Journal of Chromatography A, 1217 (2010) 136–142

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Rapid gas chromatographic analysis of less abundant compounds in distilled spirits by direct injection with ethanol–water venting and mass spectrometric data deconvolution

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ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form 20 October 2009
Accepted 2 November 2009
Available online 13 November 2009

Keywords:
Distilled spirits
Capillary GC–MS
Solvent venting
Deconvolution

ABSTRACT

The principal trace secondary compounds common to fermentation-derived distilled spirits can be rapidly quantified by directly injecting 5 μL of spirit without sample preparation to a narrow-bore 0.15 mm internal diameter capillary column. The ethanol–water is removed in an initial solvent venting step using a programmed temperature vapourization injector, followed by splitless transfer of the target analytes to the column. The larger injection facilitates trace analysis and ethanol–water removal extends column lifetime. Problems of coelution between analytes or with sample matrix were surmounted by using mass spectral deconvolution software for quantification. All operations in the analysis from injection with solvent venting to data reduction are fully automated for unattended sequential sample analysis. The synergy of the various contributory steps combines to offer an effective novel solution for this analysis. Applications include quantification of low ppm amounts of acids and esters and sub-ppm profiling of trace compounds from both the raw material malt and the ageing in wood barrels.

1. Introduction

Commercial distilled spirits are composed of hundreds of individual flavour compounds in an ethanol–water base. These compounds result from the combined production processes of raw material (cereals, fruits, etc.) extraction, subsequent fermentation and distillation, and, in many cases, ageing for periods of time in a variety of different wooden barrels [1,2]. The different compound classes found can range in concentration from high mg/L to very low ng/L. Fusel alcohols, together with fatty acids and their esters, are the most abundant secondary compounds in distilled spirits. The perceived flavour of a product cannot always be linked to the levels of the most abundant compounds, since the odour impact of low sensory threshold trace level components are often more contributory [3]. However, these compounds are often used as indices of quality and production continuity, and are also used in authentication and counterfeit investigations [4,5].

The fusel alcohols, acids and esters can be further subdivided in terms of their naturally occurring levels, which, in turn, dictate chromatographic strategies for their quantification. The fusel alcohols, together with acetaldehyde, ethyl acetate, diethyl acetal, and methanol can be quantified by split injection to a polar capillary column with flame ionization detection [6,7].

These particular compounds elute just before and after the dominant ethanol and split injection to reduce the amount of ethanol transferred to the column is necessary for acceptable peak shape for their quantification. The European reference method for the analysis of these volatile compounds in spirits is based on this technique [8]. The method has been adapted to a 0.15 mm internal diameter column using chemometric procedures to optimise both resolution and limit of quantification while achieving a substantial decrease in chromatographic run time [9].

The situation becomes more complex for quantification of the remaining target analytes (acids and esters) as their naturally occurring levels are often too low for direct split injection. Although sample preparation, extraction, and concentration techniques can be employed, direct injection is preferable due to simplicity and cost considerations. When spirits were directly injected with a low split ratio to a 50 m phenylmethyl silicone column, 23 compounds were identified, which includes most of the previously mentioned fusel and other abundant compounds [10]. However, the important lower acids were not amenable to this approach due to incompatibility with the apolar phase. Direct splitless injection to a bonded FFAP (free fatty acid phase) capillary column succeeded in profiling many of the less abundant target compounds in a full malt whiskey [11]. An undesirable consequence of this approach is that the water transferred to the column in splitless mode progressively strips

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doi:10.1016/j.chroma.2009.11.010

the acidity function from the FFAP column. The acidity function on FFAP is a weak ester bond resulting from the modification of a standard carbowax polymer with nitroterephthalic acid and is easily hydrolyzed. This results in more frequent column changes due to retention time shifts as acids begin both to elute progressively earlier with increased tailing and undergo elution order reversals with non-acidic compounds [12].

A GC–MS protocol has been described for 19 acids and phenolic compounds in distilled spirits, which involves a pre-concentration procedure based on an anion-exchange disk extraction combined with an in-vial elution and silylation step [13]. The analytes represented a cross-section of volatile and semi-volatile organics, and included wood-derived phenolic aldehydes and acids, which are usually determined together with other similar compounds by HPLC. Again, lower fatty acids were not profiled. Direct injection with a split ratio of 1–3 in an effort to quantify the trace compounds in cider spirits resulted in poor peak symmetry with pronounced tailing for the acids, probably due to the substantial amount of water transferred to the column [14].

