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Gas chromatographic–mass spectrometric analysis of acids and phenols in distilled alcohol beverages
Application of anion-exchange disk extraction combined with in-vial elution and silylation

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Abstract

A GC–MS protocol for profiling spirits, based on 19 acids and phenolic compounds, has been proposed and evaluated. The method combined a simple preconcentration procedure based on solid-phase (anion-exchange) disk extraction, and in-vial elution and silylation of the analytes. The derivatized extract was directly injected into the GC–MS system. These analytes were: $C_{6,}$ C_8, C_{10}, C_{12} acids, pyruvic acid, 2-furoic acid, succinic acid, fumaric acid, glutaric acid, lactic acid, glycolic acid, malic acid, tartaric acid, citric acid, vanillin, syringaldehyde, coniferaldehyde, vanillic acid and gallic acid. The profiles of six different spirits were found reproducible from day-to-day with <20% RSD for measurements of most of the analytes at different concentrations. Recoveries of individual analytes appear to be affected by the level of tannins in the spirits, and they varied from sample to sample. The method of standard addition was used to quantify age-related analytes. Good linearity of response with correlation coefficients in the range of 0.992–0.999 was obtained. The results of the study indicate that for spirits of the same brand but of different ages, the amounts of these analytes appear to increase with the ageing period.

Keywords: Beverages; Food analysis; Sample preparation; Organic acids; Phenol

1. Introduction

Distilled spirits are known to contain minor amounts of polar compounds such as monoacids, diacids, hydroxyacids, phenolic acids and aldehydes [1]. Aliphatic acids are mainly produced during the fermentation/distillation processes, while phenols can be formed either during fermentation, or by alcoholic extraction from the oak casks. The profile of these polar compounds, like those of fusel alcohols [2] and ethyl esters [3] is a reflection of the manufacturing process of the alcohol beverages, and is therefore useful for differentiating types or even brands of spirits. In addition, the phenolic aldehydes (vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) and acids (vanillic acid and syringic acid), being a group of compounds formed from ethanolysis of lignin during the course of maturation, have been shown to be important variables for determining the age of spirits [4]. Similarly, gallic acid which is incorporated into the spirits as a consequence of hydrolysis of tannins, is related to the ageing/maturation process. These age-related

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components, present at mg/l levels, have significant bearing on the authenticity of the beverages.

Acids and phenolics in distilled alcohol beverages are usually analyzed by high-performance liquid chromatography (HPLC) [4,5]. Due to the complexity of the sample matrix, it is very likely that there will be interfering or co-eluting peaks even under optimal chromatography conditions. It is, therefore, desirable to use a hyphenated separation and identification technique such as liquid chromatography–mass spectrometry (LC–MS) by which the peak identity can be confirmed. Since our laboratory is not equipped with an LC–MS system, the analyses were carried out using gas chromatography (GC)–MS. To be amenable to the vapor technique, these polar compounds must be converted to volatile derivatives. Silylation is the technique of choice because acid, hydroxy and phenolic functional groups present in the target analytes can be readily derivatized, and the reaction mixture can be directly injected into the gas chromatograph without further sample pretreatment. This technique, however, requires prior separation of the analytes from the sample to avoid quenching of the silylating agent by the large amount of water and ethanol present in the matrix

Due to their high solubility in the sample matrix, separation of these polar analytes cannot be efficiently accomplished by partition techniques such as conventional liquid–liquid extraction, or the more recently developed solid-phase microextraction [6]. On the contrary, solid-phase extraction (SPE) based on strong anion-exchange (SAX) disks is a more selective technique for the enrichment of the ionizable acid and phenolic analytes. Compared to the cartridge format, SPE based on disk format is faster and more reproducible because of higher sample flow-rate, more efficient mass transfer and absence of bed channeling. In addition, the physical flexibility of the disk can be exploited to reduce sample handling. For example, in an application to groundwater analysis of the herbicide Dacthal and its acid metabolites at the μg/l level, Field and Monohan performed in-vial elution and alkylation of the analytes by heating the SAX disk and the alkylation reagent together in a 2-ml autosampler vial [7]. The derivatized sample was then directly injected into a GC system.

