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# Multivariate Analysis of FTIR and Ion Chromatographic Data for the Quality Control of Tequila

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Principal component analysis (PCA) was applied to the chromatographic and spectroscopic data of authentic Mexican tequilas ($n = 14$) and commercially available samples purchased in Mexico and Germany ($n = 24$). The scores scatter plot of the first two principal components (PC) of the anions chloride, nitrate, sulfate, acetate, and oxalate accounting for 78% of the variability allowed a classification between tequilas bottled in Mexico and overseas; however, no discrimination between tequila categories was possible. Mexican products had a significantly ($p = 0.0014$) lower inorganic anion concentration (range = 1.5–5.1 mg/L; mean = 2.5 mg/L) than the products bottled in the importing countries (range = 3.3–62.6 mg/L; mean = 26.3 mg/L). FTIR allowed a rapid screening of density and ethanol as well as the volatile compounds methanol, ethyl acetate, propanol-1, isobutanol, and 2-/3-methyl-1-butanol using partial least-squares regression (precisions = 5.3–29.3%). Using PCA of the volatile compounds, a differentiation between tequila derived from "100% agave" (*Agave tequilana* Weber var. *azul*, Agavaceae) and tequila produced with other fermentable sugars ("mixed" tequila) was possible. The first two PCs describe 89% of the total variability of the data. Methanol and isobutanol influenced the variability in PC1, which led to discrimination. The concentrations of methanol and isobutanol were significantly higher (methanol, $p = 0.004$; isobutanol, $p = 0.005$) in the 100% agave (methanol, 297.9 ± 49.5; isobutanol, 251.3 ± 34.9) than in the mixed tequilas (methanol, 197.8 ± 118.5; isobutanol, 151.4 ± 52.8).

**KEYWORDS:** Agave (*Agave tequilana* Weber var. *azul*); Agavaceae; tequila; authenticity; adulteration; identity of spirits; ethanol; volatile compounds; ion chromatography; FTIR

## INTRODUCTION

The production of the Mexican spirit drink tequila is restricted to the blue agave (*Agave tequilana* Weber var. *azul*, Agavaceae) and restricted to defined geographic areas, primarily to the State of Jalisco in West-Central Mexico (*1, 2*). Two basic categories of tequila can be distinguished: "100% agave" and "mixed" tequila. For the high-quality category 100% agave, only pure agave juice is allowed to be fermented and distilled. By Mexican law, all premium 100% agave tequilas must be bottled in Mexico. A mixed tequila is manufactured by adding up to 49% (w/v) of sugar, mainly from sugar cane (*1*). This lower end tequila is usually shipped out in bulk containers for bottling in the importing countries (*3, 4*).

Tequila is protected under the North American Free Trade Agreement (NAFTA) and an agreement between the European Union and the United Mexican States on the mutual recognition and protection of designations for spirit drinks (*5*).

Adulteration with other types of alcohol (e.g., grain spirits) or the mixing of different types of tequilas is a violation of Mexican standards. Due to frequent fraud, the Mexican Tequila Regulatory Council (TRC) plans to tighten the regulations for exporters and overseas handlers, for example, by certification of tequila bottlers by Mexican government inspectors (*4*).

In official food control, efficient methods are required to prevent the wrongful use of the protected name "tequila" as well as to check the labeled category. Traditionally, tequila is characterized by the detection of volatile compounds using gas chromatography (GC) or sensory techniques (*6–14*). Recently, the classification of tequilas by near-infrared spectroscopy was reported (*13*). Concerning the tequila composition, the Mexican Official Standard (*1*) has defined wide ranges for the concentrations of volatile compounds, which are almost identical for the four tequila types, "blanco/silver/white", "gold", "reposado/rested", and "añejo/aged". No more information about the differentiation of the specifications of 100% agave and mixed

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tequila is given in the official standards. The first analytical discrimination between the two categories was reported by Aguilar-Cisneros et al. (*15*) using headspace solid-phase micro-extraction in combination with gas chromatography and isotope ratio mass spectrometry (IRMS) of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of ethanol. However, the high costs of instruments limit the possibilities for applying this method in official food control. In a recent study by Bauer-Christoph et al. (*13*), traditional gas chromatography was compared to $\delta^{13}C$-IRMS as well as SNIF-NMR of ethanol. The concentrations of methanol and 2- and 3-methyl-1-butanol as well as their ratio were found to be the most suitable analytical approach to differentiate 100% agave and mixed tequilas. By using stable isotope ratio analyses, it seemed to be more difficult to achieve a differentiation, because $\delta^{13}C$ values and (D/H) ratios of samples from 100% agave and mixed tequila only showed small differences. Preliminary results using gas chromatography and multivariate data analysis to discriminate between the two categories were reported by Aguilera-Rojo et al. (*12*).