Recent advances in GC instrumental and column technologies and MS software have now combined to offer novel alternative strategies for direct analysis of important low level compounds in distilled spirits. An important development is the increasing use of programmed temperature vapourization (PTV) injection, where the sample can be introduced at ambient or sub-ambient temperatures. This technology offers the possibility of large volume injection with removal of solvent in the injection port liner to enrich trace level analytes. When applied to distilled spirits, the solvent to be removed is the natural ethanol–water base. In this technique, the split vent is kept open for a certain period of time, with appropriate split flows and injector temperatures used to vent the solvent while retaining the analytes of interest in the liner. When the injector is switched to splitless mode, analytes are transferred to the column by rapid ballistic heating of the injector port. Moderate injection volumes (up to 20 μL on a 2.0 mm liner) can be injected "at once", i.e. without any injection speed programming [15]. As the amount injected is increased, injection speed is important because solvent must be removed in the gas phase to avoid analyte loss with liquid solvent in the split line. Normally a liner packing is used to support the solvent during its removal.

The interactions between the various parameters involved suggest an equation which calculates the maximum rate of injection for a particular solvent based on the solvent vapour pressure, split gas venting flow, PTV temperature, and other factors [16]. Water can also be vented in this way but calculated values for the optimum injection rate will be too high because some practical considerations are not considered [17–19]. In particular, the actual PTV temperature will always be lower than adjusted due to cooling within the liner by the evaporation process, and the split gas flow will have a lower than theoretical saturation with water vapour. Nevertheless, theoretical conditions can be adjusted and there are many successful publications related to solvent venting of water or water containing matrices such as alcoholic beverages. Large volume injection with speed programming of wine and wine distillates for the analysis of a 13 compound test mixtures in comparison to liquid-liquid extraction has been reported [20,21]. Surface water was also analyzed for low level contaminants after large volume injection to a Tenax packed liner held at 20 °C using an injection rate of 12 μL/min [22]. Direct solvent vent injection of distilled spirits was investigated in an attempt to quantify all target acids and esters [23]. Liners packed with either Tenax or glass wool were investigated for injection volumes of 5, 10 and 50 μL at an injection rate 5 μL/min. All target analytes were profiled but difficulties were experienced in validating a quantitative procedure. Reproducibility for the later eluting high boiling analytes was poor on Tenax because of excessive adsorptive retention due to non-specific hydrophobic interactions. Glass wool caused similar problems for lower boiling analytes due to lack of retention.

In this paper, we report an optimum set of injection conditions which allows acceptable quantification of all target analytes. An absorptive packing is used in the liner for more efficient and less stressful transfer of analytes to the column. A routine at-once injection with moderate injection volumes of 5–10 μL is used and automated injection port liner exchange extends the utility of the protocol for multiple sample analysis. Levels of target compounds in distilled spirits can vary widely depending on both specific distillation procedures used and the final blending ratios used for commercial products. The ability to tune the injection volume to specific products or compound groups is therefore important and offers an additional practical parameter before final splitless transfer of analytes to the column. Finally, the GC separation is run on a fast narrow-bore column and MS deconvolution software is applied for quantification of coeluting analytes and analytes masked by the matrix. This deconvolution software has been successfully applied in other applications [24,25]. Columns with 0.15 mm internal diameter offer circa. 40% additional plates per meter compared to a standard 0.25 mm column, allowing shorter lengths to be used without sacrificing resolution. They also have a much higher optimum gas velocity and in this work these factors combined to reduce chromatographic run time from the normal 70 min to 30 min.

2. Experimental

2.1. Standards and reagents

All target analytes as well as the internal standards methyl myristate, methyl stearate, and acetic acid $^{13}C_2$ were obtained from Sigma–Aldrich (Wicklow, Ireland), and were used as received (at least 99% pure). The propionic-3,3,3-d_3 acid, 2-methyl-d_3-propionic acid, 3-methylbutyric-d_9 acid and the decanoic-d_{19} acid internal standards were obtained from QMX Laboratories (Thaxted, Essex, UK) and had similar purity. Standard working solutions were prepared by dilution in high purity distilled ethanol. Stock solutions of acids, esters and internal standards were prepared from individual solutions by serial dilution in distilled ethanol and reagent grade water (40:60). These solutions were used to prepare calibration mixtures of analytes with levels of the individual compounds reflecting ranges found in commercial whiskey. Samples analyzed were both full malt and blended whiskies at 40% (v/v).

2.2. Gas chromatography/mass spectrometry

The system consisted of an Agilent 6890 GC connected to an Agilent 5975 Mass Selective Detector (Agilent Technologies, Palo Alto, CA). The GC was further equipped with a peltier cooled PTV injector (CIS-4, Gerstel, Mülheim an der Ruhr, Germany). The column used was a 25 m × 0.15 mm I.D. × 0.25 film thickness CP-7686 FFAP (Varian, Middleburg, The Netherlands). The oven program was 50 °C, 2 min, 10 °C/min to 165 °C, 20 °C/min to 240 °C and held for 12 min. The carrier gas was helium with a constant head pressure of 370 kPa to give a nominal initial flow of 1.4 mL/min. This initial liner flow is advantageous for splitless transfer of compounds to the narrow-bore column. A splitless time of 11 min was possible as all fusel and other low boiling compounds before this time are vented with the solvent. The mass selective detector was operated in scan mode with a range of 25–350 m/z units. The transfer line for the column was maintained at 240 °C. The quadrupole and ion source temperatures were 150 and 230 °C, respectively, and the electron multiplier voltage was 1600 eV.