The aim of this study was to determine the feasibility of combining SAX disk extraction and in-vial elution and silylation procedure as a method for sampling a wide variety of acids and phenols, at mg/l levels, in distilled alcohol beverages for GC–MS analysis. The procedure is to be used for generating reproducible fingerprints of spirits during the process of building a data bank. It will also be applied to the quantitative determination of several age-related compounds.

2. Experimental

2.1. Chemicals and materials

Citric acid >99.5%, coniferaldehyde (3-methoxy-4-hydroxycinnamaldehyde) 98%, fumaric acid >99%, glutaric acid 99%, glycolic acid 99%, malic acid >99%, malonic acid 99%, tartaric acid >99%, pyruvic acid 98%, and the surrogates: citramalic acid 98%, 3,4-dihydroxyphenylacetic acid 98%, 3,3-dimethylglutaric acid 98%, 3,5-dimethyl-4-hydroxybenzaldehyde 95%, homovanillic acid 98% and sodium tetraborate 99% were purchased from Aldrich (Sigma–Aldrich Canada, Oakville, Canada). Gallic acid monohydrate >98%, syringaldehyde (contains 2% of free acid), syringic acid >97%, vanillic acid >97% and vanillin >99%, were obtained from Fluka (Sigma–Aldrich Canada). Oxalic acid >99%, lactic acid 98% and succinic acid >99% were from Sigma (Sigma–Aldrich Canada). C_6, C_8, C_9, C_{10} and C_{12} acids, quantification grade, were supplied by Polyscience (Niles, IL, USA). Tannic acid 95% was purchased from Lancaster Synthesis (Windham, NH, USA). Acetone, acetonitrile and methanol HPLC grade were obtained from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile used in silylation was dried over molecular sieves 4 A. Methyl trimethylsilyltrifluoroacetamide (MSTFA) was supplied by Supelco (Oakville, Canada).

All spirit samples were purchased from Liquor Control Board of Ontario (Ottawa, Canada).

2.2. Standard solutions

Standard and surrogate solutions were prepared in 40% (v/v) alcohol solution, which is the alcohol strength of commercial spirits.

The working composite standard solution was composed of ~10 mg/l of lactic acid, pyruvic acid and gallic acid, ~5 mg/l of glycolic acid, all fatty acids, succinic acid, malic acid and tartaric acid, and ~2 mg/l of each of the remaining analytes.

The surrogate solution was composed of 1 mg/ml of each of the following compounds: 3,3-dimethylglutaric acid, 3,4-dihydroxyphenylacetic acid, 3,5-dimethyl-4-hydroxybenzaldehyde, citramalic acid, homovanillic acid and nonanoic acid.

2.3. Sample preparation

Unless otherwise indicated, all experiments were carried out in triplicates, with surrogates added to the sample before disk extraction to give a concentration of ~10 mg/l for each surrogate. Small 13 mm diameter disks were cut from commercially available 47 mm Empore Anion Exchange-SR disks (VWR, Mississauga, Canada) using a cork-boring tool. The 13 mm disks were installed on Gelman Disk Holder assemblies (Pall Gelman Sciences, Mississauga, Canada) attached to a J.T. Baker SPE vacuum manifold. A 10-ml disposable syringe barrel was fitted on each disk holder.

Following the instructions given by the manufacturer, the 13 mm SAX disks were preconditioned successively with 2 ml of acetone, 2 ml of methanol, 2.5 ml of deionized water, 1 ml of 1 *M* NaOH and 2×2.5 ml of water. The disks were not allowed to dry once the methanol was added to the disks. A 10-ml volume of the sample, without pH adjustment, was mixed with 100 μl of the surrogate solution. The solution was introduced to the syringe barrel and extracted under vacuum at 72.6 kPa. Once the sample had passed through the disk, the sample vial was rinsed with 5 ml of water and the rinse was drawn through the disk to dryness. A 10-ml volume of air was pushed through the syringe barrel and the disk using a syringe plunger. The disk was removed from the disk holder and dried on a Whatman ashless filter paper in the microwave oven (Parasonic, Genius model) for 2 min at maximum power.