Ion chromatography (IC) and Fourier transform infrared (FTIR) spectroscopy were recently introduced to the analysis of spirit drinks (*11, 16−18*). The characterization of vodka and rum was possible by IC (*16*). FTIR showed a great potential for the classification of brandies (*18*). Multivariate statistics has shown to be a powerful tool for the authenticity control of alcoholic beverages (*19−21*). In this work IC and FTIR are used for the first time in combination with multivariate data analysis to assess the authenticity of tequila.

## MATERIALS AND METHODS

**Samples.** Authentic tequila samples of 100% agave ($n = 7$) and mixed categories ($n = 7$) were available from controlled tequila production facilities in the Jalisco region. In addition, commercial tequila samples purchased in Mexico as well as samples submitted by local authorities to the CVUA Karlsruhe were analyzed (100% agave, $n = 5$; mixed, $n = 19$).

**Ion Chromatography.** A previously developed method (*16*) for the determination of anions in extract-free sprits such as vodka and rum was applied to tequila samples. For that purpose the sample preparation was modified to include an inline-ultrafiltration step to prevent contamination of the column, for example, by caramel or polyphenols contained in gold, reposado, or añejo tequilas.

The chromatographic analyses were performed on a Compact IC 761 system (Deutsche Metrohm, Filderstadt, Germany) equipped with an IC Filtration Sample Processor 788 and a conductometric detector including a temperature-compensated conductivity cell and an MSM packed bed suppressor. Substances were separated on an anion-exchange column (Metrosep A Supp 5, 4 × 100 mm i.d.) fitted with a guard column (RP-Guard, 4 × 25 mm i.d.). The eluent consisted of 3.2 mM sodium carbonate and 1.0 mM sodium bicarbonate per liter. The tequila samples were diluted 1:10 and injected into the sample processor. The filtration step is executed automatically prior to analysis according to the standard procedure of the manufacturer using a cellulose acetate filter with a pore size of 0.15 $\mu$m. Separations were carried out with a flow rate of 0.7 mL/min and an injection volume of 10 $\mu$L. The volume of the conductivity flow cell was 0.5 $\mu$L. The IC Net chromatography software was used for instrument control, data acquisition, and processing.

For quantification the validated procedure ISO 10304-2 (*22*) was used without modification. The calculation was carried out automatically using the standard software supplied by the manufacturer against a previously prepared calibration. Repeated analysis of authentic tequila samples was used to examine the precision of the method. Groups of cases were compared using *t* and Wilcoxon tests. Statistical significance was assumed at below the 0.05 probability level.

**Fourier Transform Infrared Spectroscopy.** A WineScan FT120 instrument (Foss Deutschland, Hamburg, Germany) was used to generate the FTIR spectra. No prior preparation of the samples is required, however, all samples were adjusted with deionized water to 38% v/v of ethanol to exclude its influence on multivariate data analysis. The samples were automatically thermostated at 40 °C in the spectrometer before analysis. The IR spectrum was scanned between 4996 and 930 cm$^{-1}$ (1054 data points per spectrum). The spectral regions of the water absorption between 1887 and 1447 cm$^{-1}$ and between 3696 and 2971 cm$^{-1}$ were excluded for data analysis. The spectra were obtained in duplicate and averaged for each sample.

**Multivariate Data Analysis.** For quantitative determination of the volatile compounds methanol, ethyl acetate, propanol-1, isobutanol, and 2-/3-methyl-1-butanol from the FTIR spectra by applying partial least-squares regression of specific spectral regions, the standard software FT 120 v 2.2.1 with a calibration provided by the manufacturer was used (Foss Deutschland, Hamburg, Germany).

The quantitative data of IC and FTIR analyses were exported to the software Unscambler v9.0 (CAMO Process AS, Oslo, Norway). The data set of the anions and the volatile compounds were preprocessed by standardization to give all variables the same variance. Then principal component analysis (PCA) was used to transform the original measurement variables into new variables called principal components (PC). The technique of cross-validation was applied to determine the number of principal components (PCs) needed. During cross-validation, one sample at a time (of *n* samples) is left out, and the prediction ability is tested on the sample omitted. This procedure is repeated *n* times, resulting in *n* models, and will give an estimate on the average prediction ability for the *n* models. This result is used to select the number of PCs needed. By plotting the data in a coordinate system defined by the two largest principal components, it is possible to identify key relationships in the data as well as to find similarities and differences.

