal desorption and a split injection at a split ratio of 1/50. Although a higher enrichment was obtained using the lowest split–split combination (1/10 TDS×1/10 PTV), the resulting chromatogram showed broad and distorted peaks (Fig. 3a), due to overloading of the capillary column.

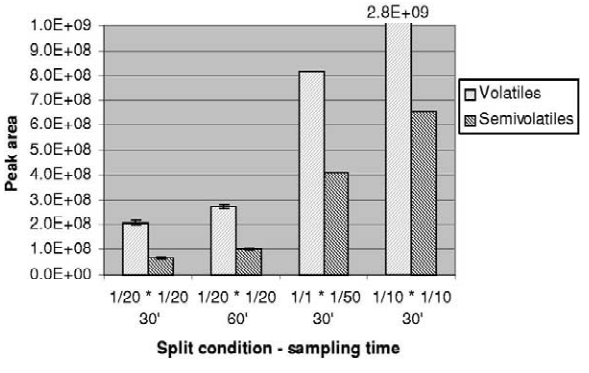


Fig. 4. Influence of sampling time (30 vs. 60 min) and split conditions applied for desorption of SBSE extracts of the whisky samples on the enrichment of the whisky volatiles during SBSE. 1/20×1/20=Split thermal desorption 1/20×split injection 1/20 (triplicate analysis). 1/1×1/50=Splitless thermal desorption× split injection 1/50. 1/10×1/10=Split thermal desorption 1/10× split injection 1/10.

## 4. Conclusions

A solid-phase microextraction method was developed to analyse the flavour volatiles of Scotch whisky. Three SPME fibres were compared in this study, namely PDMS, DVB–CAR–PDMS and PA.

The best fibres for SPME (resulting in the highest enrichment of volatiles and the highest reproducibility of the peak areas after GC–MS analysis) were PDMS and DVB–CAR–PDMS. The best condition was 30 min extraction by liquid immersion of a stirred sample at 25 °C without salt addition.

The newer technique, SBSE is also an appropriate method for the extraction of whisky volatiles but its major drawback in comparison with SPME is the need of a dedicated thermal desorption device. When applied in the split desorption–split injection mode, it does not improve significantly the results obtained by SPME. However when splitless desorption–split injection is applied, SBSE results in a higher enrichment of the whisky volatiles and good chromatograms with well resolved sharp peaks are obtained.

For ultratrace analysis on the other hand, the use of SBSE in splitless desorption–splitless injection is advised to achieve the highest sensitivity.

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