Direct injection of spirit was performed with a Gerstel MPX-2 autosampler equipped with a 10 μL syringe. The autosampler was

also equipped with an ALEX automatic liner exchange device (Gerstel, Mülheim an der Ruhr, Germany), which allowed both standard liquid injection and automatic liner exchange at any predetermined frequency during a multisample analysis sequence. Essentially, liners can be replaced before overload with non-volatile material and this function is especially useful when injecting larger volume.

2.2.1. Solvent vent injection

Injection port liners were 2 mm-deactivated glass and two different packings were investigated. These were 3% Rxi-1 (polydimethylsiloxane) on 80/100 Silcoport W and 10% Stabilwax DA (polyethylene glycol) on 100/120 Silcoport W (Restek, Bellefonte, PA). Liners were packed with 2 cm of material and packings were supported on a small plug of deactivated quartz wool inserted into the bottom of the liner. The autosampler was programmed to inject 5 μL (acids and esters) or 10 μL (trace phenolics and lactones) of a standard or sample without speed programming at an injection penetration of 45 mm. This resulted in the injected liquid depositing on top of the liner packing. The injector temperature was held at 20 °C for 2.2 min, then ramped at 10 °C/s to 230 °C (Stabilwax) or 320 °C (Rxi-1) and held for 10 min. The venting flow for ethanol–water removal at the initial injector temperature was controlled by the GC pneumatics and was set to 200 mL/min for 2 min. After this 2 min, the inlet pneumatics automatically reverts to splitless mode and the injector heating program commences at 2.2 min to transfer analytes to the column. Liners were changed after a maximum of five injections during analysis.

2.2.2. Split injection

The autosampler was programmed to inject 1 μL of a standard or sample solution without speed programming. Liners used were 2.0 mm glass packed with deactivated glass wool (Restek, Bellefonte, PA). The injector temperature program was 60 °C then immediately ramped at a rate of 10 °C/s to 320 °C and held for 10 min. The injector was operated at a 1:20 split ratio.

2.3. Identification and quantitation of whiskey target compounds

Each data file for standards and samples was analyzed by the IFD™ mass spectral deconvolution algorithms (Ion Signature Technology, North Smithfield, RI). The software identifies and quantifies compounds based on the mass spectral patterns of at least three ions per compound. In this study, the main (quant) ion and two or three of the most abundant qualifier ions were used. These patterns exclude, for the most part, typical hydrocarbon ions. By proper choice of ions, compounds coeluting with non-target matrix and even coeluting analytes can be deconvolved and individually quantified. Interactive features are also available in the software to allow the analyst to increase the acceptable accuracy of the expected mass spectral pattern when required for exclusion of non-target material. Response factors (RFs) were used to estimate analyte concentrations in the whiskey and for comparing split versus solvent vent results:

$$\mathrm{RF} = \frac{\mathrm{response}_{\mathrm{target}}/\mathrm{response}_{\mathrm{internal\ standard}}}{\mathrm{concentration}_{\mathrm{target}}/\mathrm{concentration}_{\mathrm{internal\ standard}}}.$$

Standards of 200 ppm for each internal standard and 500 ppm of each target compound for the comparative split injection method were made in an 80% (v/v) ethanol solution. This solution was then diluted 1:100 with 40% (v/v) ethanol to use for the solvent vent method resulting in 5 ppm of each compound and 2 ppm of each internal standard.

The samples for the calibration curves were created separately in a three level calibration curve ranging from 0.2 to 1.0 ppm (higher esters), 1.0 to 5.0 ppm (lower acids), 2.0 to 10.0 ppm, 4.0 to 20.0 ppm (selected acids and esters) and 39.0 to 192.0 ppm (acetic acid). For the internal standard concentrations, methyl myristate, d_3-propionic acid, d_3-isobutyric acid, d_9-isovaleric acid and d_{19}-decanoic acid were 5 ppm, acetic acid $^{13}C_2$ was 50 ppm and methyl stearate was 2 ppm. Each concentration level was run in triplicate. The average response factor %RSD at each concentration was <5% for each target analyte and <10% over the calibration range analyzed.