To perform in-vial disk elution and derivatization, the dried disk was placed in a 2-ml autosampler vial with 500 μl acetonitrile and 500 μl MSTFA. The vial was then capped and heated at 80°C for 20 min. It was cooled to room temperature before being transferred to the autosampler carousel for GC–MS analysis.

For a smaller sample volume of 2.5 ml, the same procedure was followed except that 25 μl of the surrogate solution was used and the vial was rinsed with 2 ml of water after the sample has passed through the disk.

2.4. Recovery

Recovery data were obtained from spike and recovery experiments using the working composite standard solutions (40%, v/v, alcohol) with and without tannic acid, a whisky and a cognac samples. Analyte standards at two concentrations (low and high) were spiked into three replicate 2.5-ml samples of the working solutions and the spirits. The samples were processed through extraction, elution and silylation as described above before GC–MS analysis. The calibration curves used to predict the amounts of individual analytes were constructed by analyzing four different composite standard solutions in acetonitrile, which were treated with silylation reagent but did not go through the disk extraction process. From the difference in the amounts of analyte in the spirit determined before and after spiking, and the known amount spiked to the matrix, the recovery (%) of each analyte was calculated.

2.5. Instrumentation

The extracts were analyzed using a Hewlett-Packard gas chromatograph 6890 series II equipped with a 6890 mass selective detector (Hewlett-Packard, North Hollywood, CA, USA). The column used was a 30 m×0.25 mm I.D. DB5 ms (5% diphenyl and 95% dimethylpolysiloxane) fused-silica, 0.5 mm film thickness (J&W Scientific, Folsom, CA, USA). The carrier gas was helium and the flow-rate was kept at 1.1 ml/min throughout the run with an electronic pressure controller. The head pressure was kept at 8.8 p.s.i. throughout the run with an electronic pressure controller (1 p.s.i.=6894.76 Pa), because if the flow is constant, the pressure must vary. The split/splitless injector was operated in the splitless mode for 1 min after injection of sample. The injector port and the interface were set at 250°C and 290°C, respectively. The oven temperature was

programmed as follows: 75°C for 2 min, then ramped at 25°C/min to 100°C followed by 10°C/min to 300°C, hold for 5 min. All mass spectra were acquired in the electron impact (EI) mode at 70 eV. The mass spectrometer was scanned in the range of 39 to 450 u at a rate of 1.66 scans/s. All other mass spectrometer parameters are set by the Autotune software included in the Chemstation except the electron multiplier voltage which was set 200 V higher relative to the value determined by the Autotune.

Quantification was performed on the extracted ion chromatograms using auto-integration. Qualifiers were used to confirm the identity of individual components before quantification was carried out.

3. Results and discussion

Higher capacity is associated with larger SAX disks, and therefore more analytes will be extracted from the sample. However, the method development strategy was aimed at reducing sample handling by carrying out the elution and the derivatization steps together in an autosampler vial. This approach requires the disk to fit snugly in the 2-ml vial and be fully immersed in 1 ml silylation solution. Therefore, disks of 13 mm diameter were used in this study.

The acids and phenols commonly identified in distilled spirits are listed in Table 1, together with their molecular masses, pK_a values, the upper concentration limits and target ions used for quantification. Data for the surrogates are also listed right above the corresponding analytes. The analytes vary widely in acidity, with pK_a values in water ranging from 2.4 for pyruvic acid to 7.4 for vanillin (Table 1). These values are likely to be higher in 40% (v/v) alcohol solution because alcohol has a lower dielectric constant than water. For the purpose of discussion, these values are assumed to be the same in the aqueous alcohol matrix. The pH values of distilled spirits usually lie in the range of 3.7 to 4.8. It is recommended by the disk manufacturer to raise the sample pH at least two units above the pK_a value of the weakest acid to ensure all analytes are ionic. However, due to competition for the exchange sites by the buffer anion [8], the responses of individual analytes were found to decrease when sodium tetraborate was added to raise the sample pH to 9.1. All subsequent analyses were conducted without adjusting the sample pH.