## RESULTS AND DISCUSSION

**Ion Chromatography.** Spirits are diluted to bottling strength with water from high-proof distillates. The ionic content of the water and brand-specific water additives used gives rise to differences in the ionic composition of the product. Therefore, the simple, cost-saving, and reliable method of ion chromatography, which is already approved in water analysis (*23*), can be used for the determination of anions in spirits. A major advantage of ion chromatography as an analytical technique is that most of the time it requires little or no sample preparation and it uses only a small amount of the sample. Even in the analysis of a matrix as complex as spirit drinks, it shows high selectivity, sensitivity, and reproducibility (*16*). To optimize the sample presentation before ion chromatography, the sample was filtered by an automated unit included in the autosampler of the ion chromatograph, resulting in interference-free chromatograms (**Figure 1**). During routine analyses of 100 authentic samples, no interfering peaks of the matrix were observed. In blank analyses between the sample runs no carry-over from the filtration unit, which was automatically cleaned after each run, could be detected. The cellulose acetate filter could be used for more than 200 samples without degradation of the filter material and without break-through or carry-over of the filter residue. The RSD, determined by multiple analyses of different tequila samples, did not exceed 7.07%, indicating good assay precision (**Table 1**). In addition to the inorganic anions chloride, nitrate, and sulfate, the simultaneous determination of acetate and oxalate was possible. The analysis results of 38 anonymized tequila samples are given in **Table 2**. Phosphate could not be detected in any of the samples.

Using descriptive statistics, significant differences between the tequila samples were recognizable. In particular, large differences exist between spirits bottled in Mexico and overseas. The Mexican-bottled products had a significantly ($p = 0.0014$) lower inorganic anion concentration (range = 1.5−5.1 mg/L;

Quality Control of Tequila

*J. Agric. Food Chem.,* Vol. 53, No. 6, 2005 **2153**
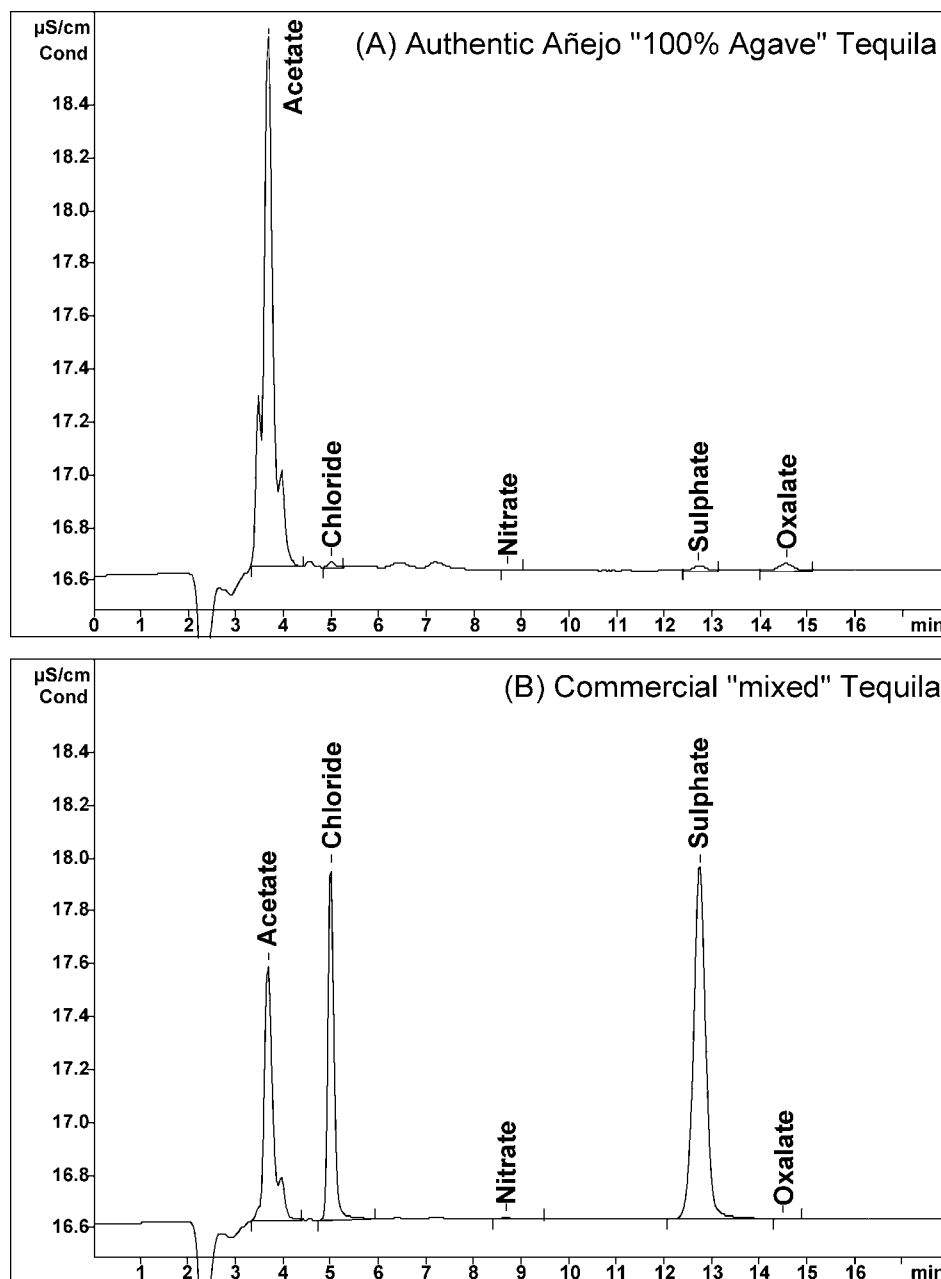


**Figure 1.** Ion chromatograms of tequila samples: (**A**) authentic Añejo 100% agave tequila 9; (**B**) commercial mixed tequila 31. Quantitative data are presented in **Table 2**.