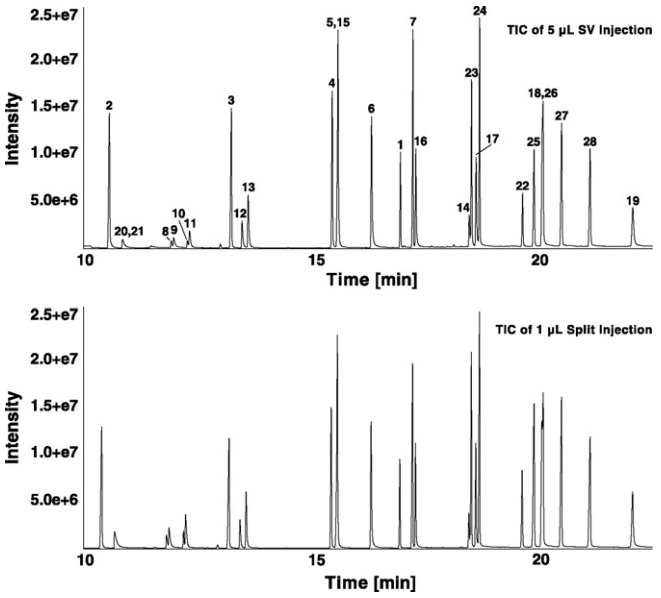

Fig. 1. Total ion chromatograms of a 5 μL solvent vent injection, top, and a standard 1 μL split injection, bottom.

3. Results and discussion

3.1. Evaluation of solvent vent in comparison to split injection

Table 1 lists the analytes and their corresponding internal standards, retention times, target ions and expected abundances used for deconvolution. Seven internal standards in total were used, with each of the five lower acids having its own internal standard. This was found to be necessary for acceptable recovery and quantification. The reason is most likely related to the two minutes these active compounds reside on the inlet packing as the solvent is being removed.

Fig. 1 compares the 5 μL solvent vent injection (SV, top) and the standard 1 μL split injection (bottom) total ion chromatograms (TIC). For purposes of comparison, 25 ng of each compound and 10 ng of each internal standard should be on-column for both injection modes. Visually, each compound pair appears to have the same approximate peak height. The slightly earlier retention times for initial peaks in split mode occurs because the transfer of sample to the column occurs without the 2 min solvent vent delay. Table 2 lists the average response factors (RF_{Ave}) for the split and solvent vent injections and their ratios ($RF^{Split}_{Ave}/RF^{SV}_{Ave}$). This response factor is based on a single calibrant concentration standard. Included in the solvent vent data are the RF_{Ave} for both the Rxi-1 and Stabilwax packings. Measurement precision is excellent for the Rxi-1 packing, with the exception of propionic acid and isovaleric acid, where percent relative deviations (%RSD) are 13.2% and 25.8% when d_3-isobutyric acid is the common internal standard for these three acids. By adding d_3-propionic acid and d_9-isovaleric acid as internal standards for corresponding non-deuterated compounds (progressing from five to seven internal standards in total), all RSD's are less than 10% on Rxi-1. Although the SV %RSD's are marginally higher than for the split injection, they are well within the criterion for quantitative data. In contrast, five compounds yield RSD's

Table 1
Whiskey target compounds and their internal standards (italicized) with retention times, ions and relative abundances.

No.	Compound	RT, min	Main Ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)
1	*Methyl myristate*	16.97	74	87(70)	143(24)	
2	Ethyl caprylate	10.54	88	101(39)	127(32)	
3	Ethyl caprate	13.23	88	101(46)	155(23)	
4	β-Phenyl ethyl acetate	15.46	104	105(12)	91(18)	43(30)
5	Ethyl laurate	15.59	183	228(41)	185(76)	157(96)
6	β-Phenyl ethyl alcohol	16.33	91	92(57)	122(29)	
7	Ethyl myristate	17.25	88	256(10)	213(15)	157(19)
8	*d_3-Propionic acid*	11.9	77	76(35)	75(20)	60(42)
9	Propionic acid	11.92	74	73(70)	45(95)	
10	*d_3-Isobutyric acid*	12.28	91	76(97)	46(550)	
11	Isobutyric acid	12.31	88	73(418)	43(663)	
12	*d_9-Isovaleric acid*	13.47	63	64(24)	93(25)	50(23)
13	Isovaleric acid	13.56	60	87(24)	45(22)	
14	*d_{19}-Decanoic acid*	18.49	77	191(12)	141(72)	157(9)
15	Caproic acid	15.59	87	99(16)	74(56)	
16	Caprylic acid	17.31	85	84(95)	87(72)	
17	Decanoic acid	18.6	129	143(18)	172(11)	
18	Lauric acid	20.06	73	60(80)	129(45)	157(35)
19	Myristic acid	22.07	73	228(27)	185(40)	129(57)
20	*Acetic acid $^{13}C_2$*	10.83	46	44(32)	62(77)	
21	Acetic acid	10.83	60	43(148)	42(26)	
22	*Methyl stearate*	19.62	74	87(72)	199(11)	298(22)
23	Ethyl hexadecanoate	18.54	88	101(60)	157(18)	
24	Ethyl-9-hexadecenoate	18.7	55	194(27)	236(27)	237(22)
25	Ethyl stearate	19.92	88	101(60)	157(19)	312(20)
26	Ethyl oleate	20.12	264	265(84)	222(61)	180(48)
27	Ethyl linoleate	20.53	67	95(72)	263(21)	308(14)
28	Ethyl linolenate	21.12	79	95(62)	108(41)	261(6)