At the pH of the spirit samples, phenolic aldehydes (vanillin, syringaldehyde and coniferaldehyde) exist predominantly in the non-ionized state because of their relatively high pK_a (~7.4). Diacids (succinic, fumaric and glutaric acids) and hydroxy polyacids (malic, tartaric and citric acids) which have low pK_a values will dissociate partially to form monoanions and lesser amounts of dianions, while the other monoacid analytes will exist in neutral form in equilibrium with the corresponding monoanions. It is reasonable to assume that on the average, each analyte has only one anion site which undergoes exchange with the disk, and it is 50% ionized at the sample pH. Based on an average M_r of 150 for each analyte, about 7 ml of a composite solution containing analytes at the levels corresponding to the upper concentration limit shown in Table 1 will saturate the exchange sites of the 13 mm SAX disk, which has a limited capacity of 0.015 mequiv. For quantitative determination of the age-related analytes, disk saturation was avoided by using a sample volume of less than 7 ml. However, for fingerprinting purposes, 10-ml samples were used to gain sensitivity since saturation of the disk can be tolerated as long as the chemical patterns generated are reproducible, and there are enough differences in the fingerprints to differentiate various spirit samples.

MSTFA was selected as a derivatization agent for this study. Other than being a versatile reagent which silylates hydroxy, carboxyl and phenol groups present in the analytes, MSTFA and its by-products do not interfere with the chromatography of the target analytes because they, being highly volatile, elute at the solvent front.

Elution and derivatization were carried out together by heating the autosampler vial containing the SAX disk in the silylating solution. The time required for the combined process of elution and derivatization was determined by monitoring the responses of the analytes extracted from the composite standard solution after 10, 15, 20 and 30 min of heating the vial. No significant changes in the responses of all analytes were observed after 15 min. For all the experiments described in this report, the in-vial elution and silylation process was conducted

Table 1
List of analytes with their pK_a values, concentration and target ions for GC–MS quantitation

Analytes[a]	M_r	pK_a[b] 25°C	Upper concentration limit[c] (mg/l)	Target ion (m/z)
Mono acids				
C_9 acid (surrogate)	158	4.9		215
Glycolic acid	76	3.8	20	205
Pyruvic acid	88	2.4	21	217
Lactic acid	90	3.8	120	117
2-Furoic acid	112	3.2	10	169
C_6 acid	116	4.8	4	173
C_8 acid	144	–	20	201
C_{10} acid	172	–	35	229
C_{12} acid	200	5.3	20	257
Diacids				
3,3-Dimethylglutaric acid (surrogate)	160	3.8, 6.4		289
Malonic acid	104	2.8, 5.7	7	147
Fumaric acid	116	3.0, 4.4	3	245
Succinic acid	118	4.2, 5.7	10	147
Glutaric acid	132	4.3, 5.4	1	147
Hydroxy polyacids				
Citramalic acid (surrogate)	148	–		247
Malic acid	134	3.4, 5.1	40	233
Tartaric acid	150	3.9, 4.3	20	292
Citric acid	192	2.8, 4.3	4	273
Phenolic aldehydes				
3,5-Dimethyl-4-hydroxybenzaldehyde (surrogate)	150	–		222
Vanillin	152	7.4	3	194
Coniferaldehyde	178	–	1	220
Syringaldehyde	182	–	6	224
Phenolic acids				
Homovanilic acid (surrogate)	182	–		326
Vanillic acid	168	4.5, 9.4	4	297
Syringic acid	198	4.3, 9.5	5	327
3,4-Dihydroxyphenylacetic acid (surrogate)	168	4.4		384
Gallic acid	170	4.5, 8.8	40	281

[a] Oxalic acid was identified but not included in this study because it was found unstable in the standard solution.
[b] Values taken from Refs. [11,12]. Only the first two acidity constants are listed.
[c] Estimation of concentrations was obtained by comparing the GC–MS responses of the samples with the working standard solution using the initial SAX disk extraction procedure before optimization.

for 20 min. Varying the MSTFA–acetonitrile volume ratio from 1:9 to 1:1 while keeping the total volume at 1 ml did not change the responses significantly. For this study, equal volumes of MSTFA and acetonitrile were used to ensure that excess silylating agent was present, which is a condition required for complete derivatization [9].