**Table 1.** Validation Data of Ion Chromatography (Acetate, Chloride, Nitrate, Sulfate, and Oxalate); Comparison between Intra- and Interday Precisions

|  | intraday precision[a] (%) | | interday precision[a] (%) | |
|---|---|---|---|---|
|  | tequila 36 | tequila 31 | tequila 36 | tequila 31 |
| acetate | 0.88 | 1.45 | 1.94 | 1.81 |
| chloride | 6.37 | 1.79 | 6.99 | 1.99 |
| nitrate | 1.18 | 6.55 | 4.08 | 7.07 |
| sulfate | 1.75 | 1.91 | 3.27 | 1.92 |
| oxalate | nd | nd | nd | nd |

[a] Precision is expressed as RSD (%), $n = 20$. nd, not detected.

mean = 2.5 mg/L) than the tequilas bottled overseas (range = 3.3−62.6 mg/L; mean = 26.3 mg/L) (**Figure 2**).

This confirms our previous results of premium vodka or rum brands that also had particularly low inorganic anion concentrations (*16*). Such products are usually manufactured using ion

exchange or reverse osmosis for deionization. An explanation for the lower concentrations of anions in Mexican products may be the fact that they are distilled directly to 40% v/v if they are intended to be bottled in the country of origin. To reduce unitary transportation costs, bulk products are distilled to 55% v/v (*2*). In the importing country, those products are obviously diluted with drinking water containing relatively high amounts of anions.

Considering the organic acid anions, no significant differences in the acetate concentration of the tequila groups could be found; for example, both 100% agave and mixed tequilas encompass wide ranges of 62.1−363.5 and 39.8−345.8 mg/L, respectively. However, oxalate was found predominantly in the 100% agave tequilas. Calcium oxalate crystals are found abundantly in all tissues of *Agave tequilana* plants (*24*). The acidic pH of the agave matrix (*2, 25, 26*) apparently liberates the free oxalic acid in part, which is then transferred into the alcoholic distillate. However, due to the low oxalate concentrations detected, further
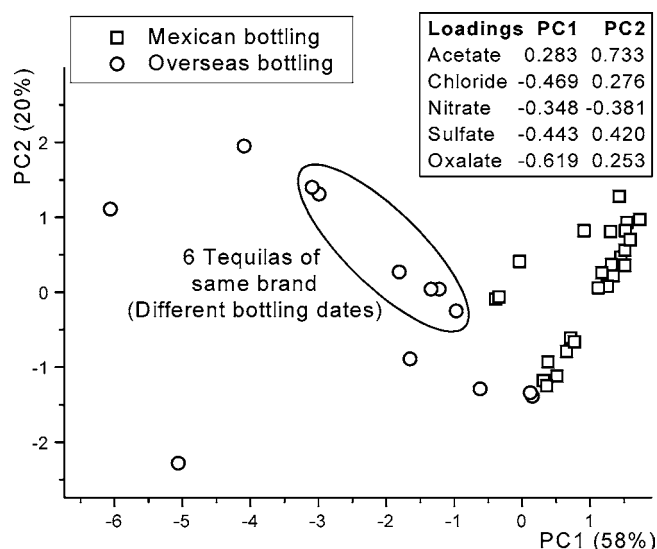
**Table 2.** Results of Ion Chromatography and FTIR Spectroscopic Analyses for Authentic and Commercial Tequila Samples