Table 2
Average response factors (RF_{Ave}) from 1 μL 1:20 split injection and 5 μL solvent vent (SV) injection method and their ratios. The compounds are listed with their corresponding internal standards, which are italicized.

Compound	Split injection ($n=7$)		SV injection on Rxi-1 ($n=42$)			SV injection on Stabilwax ($n=42$)		
	RF_{Ave}	%RSD	RF_{Ave}	%RSD	$RF_{Ave,Split}/RF_{Ave,SV}$	RF_{Ave}	%RSD	$RF_{Ave,Split}/RF_{Ave,SV}$
Methyl myristate	–	–	–	–	–	–	–	–
Ethyl caprylate	0.68	0.8	0.48	8.8	1.41	0.03	18.3	25.45
Ethyl caprate	0.66	0.6	0.61	5.8	1.09	0.11	10.6	6.25
β-Phenyl ethyl acetate	1.64	0.9	1.69	4.3	0.97	1.55	5.3	1.06
Ethyl laurate	0.13	1.7	0.12	3.9	1.08	0.13	3.8	1.04
β-Phenyl ethyl alcohol	1.41	0.6	1.34	9.4	1.05	1.58	2.9	0.90
Ethyl myristate	0.91	0.5	0.87	1.9	1.04	0.88	0.8	1.03
d_3-Isobutyric acid	–	–	–	–	–	–	–	–
Propionic acid	2.84	2.0	2.27	13.2	1.25	2.67	5.1	1.06
Isobutyric acid	0.89	1.2	0.83	10.1	1.07	0.94	2.4	0.95
Isovaleric acid	9.76	1.1	20.74	25.8	0.47	10.62	2.7	0.92
d_{19}-Decanoic acid	–	–	–	–	–	–	–	–
Caproic acid	2.20	1.8	1.94	6.6	1.13	2.11	20.8	1.04
Caprylic acid	0.42	1.2	0.40	5.5	1.06	0.42	14.2	1.02
Decanoic acid	0.92	0.8	0.85	4.0	1.08	0.80	3.9	1.15
Lauric acid	1.52	0.9	1.34	3.5	1.14	1.15	8.9	1.32
Myristic acid	1.10	3.4	0.95	7.5	1.16	0.63	29.0	1.76
Acetic acid $^{13}C_2$	–	–	–	–	–	–	–	–
Acetic acid	1.04	1.6	1.17	4.7	0.89	1.27	5.2	0.82
Methyl stearate	–	–	–	–	–	–	–	–
Ethyl hexadecanoate	0.52	0.8	0.58	2.1	0.91	0.58	4.3	0.91
Ethyl-9-hexadecenoate	0.35	0.7	0.41	3.5	0.87	0.42	4.1	0.84
Ethyl stearate	0.89	2.0	0.80	1.3	1.10	0.82	3.8	1.07
Ethyl oleate	0.15	2.5	0.14	4.2	1.07	0.16	4.9	0.94
Ethyl linoleate	0.36	2.1	0.40	4.0	0.91	0.46	3.1	0.79
Ethyl linolenate	0.44	1.0	0.44	5.5	0.99	0.56	3.7	0.78
d_3-Propionic acid	–	–	–	–	–			
Propionic acid	0.698	1.7	0.722	2.6	0.97			
d_9-Isovaleric acid	–	–	–	–	–			
Isovaleric acid	0.896	0.8	0.920	1.9	0.97			

greater than 10% on Stabilwax. Higher acids seem to have particular difficulty desorbing from this packing and this is most likely related to the lower maximum allowable temperature. These analyses, $n = 42$, were performed over several months using at least six different liners per packing.

The $RF_{Ave,Split}/RF_{Ave,SV}$ ratio was used to compare on-column amounts for the SV injection technique to standard split injection. The ratio for all target compounds on Rxi-1 packing was excellent except for ethyl caprylate, presumably due to small differences between its boiling point and the solvent vent injector temperature, resulting in poorer recovery. In any case, this compound is usually at a level in spirits where it can be adequately quantified by the simple split injection [7]. The average ratio for all whiskey target compounds was $1.05 \pm 11.3\%$. Poorer results were obtained on the Stabilwax; if ethyl caprylate and ethyl caprate are excluded, the average ratio for all remaining target compounds is $1.02 \pm 21.8\%$. On the Stabilwax packing, ethyl caprylate and ethyl caprate are statistical outliers and are badly under-recovered while ethyl linoleate and ethyl linolenate are over-recovered. It is unclear why these two compounds behaved badly on this packing. Given these quantitative differences, the Rxi-1 packed inlet was selected for routine use to estimate compound and spiked concentrations in whiskey.