Fig. 1 shows the total ion chromatogram (TIC) of the extract obtained from the working standard solution with added surrogates. All analytes were detected as derivatives with the labile protons of all the hydroxy, carboxyl and phenolic functions replaced by trimethylsilyl (TMS) groups. Pyruvic acid, a keto monoacid, was derivatized in its enol form to

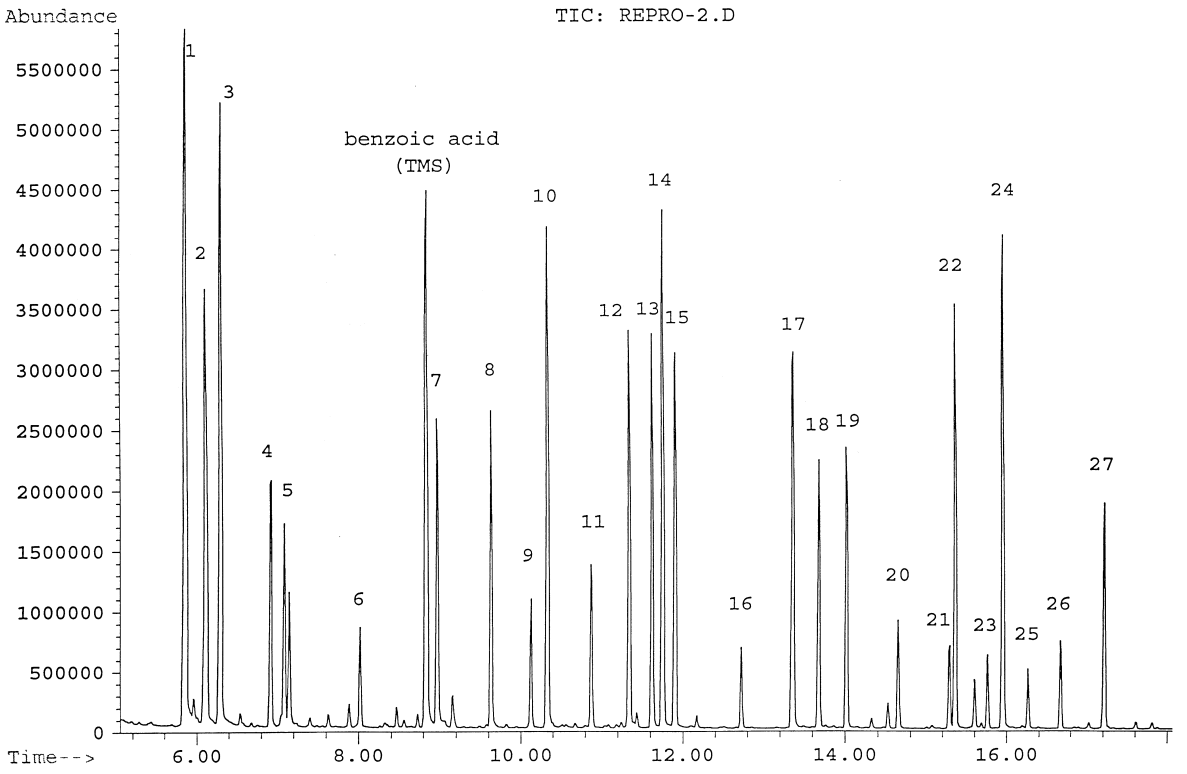

Fig. 1. TIC of the working standard solution with peak identity and concentration (mg/l). 1=Lactic acid $(TMS)_2$ (13.3); 2=glycolic acid $(TMS)_2$ (4.1); 3=C_6 acid (TMS) (4.1) and pyruvic acid $(TMS)_2$ (13.7); 4=oxalic acid $(TMS)_2$ (14.4); 5=2-furoic acid (TMS) (2.6); 6=malonic acid $(TMS)_2$ (1.9); 7=C_8 acid (TMS) (4.7); 8=succinic acid $(TMS)_2$ (5.1); 9=fumaric acid $(TMS)_2$ (1.3); 10=C_9 acid (TMS) surrogate, (11); 11=glutaric acid $(TMS)_2$, (2.6); 12=3,3-dimethylglutaric acid $(TMS)_2$, surrogate (10.7); 13=C_{10} acid (TMS) (6.8); 14=citramalic acid $(TMS)_3$, surrogate (12.2); 15=malic acid $(TMS)_3$ (7.4); 16=vanillin (TMS) (1.2); 17=3,5-dimethyl-4-hydroxybenzaldehyde (TMS), ISTD4 (9.6); 18=tartaric acid $(TMS)_4$ (4); 19=C_{12} acid (TMS) (4.6); 20=syringaldehyde (TMS) (1.9); 21=vanillic acid $(TMS)_2$ (2.2); 22=homovanillic acid $(TMS)_2$, surrogate (9.9); 23=citric acid $(TMS)_4$ (2.2); 24=3,4-dihydroxyphenylacetic acid $(TMS)_3$, surrogate (13.5); 25=coniferaldehyde (TMS) (1.3); 26=syringic acid $(TMS)_2$ (2.6); 27=gallic acid $(TMS)_4$ (11.6).

give a disilylated derivative. All peaks were well separated except the derivatives of pyruvic acid and C_6 acid. However, integration of these two compounds was not a problem because they have characteristically different target ions. Benzoic acid, which was always detected in the blank, is a manufacturing contaminant on the Empore disk. For practical reason, the peak was ignored in all the chromatograms.