| no. | sample name | origin | acetate (mg/L) | chloride (mg/L) | nitrate (mg/L) | sulfate (mg/L) | oxalate (mg/L) | methanol (mg/100 mL of ethanol) | ethyl acetate (mg/100 mL of ethanol) | propanol-1 (mg/100 mL of ethanol) | isobutanol (mg/100 mL of ethanol) | 2-/3-methyl-1-butanol (mg/100 mL of ethanol) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Blanco 100% Agave | authentic | 239.0 | 0.7 | 0.2 | 0.5 | nd[a] | 314 | nd | nd | 228 | 81 |
| 2 | Blanco 51% Agave | authentic | 223.6 | 1.2 | 0.7 | 0.7 | nd | 221 | 2 | nd | 152 | 75 |
| 3 | Reposado 100% Agave | authentic | 327.0 | 0.8 | 0.3 | 0.6 | 0.8 | 351 | nd | nd | 295 | 99 |
| 4 | Reposado 51% Agave | authentic | 315.2 | 1.7 | 0.6 | 0.7 | nd | 229 | 1 | nd | 205 | 89 |
| 5 | Añejo 100% Agave | authentic | 363.5 | 1.4 | 0.3 | 0.7 | 0.9 | 318 | 13 | nd | 279 | 111 |
| 6 | Añejo 51% Agave | authentic | 345.8 | 0.7 | 0.7 | 0.6 | 0.8 | 228 | 14 | nd | 218 | 101 |
| 7 | Reposado 100% Agave | authentic | 273.2 | 0.8 | 0.3 | 0.6 | 0.8 | 310 | nd | nd | 243 | 96 |
| 8 | Reposado 51% Agave | authentic | 253.1 | 1.5 | 0.3 | 0.7 | nd | 234 | 14 | nd | 174 | 87 |
| 9 | Añejo 100% Agave | authentic | 295.6 | 1.8 | 0.3 | 0.6 | 1.0 | 305 | 2 | nd | 265 | 106 |
| 10 | Añejo 51% Agave | authentic | 279.8 | 0.8 | 0.2 | 0.6 | nd | 224 | 31 | nd | 175 | 97 |
| 11 | Reposado 100% Agave | authentic | 314.4 | 1.1 | 0.7 | 0.7 | 0.9 | 327 | nd | nd | 279 | 106 |
| 12 | Reposado 51% Agave | authentic | 298.4 | 0.7 | 0.3 | 0.6 | nd | 229 | 10 | nd | 196 | 101 |
| 13 | Reposado 100% Agave | authentic | 268.7 | 0.9 | 0.6 | 0.6 | 0.8 | 329 | nd | nd | 269 | 98 |
| 14 | Reposado 51% Agave | authentic | 248.2 | 1.8 | 0.8 | 0.7 | nd | 240 | 5 | nd | 186 | 90 |
| 15 | Añejo 100% Agave | commerical | 231.3 | 2.7 | 1.2 | 1.1 | 1.3 | 235 | 5 | nd | 222 | 830 |
| 16 | Blanco | commerical | 122.4 | 0.9 | 0.8 | 0.6 | nd | 206 | nd | nd | 129 | 51 |
| 17 | Blanco | commerical | 70.8 | 1.0 | 0.2 | 0.6 | nd | 208 | 51 | 29 | 100 | 22 |
| 18 | Blanco 51% Agave | commerical | 95.8 | 1.7 | 0.9 | 0.9 | nd | 174 | 21 | 16 | 252 | 26 |
| 19 | Blanco 100% de Agave | commerical | 68.8 | 1.2 | 0.9 | 0.8 | nd | 298 | 63 | 39 | 189 | 39 |
| 20 | Blanco 100% Agave | commerical | 62.1 | 0.9 | 0.7 | 0.6 | nd | 317 | 66 | 9 | 209 | 5 |
| 21 | Blanco 51% Agave | commerical | 221.4 | 0.7 | 0.2 | 0.6 | nd | 203 | 39 | 9 | 156 | 21 |
| 22 | Añejo 100% Agave | commerical | 160.5 | 1.1 | 0.8 | 1.2 | 1.3 | 167 | 38 | 45 | 239 | 74 |
| 23 | Añejo 51% Agave | commerical | 152.8 | 1.9 | 0.8 | 1.4 | 1.3 | 108 | 39 | 50 | 208 | 64 |
| 24 | Reposado 100% Agave | commerical | 126.1 | 0.6 | 0.2 | 0.7 | nd | 304 | 61 | nd | 298 | 20 |
| 25 | Reposado 51% Agave | commerical | 138.6 | 1.2 | 0.7 | 0.7 | nd | 192 | 38 | nd | 217 | 3 |
| 26 | Agave Brandy | commerical | 21.2 | 21.9 | 15.4 | 4.4 | nd | 39 | nd | nd | 66 | 19 |
| 27 | Licor de Agave | commerical | 138.7 | 6.0 | 2.0 | 1.4 | 1.4 | 725 | 903 | 437 | 16 | nd |
| 28 | Gold | commerical | 128.9 | 22.2 | 0.8 | 9.0 | nd | 141 | 15 | 33 | 126 | 38 |
| 29 | Silver | commerical | 139.4 | 21.7 | 1.2 | 9.4 | nd | 177 | nd | 27 | 137 | 39 |
| 30 | Silver | commerical | 146.5 | 44.4 | 8.8 | 9.4 | nd | 206 | 8 | 41 | 132 | 55 |
| 31 | Silver | commerical | 141.2 | 34.7 | 0.8 | 10.1 | nd | 121 | nd | nd | 116 | 12 |
| 32 | Silver | commerical | 168.8 | 9.3 | 8.5 | 2.4 | nd | 179 | 6 | 11 | 155 | 51 |
| 33 | Silver | commerical | 115.7 | 25.7 | 1.1 | 3.1 | nd | 185 | nd | 1 | 135 | 28 |
| 34 | Gold | commerical | 119.1 | 19.4 | 1.0 | 2.7 | nd | 130 | 9 | 24 | 175 | 48 |
| 35 | Silver | commerical | 107.9 | 22.3 | 0.7 | 2.4 | nd | 115 | 20 | 51 | 112 | 19 |
| 36 | Silver 100% Natural | commerical | 59.7 | 8.4 | 2.8 | 0.9 | nd | 120 | nd | nd | 95 | 11 |
| 37 | Gold | commerical | 39.8 | 1.7 | 0.8 | 0.8 | nd | 145 | 7 | 22 | 195 | 21 |
| 38 | Blanco | commerical | 63.3 | 1.9 | 1.7 | 0.6 | nd | 164 | nd | nd | 108 | 19 |