3.2. Target compound spiked whiskey analysis

To further explore the ability of the deconvolution software to quantify target analytes, each compound's concentration was estimated in an original whiskey sample. Prior to analysis, a three-point calibration curve of target compounds in 40% ethanol (v/v) produced an average RSD for RF_{Ave} of 6% for all compounds except for ethyl caprylate and β-phenyl ethyl alcohol which yielded a RF_{Ave} RSD of 16% and 20%, respectively. For these two compounds, the RF_{Ave} of the concentration level closest to the sample concentration was used. When the RF_{Ave} RSD for each compound was less than 10%, the correlation coefficient was 0.999. The concentration levels chosen roughly mirror levels found in blended whiskey.

Additionally, the two lowest calibration standard levels used to make the calibration curve were individually spiked into two samples of the original whiskey. Table 3 lists the results of the average concentration for each compound found in the original and spiked whiskey samples. The average RSD for all compounds found naturally in the test whiskey was 3.7%, with all target compounds less than 15%. These results are impressive when one considers that more than two-thirds of the target compounds were below the lowest calibration point and many at the instrument detection level. Measurement accuracy was also excellent, despite the fact that one-third of the target compounds was spiked at concentrations below the measured amount in the sample. The average precision for the Level 1 spiked sample was 2.6%, with an average recovery of $105 \pm 10\%$. Similarly, Level 2 precision and accuracy were 2.7% and $108 \pm 12\%$, respectively, with caproic acid over-recovered and outside of the acceptable range for quantitative data, i.e. ±20%.

Fig. 2 displays the total and reconstructed ion chromatograms for two sets of coeluting compounds. Even when compounds are perfectly coeluting, the software is able to identify and quantify target compounds. For example, compounds pair peaks for acetic acid $^{13}C_2$ (20) and acetic acid (21) completely overlap with each other and the matrix, see Fig. 2a, as do compound pair peaks for lauric acid (18) and ethyl oleate (26), see Fig. 2b. Note that the signals for the compound pair 20 and 21 differ by a factor of 10 as expected, since the internal standard (20) is approximately 10 times that of the acetic acid found naturally in this sample. Fig. 2c depicts the results of ethyl oleate deconvolution in the presence of lauric acid, which is 13 times greater in concentration. The figure shows a peak height signal of 2500 counts, with a peak area at 4.2 million. Only those peak scans that have been converted to

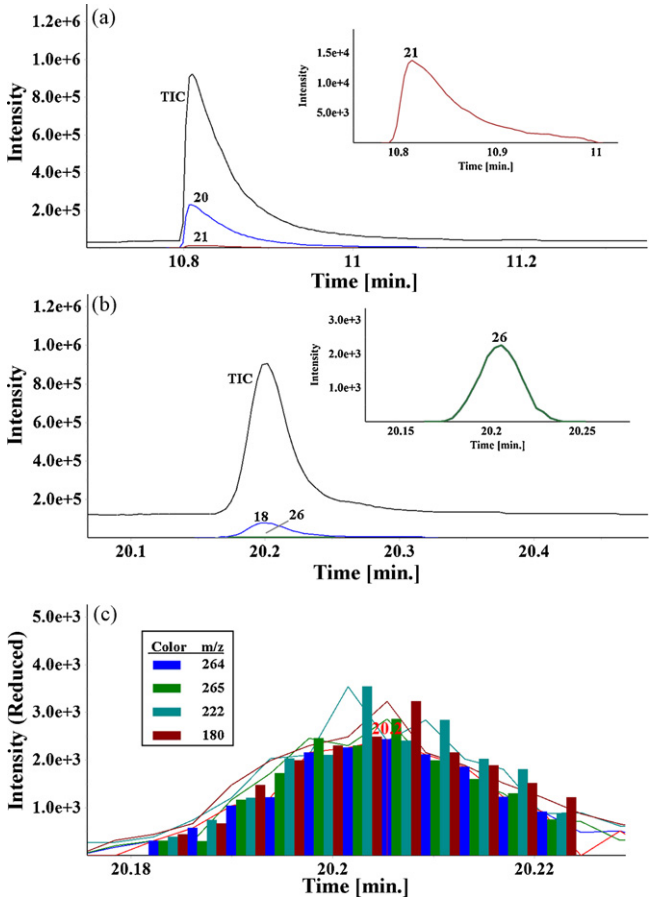

Fig. 2. TIC and RIC for target compounds in a whiskey, refer to Table 1 for peak compound information. (a) Acetic acid $^{13}C_2$ (20) and acetic acid (21), (b) lauric acid (18) and ethyl oleate (26) and (c) deconvolved RIC for ethyl oleate as visualized in the Ion Signature deconvolution software.