At room temperature, elution of analytes from the disk and subsequent derivatization might continue, or the silylated derivatives might degrade. These changes are undesirable because the reproducibility of the measurements cannot be ensured. A study was carried out to determine the stability of response ratios as a function of waiting time at 5, 20, 28 and 44 h on the autosampler, using a standard solution, a cognac and a whisky samples. For the three solutions, no significant changes in the responses of the surrogates were observed over the 44 h period. The response ratios for most of the analytes were constant within 20 h, while that of malonic acid showed a marked decrease and that of syringic acid increased significantly in the three solutions over the same period of time. As a result of this study, malonic acid and syringic acid were removed from the list of the

target analytes. All samples were analyzed within the first 20 h after silylation to minimize the effect of waiting time on the responses.

3.1. Precision and recoveries

The precision of the method was determined using the composite standard solution. Three extractions were carried out and each extract was analyzed once by GC–MS. Measurements of the responses or response ratios of individual analytes to the corresponding surrogates were repeatable with <6% RSD.

As shown in Table 2, the recoveries of individual analytes from 2.5 ml of each of the three samples were different, suggesting the presence of matrix effects. For most of the analytes, the recoveries decreased in the order: standard solution>whisky CC>cognac RM. The trend may be related to the presence of tannins in the spirits. Hydrolyzable tannins, which are highly soluble in spirits [10] are derived from oak barrel during ageing. They are macromolecules containing multiple acidic phenolic functions which compete for the exchange sites on the SAX disk. Their presence, therefore, has an adverse effect on the extraction efficiencies of the target analytes. Cognac RM is likely to have a higher