[a] Not detected.
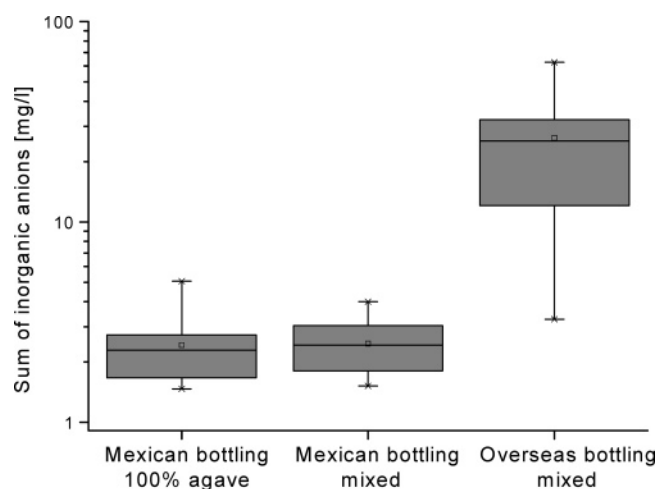


**Figure 2.** Box charts of the analysis results of tequila samples in a logarithmic scale (box, 25th, 50th, and 75th percentiles; whiskers, 5–95% range). The Mexican tequilas have significantly lower anion concentrations than the exported ones ($p = 0.0014$).



**Figure 3.** PCA scores scatter plot of the concentrations of the anions acetate, chloride, nitrate, sulfate, and oxalate of 38 tequila samples under study.

studies using preconcentration are required to evaluate this parameter as a discriminating factor between the tequila categories.

The findings of the descriptive statistics are verified by the multivariate analysis of all anions. The scores scatter plot of the first two PCs, which together express 78% of the total variability, is shown in **Figure 3**. A clustering of the authentic tequilas bottled in Mexico can be observed; however, no discrimination between 100% agave and mixed tequilas is possible. The exported tequilas seemed to separate on PC1 in the score plot, which could be mainly attributed to the negative

Quality Control of Tequila

*J. Agric. Food Chem.,* Vol. 53, No. 6, 2005    **2155**

influence of chloride, sulfate, and oxalate in the PC1 loadings. The subclusters in the Mexican tequila group may be explained by differences in the water quality of the respective bottling sites. Furthermore, some of the exported tequilas closely cluster with the Mexican ones, showing that the model has its limitations in determining the origin of tequila. However, it was found that six tequilas of the same manufacturer are clustered in the scores plot (**Figure 3**). Therefore, samples of the same brand but with different dates of bottling appear to have very similar anion concentrations. This is verified by previous findings (*16, 17*) describing very stable anion compositions in spirit drinks bottled at the same site. Therefore, tequila can be characterized by the ionic composition of the water used in its production. An allocation or a differentiation of tequilas, for example, in the context of food and restaurant controls or charges against restaurant operators and barkeepers, may be possible. Sometimes, instead of a high-quality brand shown on the menu, cheap or possibly inferior quality products may be sold. Apart from organoleptic evidence and the analysis of volatile compounds, ion chromatography can be used to determine the brand of tequila. However, in addition to the adulterated product, authentic comparison samples should be confiscated and analyzed if possible.

**Fourier Transform Infrared Spectroscopy.** As opposed to ion chromatography, which characterizes the water quality used in spirit manufacturing, FTIR characterizes the alcoholic distillate as the other major constituent of tequila. Recent developments in design and performance of FTIR spectrometers combined with advances in chemometrics software have provided an interesting analytical tool that is suitable for rapid product screening and process control (*21*). Compared to a conventional analysis of spirit drinks, which uses different methods such as distillation and pycnometry or oscillation-type densimetry (*27*) as well as gas chromatography, the method is substantially faster (only 1 min per sample) and easier to use. No time-consuming sample preparation (such as distillation or extraction for traditional volatile compound analysis) is required at all.