bars (four per scan) are integrated. Identification occurs when ion abundance ratios for main (blue) and confirming ions (green, aqua blue and brown), after normalization to the main ion at each scan, are within the acceptance criterion set by the analyst. The figure shows higher than expected ion signals at m/z 180 on the right-hand side of the peak. The algorithms mathematically adjust ion signals by comparing scan-to-scan difference across the peak. Only those scans that fall within the acceptance criterion appear as bars and are integrated, which makes the compound identification and quantification easy to inspect.

3.3. Trace phenolics and lactones

The ability to tune the injection volume when solvent venting can be productively applied to detect sub-ppm compounds of importance in distilled spirits. The only prerequisite is that correct conditions are used to allow target analytes to remain in the liner during solvent removal. If the solvent is not removed totally as vapour, then the analytes can be lost with liquid solvent in the vent line. For an at-once injection this requires an injection volume compatible with the liner packing capacity together with suitable venting conditions for removal of this volume as vapour.

Table 4 lists 21 compounds with retention times and target ions. These compounds represent contributions from two important traditional operations in whiskey production, namely, the drying procedure used in raw material malt production, and the ageing step in oak barrels. When peat smoke is used in the dry-

Table 3
Concentrations of whiskey target compounds and their recoveries for a test whiskey and two spiked samples measured in ng/μL.

Compound	Whiskey ($n=3$)	%RSD	Whiskey with spike Level 1 ($n=3$)				Whiskey with spike Level 2 ($n=3$)			
	$Conc_{Ave}$		Spike 1	$Conc_{Ave}$	%RSD	%Recovery	Spike 2	$Conc_{Ave}$	%RSD	%Recovery
Ethyl caprylate	1.17	6.3	2	2.93	2.4	93	6	6.17	11.7	86
Ethyl caprate	3.97	1.5	4	8.27	0.7	104	12	16.00	0.0	100
β-Phenyl ethyl acetate	0.61	1.0	0.4	1.00	0.0	99	1.2	1.77	3.3	98
Ethyl laurate	2.40	0.0	2	4.50	0.0	102	6	8.63	1.3	103
β-Phenyl ethyl alcohol	4.44	2.1	4	9.29	1.0	110	12	13.41	3.5	82
Ethyl myristate	0.27	0.0	0.4	0.71	1.6	106	1.2	1.60	0.0	109
Propionic acid	0.14	7.1	1	1.33	15.6	117	3	3.23	7.8	103
Isobutyric acid	0.43	15.2	1	1.67	3.5	117	3	3.77	11.1	110
Isovaleric acid	0.20	2.8	1	1.37	4.2	114	3	3.77	1.5	118
Caproic acid	0.84	2.1	1	2.00	0.0	109	3	5.07	3.0	132
Caprylic acid	3.17	1.8	2	6.10	1.6	118	6	11.00	0.0	120
Decanoic acid	3.90	0.0	4	9.07	0.6	115	12	19.00	0.0	119
Lauric acid	1.90	10.5	4	6.83	0.8	116	12	16.00	0.0	115
Myristic acid	0.35	15.1	2	2.37	8.8	101	6	7.20	6.4	113
Acetic acid	5.27	2.2	39	50.00	2.0	113	115	140.00	0.0	116
Ethyl hexadecanoate	0.77	2.0	0.4	1.10	0.0	94	1.2	2.00	0.0	101
Ethyl-9-hexadecenoate	0.83	0.7	0.4	1.10	0.0	89	1.2	1.93	3.0	95
Ethyl stearate	0.12	4.9	0.2	0.33	1.8	103	0.6	0.80	1.9	112
Ethyl oleate	0.15	3.8	0.2	0.36	1.6	101	0.6	0.80	3.1	107
Ethyl linoleate	0.12	0.0	0.2	0.34	1.7	107	0.6	0.82	1.2	114
Ethyl linolenate	0.24	2.4	0.2	0.44	4.7	102	0.6	0.98	0.6	117

%Recovery = $Conc_{Ave}$ of whiskey with spike/($Conc_{Ave}$ of whiskey + spike).

Table 4
Trace compounds representing contributions from the important traditional operations of malt drying and ageing in whiskey production.