Table 2
Recoveries of analytes from different matrices

	Recovery (%)			
	Standard+0.05% tannic acid[a]	Standard	Whisky	Cognac
Nonoanoic acid (surrogate)		86.1		71.3
Glycolic acid	101.0	104.0	99.0	78.6
Pyruvic acid	22.8	49.0	46.0	19.9
Lactic acid	53.2	63.2	61.0	58.5
2-Furoic acid	73.1	98.6	89.6	95.8
C_6 acid	69.3	80.0	87.0	74.3
C_8 acid	49.0	85.4	71.4	74.8
C_{10} acid	45.6	91.6	71.6	66.8
C_{12} acid	40.5	86.3	55.4	51.8
3,3-Dimethylglutaric acid (surrogate)		46.0		14.5
Succinic acid	34.2	42.4	23.0	14.1
Fumaric acid	20.9	59.1	22.9	18.1
Glutaric acid	25.8	32.2	25.2	13.2
Citramalic acid		34.0		14.4
Malic acid	15.1	49.1	23.3	17.1
Tartaric acid	11.3	36.9	22.0	15.5
Citric acid	7.4	22.1	6.8	5.7
3,5-Dimethyl-4-hydroxybenzaldehyde (surrogate)		99.1		50.5
Vanillin	61.8	80.9	82.5	51.4
Syringaldehyde	52.1	104.3	101.6	66.6
Coniferaldehyde	46.8	94.5	60.7	51.2
Homovanillic acid (surrogate)		40.1		13.2
Vanillic acid	12.2	36.2	20.4	11.8
3,4-Dihydroxyphenyacetic acid (surrogate)		9.7		5.3
Gallic acid	–[b]	3.3	2.1	2.4

[a] Tannins usually present at 0.01 to 0.05% in spirit samples [13].
[b] Recovery cannot be determined because the tannic acid added also contains a small amount of gallic acid.

concentration of tannins than Whisky CC, as indicated by the presence of higher level (6.7 vs. 1.7 mg/l) of gallic acid, which is the hydrolysis product of tannins. The standard solution is devoid of tannins and therefore shows higher recoveries. The role of tannins was demonstrated by the lower recoveries obtained from the standard solution fortified with tannic acid at a level normally found in spirits. However, it should be noted that since spirits contain myriad of other components, tannic acid may not be the only factor that affects the recoveries.

Based on the recovery data obtained from the working standard solution, three classes of compounds were identified (Table 2): fatty acids, phenolic aldehydes, 2-furoic acid and glycolic acid were recovered in high yields (>80%); gallic acid was obtained in extremely low yield (3%) and the remaining analytes were recovered in relatively low yields (22–65%). The standard solution that had passed through the disk was examined for analytes by passing through a second 13 mm SAX disk. Little or no breakthrough was observed for the analytes. Inefficient elution was considered to be the cause of the low recoveries. The disk was removed from the vial after the elution/silylation process, and after being rinsed with acetonitrile, it was heated in a fresh MSTFA solution for repeated derivatization. More analytes, about 5–20% of the amounts recovered in the first elution/silylation step, were obtained from the second treatment of the disk. Evidently, the in-vial elution and silylation process was not completed in the first treatment. The fact that prolonged heating of the disk in the presence of excess silylating reagent did not increase the response suggests that the in-vial elution/silylation could be an equilibrium process. Phenolic aldehydes, which exist predominantly in the non-ionized state at the sample pH, were recovered at high yields possibly due to more efficient elution since they were retained on the disk through interaction with the styrene–divinylbenzene matrix of the SAX, and not through ion-exchange mechanism. Comparison of the data of the fatty acids and the diacids also suggests that the extraction process is not purely ionic, but may also involve reverse phase absorption of the alkyl chain, resulting in higher recoveries of the fatty acids than the diacids.

3.2. Chemical profiling of spirits

In our laboratory, the method is intended to be used for populating a data bank, from which the acid and phenol profiles of standard spirits are retrieved whenever necessary to compare with that of the sample to determine its authenticity. It is, therefore, required that the method be reproducible from day to day to obtain the same fingerprint any time the spirit sample is analyzed. The recovery data in Table 2 indicate that the surrogates chosen resemble most, though not all, of the corresponding analytes in the extraction and GC–MS characteristics. For these compounds, prediction of concentrations from calibration curves based on analyte/surrogate response ratios will be more accurate than that based on the analyte responses. However, no attempts were made to assure the accuracy of the analyte concentrations used to characterize the spirits, because reproducibility, rather than accuracy, is the important performance criterion of a method used for fingerprinting purpose.