Ethanol and the volatile compounds as well as polyphenolic components extracted during aging contain absorptions of various functional groups in the infrared spectra. However, the constituents of spirit drinks are chemically very similar and therefore display similar and overlapped absorptions, which cannot be assigned to individual compounds (**Figure 4**). Therefore, chemometric techniques have to be used to interpret the spectra. For the quantitative determination of specific components in spirit drinks, a ready-to-use PLS calibration was made available by the manufacturer, which was previously validated in our laboratory. The analysis results of the tequila samples and validation data are given in **Tables 2** and **3**.

Good assay precision was determined for the parameters density, ethanol, and methanol, whereas the volatile compounds showed inferior precisions up to 29.3%. It should be noted, however, that FTIR is an indirect method, which uses a calibration based on previously determined wet-chemical results. In addition, in comparison to other spirit drinks, tequila contains only relatively low amounts of higher alcohols according to Mexican standards (*1*); for example, ethyl acetate, propanol-1, and 2-/3-methyl-1-butanol could not be detected in all samples using FTIR spectroscopy. FTIR should therefore be treated as a fast, reliable screening method. Due to the calibration sets and not to the FTIR technique itself, the quantitative results have not enough confidence for official complaints against manu-
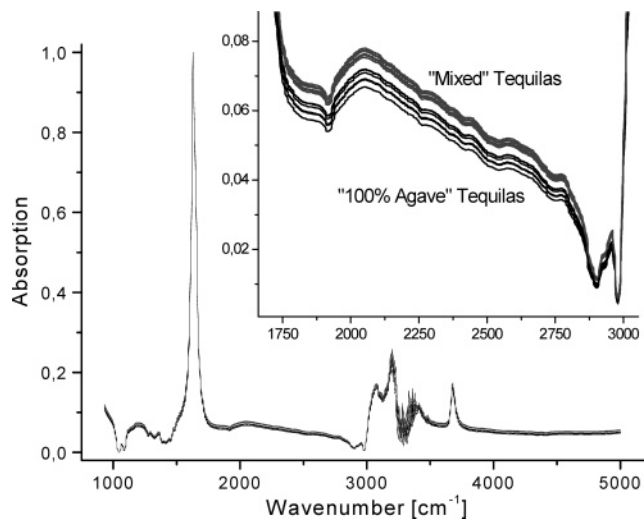


**Figure 4.** FTIR spectra of seven authentic 100% agave and seven authentic mixed tequila samples. The inset shows a characteristic region of the spectra with a strong vertical expansion.

**Table 3.** Validation Data of FTIR Spectroscopy Data

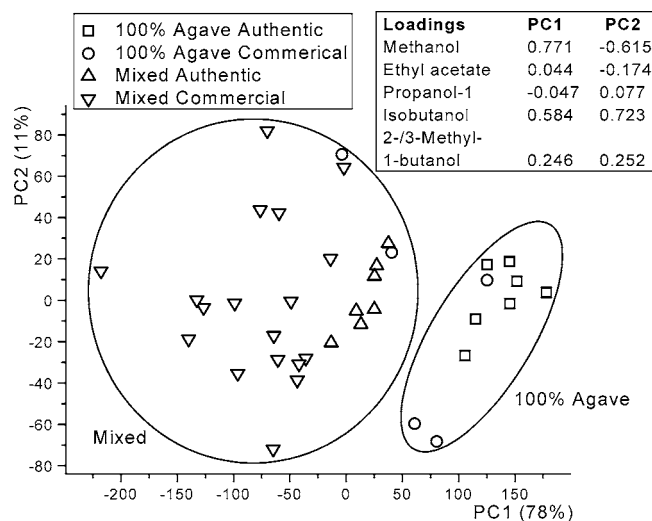| *n* = 186 | *R* | SEC[a] (mg/100 mL of ethanol) | precision[b] (%) |
|---|---|---|---|
| rel density | 0.9895 | 0.0007 | 0.07 |
| ethanol (% v/v) | 0.9975 | 0.17 | 0.42 |
| methanol | 0.9974 | 23.5 | 5.3 |
| ethyl acetate | 0.9943 | 29.9 | 13.5 |
| propanol-1 | 0.9894 | 40.8 | 27.8 |
| isobutanol | 0.9745 | 22.4 | 21.8 |
| 2-/3-methyl-1-butanol | 0.9397 | 36.0 | 29.3 |

[a] Standard error of calibration. [b] Precision is expressed as coefficient of variation (SEC/mean × 100).

facturers. In this regard, the results should be confirmed using reference methods such as gas chromatography (*28*).