No.	Compound	RT, min	Main ion	Ion 1 (%RA)	Ion 2 (%RA)	Ion 3 (%RA)
1	Guaicol	15.92	109	124(84)	81(59)	53(15)
2	Lactone-1	16.26	99	87(16)	71(28)	69(23)
3	Homoguaicol	16.61	138	123(88)	95(29)	
4	Lactone-2	16.85	99	87(23)	71(25)	69(27)
5	m-Cresol	17.06	108	107(87)	77(33)	79(32)
6	Phenol	17.07	94	95(12)	66(29)	65(22)
7	2-Ethyl phenol	17.54	107	108(9)	122(45)	77(25)
8	2,4-Dimethyl phenol	17.55	122	107(102)	121(56)	77(27)
9	p-Cresol	17.6	107	108(102)	77(29)	79(30)
10	o-Cresol	17.6	108	107(98)	77(29)	79(29)
11	4-Ethyl phenol	18.15	107	108(8)	122(34)	77(16)
12	2,6-Dimethoxy phenol	18.78	154	139(46)	111(22)	96(24)
13	Vanillin	21.58	152	151(109)	81(22)	109(18)
14	Ethyl vanillate	21.85	151	196(52)	168(22)	123(15)
15	Acetovanillone	22.1	151	166(49)	123(22)	
16	Ethyl homovanillate	22.6	137	210(24)	138(14)	
17	Syringaldehyde	26.92	182	181(62)	167(12)	183(11)
18	Ethyl syringate	27.6	181	226(76)	198(25)	211(11)
19	Acetosyringone	28	181	196(43)	153(12)	
20	Syringyl acetone	28.4	167	210(25)	168(12)	
21	Ethyl homosyringate	29.07	167	240(30)	168(17)	

ing procedure various low threshold phenolic compounds (phenol, cresols, etc.) are subsequently transmitted to the spirit [26,27]. On the other hand, the ageing step produces important wood extractive organoleptic compounds such as the isomeric lactones and compounds 13–21.

Fig. 3 shows the TIC and RIC after a 10 μL injection of a malt whiskey. Initially, these compounds were found by adding the known ion ratio information into the deconvolution method and searching for them. Once found, known standards were purchased except for syringyl acetone and spiked into the sample to confirm identity. Ethyl homovanillate, ethyl syringate and ethyl homosyringate were available as synthetic standards from a previous work [28]. All targets were successfully extracted as clear peaks from the complex overlapping matrix spectra. This example clearly shows the synergy between large volume injection and spectral deconvolution of the data: as the injection volume increases both target and non-target matrix signal increase in tandem, making deconvolution an important analytical tool when detecting trace compounds [29,30]. The example also illustrates how the software can be used with library data obtained from NIST, Wiley, Adams, etc. to assign tentative identification to unknowns in the sample. In this case, we confirmed the tentative assignments by spiking the sample.

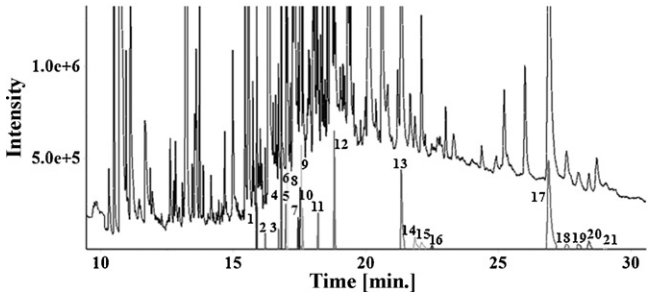

Fig. 3. TIC and RIC of trace compounds identified in a malt whiskey sample, see Table 4 for compound identities.

4. Conclusions

Trace and ultra-trace secondary compounds in aged distilled spirits can be routinely detected and quantified by GC injection without sample pre-treatment and using MS detection. The success of the approach utilises the synergistic benefits between solvent venting injection of suitable sample volumes with ambient temperature retention of target analytes in the liner, and application of mass spectral deconvolution of the target analytes in the data file. A disadvantage is that more frequent liner changing is required to accommodate larger injection volumes. Multiple internal standards (especially lower acids) are used for accurate quantification. These standards are readily available and represent lower cost when compared to personnel and material costs for off-line classical sample pre-treatment and concentration. Short narrow-bore columns can be used to substantially reduce GC run times as the deconvolution algorithms extract target compound profiles and areas from both other coeluting target and non-target matrix. This general concept can be effectively applied to other volatility compound ranges by proper choice of solvent vent injection and chromatographic conditions followed by MS deconvolution. There are many more wood-originating compounds in aged spirits that will not chromatographically elute from the polar phase used in this study. However, current results immediately suggest an extension of this work to high temperature apolar columns and using much larger injection volumes with speed programming. Apolar phases will not be suitable for acids and other compounds, but a number of individual analyses with different columns and different solvent venting conditions could be envisaged for quantification of specific compound groups, allowing a stepwise build up of useful data.

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