Long-term reproducibility of the profile based on analyte concentrations were determined using six commercial spirit samples containing high and low levels of analytes. A 10-ml volume of each sample was extracted and analyzed for five different days at about two-week intervals over a period of about three months. One extraction and one GC–MS analysis were carried out each day for each spirit and the working standard solution. The determination of analyte contents was generally reproducible from day-to-day within <20% RSD for most of the analytes at different concentrations. These results show that one analysis per sample at any time would give a reliable quantitative profile, and the method is suitable for use in establishing a reference data bank. The TICs of these six spirits are distinctly different from one another by visual examination, indicating the analytes chosen in this study are useful variables for characterizing distilled spirits. Multivariate statistical analysis technique will be used to evaluate a larger set of data when the data bank is established.

For routine profile analysis, normally a large volume of the standard solution is prepared and used for several months before it is exhausted. During this period of time, acids might react with ethanol in the

medium to form ethyl esters. A study was carried out to determine the stability of individual analytes in the solution. A working standard solution was analyzed as a sample immediately after preparation on three different days at one month interval over a period of three months. Each day the test solution was extracted twice and each extract was analyzed once. The average analyte concentrations of the test solution were predicted from the one-point calibrations obtained from a freshly prepared standard solution. Over the three month period, the concentrations of individual analytes varied randomly with <10% RSD, indicating no significant degradation of the analytes in the standard solution.

3.3. Determination of age-related analytes

Due to the presence of matrix effects on response as described earlier, the quantification of analytes in spirits was determined by the method of standard addition. To avoid saturation of the disk which will adversely affect the linearity of the response, only 2.5 ml of the sample was used. Two spirit samples, Cognac RM VSOP and Whisky CG 6 years, were spiked at three levels of the age-related analytes: vanillin, syringaldehyde, coniferaldehyde, vanillic acid and gallic acid. For most of the compounds, the highest level corresponds to approximately twice the analyte concentration in the unspiked sample. For both spirits, good linearity of response or response ratio was observed for the analytes, with correlation coefficients in the range of 0.992 to 0.999, and the residual plots did not show any trend.

To demonstrate the utility of the standard addition method described above, three sets of spirits were analyzed. In each set, there were two or three spirits of the same brand name but of different degree of maturation. As shown in Table 3, for Cognac RM, the VS and VSOP samples are very similar in the analyte contents, while the XO only shows significantly higher levels of vanillic acid and gallic acid than the VS and the VSOP counterparts. The 30-year-old Scotch BW is richer in all analytes than its 12- and 17-year-old counterparts, which are very similar in analyte composition except in gallic acid content. For Whisky CC, the 12-year-old sample shows slightly higher levels of all the target analytes. It should be noted that the correlation between the levels of the phenolic compounds and the ageing period is not a simple relationship. A previous study [4] on Grappa has indicated that the levels of phenolic aldehydes are not only dependent on the ageing duration, but are also affected by the storage conditions and characteristics of the barrels. This may also explain why Cognac RM VSOP and 6-year-old Whisky CC which have undergone comparable maturation periods, do not have comparable levels of the analytes.

4. Conclusions

This SAX disk-based SPE protocol provides a new sample preparation method for the GC–MS analysis of acids and phenols in distilled alcohol beverages. The adoption of in-vial elution and silylation approach reduces sample handling. This technique yields reproducible chemical profiles of spirits. It also allows quantitative analysis of selected analytes.

Table 3
Concentrations of age-related compounds in spirits

Compound name	Cognac RM[a]			Scotch BW			Whisky CC	
	VS	VSOP	XO	12 years	17 years	30 years	6 years	12 years
Vanillin	2.2	2.1	2.5	1.6	2.1	6.1	1.2	1.9
Syringaldehyde	6.7	4.9	5.9	3.5	4.0	15.8	3.0	5.4
Coniferaldehyde	1.0	0.7	0.7	0.8	0.4	1.5	0.6	1.1
Vanillic acid	0.9	0.8	1.6	0.6	1.0	3.9	0.7	0.9
Gallic acid	6.4	6.7	11.8	2.4	7.0	37.6	1.8	2.4

[a] Numbers are quoted in mg/l. VS: A blend of 2–4-year-old cognacs; VSOP: contains a very high percentage of 4–6-year-old cognacs; XO: contains a very high percentage of >6-year-old cognacs.

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