Using PCA of the volatile compounds determined with FTIR (methanol, ethyl acetate, propanol-1, isobutanol, and 2-/3-methyl-1-butanol), two groups of samples are noted: a first group of mixed tequilas, which shows a considerable dispersion especially within commercial tequilas, and a second group comprising the 100% agave tequilas (**Figure 5**). The first two PCs describe 89% of the total variability of the data; the variability in PC1, which leads to the discrimination, is influenced by methanol and isobutanol. The concentrations of these compounds are significantly higher (methanol, $p = 0.004$; isobutanol, $p = 0.005$) in the 100% agave tequilas (methanol, $297.9 \pm 49.5$; isobutanol, $251.3 \pm 34.9$) than in the mixed ones (methanol, $197.8 \pm 118.5$; isobutanol, $151.4 \pm 52.8$). This can be related to the amount of agave used, because larger amounts of methanol are mainly due to the presence of pectins in the agaves.

The other compounds leading to the variability in PC2 have no discriminating power. Interestingly, the PCA scores plot shows that two of the five commercial samples labeled 100% agave do not fit in the group of authentic 100% agave samples. Adulteration seems to be possible in these cases.

**Food Regulatory Viewpoints.** One sample declared as "Agave brandy" of unknown origin totally diverges in the ion chromatographic as well as in the FTIR spectroscopic analysis from the other samples. The sample had the lowest concentration of methanol and the highest concentration of nitrate of all samples analyzed. In the organoleptic examination only a flat

| Loadings | PC1 | PC2 |
|---|---|---|
| Methanol | 0.771 | -0.615 |
| Ethyl acetate | 0.044 | -0.174 |
| Propanol-1 | -0.047 | 0.077 |
| Isobutanol | 0.584 | 0.723 |
| 2-/3-Methyl-1-butanol | 0.246 | 0.252 |

**Figure 5.** PCA scores scatter plot of the alcoholic compounds methanol, ethyl acetate, propanol-1, isobutanol, and 2-/3-methyl-1-butanol, determined in 38 tequila samples using FTIR spectroscopy.

aroma was found. Therefore, the serving of this brandy in a bar as tequila is a deception of the consumer.

Another sample declared as "tequila 100% natural" was also conspicuous, because it was found to be of the mixed category. The declaration "100% natural" can easily be confused with the declaration "100% agave" and is also a deception of the consumer.

From our experience, products of the mixed category, which have by far the greatest market share in Europe, are often advertised on the label as of "premium" quality. Gold tequilas colored with food dyes are frequently advertised as of "special quality due to aging".

Considering all of these aspects, a clear labeling of the tequila category is required to give better information to the consumer. In Europe, an addition of sugar to the mash before fermentation is not permitted in the production of spirit drinks. Therefore, the consumer expects to get 100% agave tequila, especially if a high quality is announced on the label. The implementation of an ingredient list in the labeling of tequila would be adequate.

A revision of the Mexican Official Standard with respect to giving distinct analytical specifications for the two categories would also be advisable. Apart from control of the Certificates of Export of the CRT, the official food control in the importing countries would then have a better potential to detect fraudulent or wrongly designated tequilas.

**Applicability in Routine Analysis.** Increasing requirements and cost-pressures force governmental and commercial food-testing laboratories to replace traditional reference methods with faster and more economical methods. For this purpose, screening methods, which ensure a very high sample throughput, seem to be most advantageous. Obviously, the combination of chromatography or spectroscopy with PCA achieves a much higher level of discrimination than simple consideration of individual compounds.

The rapidity with which information can be obtained about a large number of alcoholic compounds within the tequila sample and the requirement of no sample preparation indicate that FTIR is unique in its ability to comprehensively survey a large number of samples. There are considerable advantages in comparison with conventional methods of analysis. Only conspicuous results of analysis, which may lead to an official rejection of the tequila, must be assured by complex and labor-intensive reference analytic (e.g., distillation and gas chroma-

tography). FTIR will attain increasing importance as a routine method in beverage analysis.

Compared to the results in headspace gas chromatography isotope ratio mass spectrometry analyses using the combustion and pyrolysis mode (HRGC-C/P-IRMS) developed previously (*15*), the methods described in this paper using ion chromatography and FTIR spectroscopy are much easier to perform in routine analysis. Headspace HRGC-IRMS analysis is time-consuming and requires special instrumentation. Only differentiation between 100% agave and mixed tequilas depending on the origin of sugars used (C3, C4, or CAM) was possible.

In the future, multivariate analysis in combination with other spectroscopic techniques such as ^{1}H NMR may provide further information about the tequila composition, especially in regard to the discrimination between blanco, gold, reposado, and añejo tequilas, which could be solved by neither ion chromatography nor FTIR spectroscopy.

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Quality Control of Tequila

*J. Agric. Food Chem.,* Vol. 53, No. 6, 2005 **2157**

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