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## Development and validation of a High Performance Liquid Chromatography-Diode Array Detection method for the determination of aging markers in tequila

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## ABSTRACT

A solid-phase extraction procedure followed by analysis by high performance liquid chromatography (HPLC) with UV-vis photodiode array detection (DAD) is proposed to simultaneously determine 11 aging markers in tequila. The method showed good intraday ( $n = 5$ ) and interday ( $n = 3$ ) precision, RSD < 1.6% in both cases, for each of the identified compounds. The calibration curves were linear at the tested ranges ( $R^2 > 0.999$ ). Good recoveries (84.2–108.5%) were obtained for 10 of the 11 compounds studied; and the LOD and LOQ ranged from 0.62 to 4.09  $\mu\text{g/mL}$  and 1.9–12.4  $\mu\text{g/mL}$ , respectively. The proposed methodology was applied to a set of 15 authentic tequila samples grouped by aging state (*blanco*, *reposado* and *añejo*). An ANOVA analysis combined with discriminant analysis with stepwise backward variable selection was used to differentiate between the various aging groups based on their oak related compounds content.

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### 1. Introduction

Tequila is a Mexican alcoholic beverage recognized worldwide for its unique flavor. According to the Mexican regulations, Tequila must be made exclusively from *Agave tequilana* Weber blue variety [1]. During tequila elaboration, the agave steams are cooked in ovens or autoclaves with the purpose of converting the existing complex carbohydrates into fermentable sugars. The cooked juice is then fermented and distilled twice in order to obtain a clear spirit. Tequila is classified according to the percentage of sugars used during the fermentation process that are from *A. tequilana* Weber blue variety. For the case of 100% tequilas, all sugars are from the *A. tequilana* Weber blue variety, whereas for mixed tequilas, up to 49% of the sugars can be from different sources, typically sugar cane. Tequila can also be classified according to its aging time in oak casks. In this way, the silver variety or *tequila blanco* is the name for unaged tequila; aged tequila or *tequila reposado* is the tequila that has been aged for at least 2 months in oak casks; and extra aged

tequila or *tequila añejo* is the tequila that has been aged for at least 1 year in oak casks [1].

Currently, some methodologies have been reported in the literature for the differentiation between 100% and mixed tequilas based on the concentration of methanol, as well as 2- and 3-methyl-1-butanol as measured by gas chromatography [2], or in other reports by a comparison of their infrared spectra [3]. For discrimination among different aging states, some studies have investigated the use of ion chromatography and Fourier transform infrared (FTIR) spectroscopy [3], as well as stable isotope analysis [4], however, these methodologies have not been able to differentiate among the common age classifications of tequilas. It is worth mentioning that the Mexican regulation for tequila does not establish any analytical tests for verifying the authenticity of tequila type or age.

During tequila aging, complex chemical changes occur in the oak casks, wood constituents are extracted into the tequila, providing it color and a characteristic taste [5]. The aging step, however, is one of the most susceptible to adulteration, as oak extracts or caramel coloring may be added to tequila *blanco* during production to simulate the color of tequilas that have been aged in oak barrels.

In aged alcoholic beverages such as wine, brandy and cachaça, the presence of low molecular weight phenolic compounds such as gallic acid, vanillin, syringaldehyde, sinapinaldehyde,

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coniferaldehyde, syringic acid, ferulic acid, esculetin, scopoletin, and furanic compounds has been reported [6–10]. The occurrence of these compounds in alcoholic beverages is the result of oak lignin degradation and oxidation processes [11] and is considered evidence that the alcoholic beverage has been aged authentically in an oak cask [9,12]. By identifying the compounds that tequila acquires during aging, it may be possible to authenticate this production stage.

Sample preconcentration, through techniques such as liquid–liquid extraction (LLE) [13,14] and solid-phase extraction techniques (SPE) [10,15] has been used to enhance the analysis of phenolic compounds in other alcoholic beverages. In these studies, SPE has shown to be more effective and yield shorter extraction times as compared to LLE. In particular, SPE cartridges with polymeric sorbents have been preferable due to higher recoveries of phenolic compounds [7]. No study yet exists, however, which presents a technique for the simultaneous determination of age-marking compounds in tequila.

The aim of this study was to develop and validate a method for the identification and quantification of low molecular weight phenolic compounds that tequila acquires during aging time in oak barrels, using high performance liquid chromatography with UV–vis photodiode array detection (HPLC–DAD). This method was then used to statistically classify certified tequila samples according to their aging time by discriminant analysis.

## 2. Experimental

### 2.1. Reagents and materials

Gallic acid, 5-(hydroxymethyl)-furfural, protocatechuic acid, vanillin, syringaldehyde, sinapinaldehyde, coniferaldehyde, vanillic acid, syringic acid, ferulic acid, scopoletin, were used as chromatographic standards as purchased from Sigma–Aldrich (Toluca, México). Two-gram Discovery DSC-18 SPE cartridges of polymeric material (polypropylene), were used as purchased from Sigma–Aldrich. HPLC grade methanol and acetonitrile were purchased from Caledon (Georgetown, Canada). Ultrapure water from Milli-Q system (Millipore, Bedford, MA, USA) was used in all experiments. All solutions used as mobile phases were filtered through 0.45  $\mu\text{m}$  membranes (Millipore) and degassed prior to use.

### 2.2. Tequila samples

The analytical procedure was tested on authentic tequila samples obtained directly from a diverse group of major Mexican tequila producers. Three samples from three different tequila producers were obtained for each of the common 100% agave tequila age classifications (*blanco*, *reposado*, and *añejo*). Three samples from three different tequila producers were also obtained for each of the two common age classifications for mixed tequilas (*blanco*, and *reposado*). It must be noted here that mixed tequila of the *añejo* age classification is rarely encountered in the industry and was thus not used in this study. Additional age classifications for tequila such as gold (*joven/oro*) or ultra aged (*extra añejo*) were deemed to be outside the scope of this study.

### 2.3. Sample preparation and SPE extraction

In order to optimize recovery and detection of the phenolic compounds identified as aging markers, tequila samples were prepared as follows: 50 mL of sample was placed in a round-bottomed flask, and attached to a Büchi rotavapor R-120 (Flawil, Switzerland) operating at a vapor temperature of 28 °C, a vapor pressure of 120 mbar and rotating at 2 rpm. The sample was concentrated until it reached

approximately 50% of its initial volume in order to remove the ethanol from the sample. The resulting solution was then passed through a preconditioned Discovery DSC-18 cartridge, making use of a Varian SPE vacuum chamber (Varian, USA) that allows for the processing of up to 20 samples simultaneously. Cartridge preconditioning followed the manufacturer recommendations: 12 mL of HPLC grade methanol was passed through the cartridge followed by an additional 12 mL of water adjusted to pH 2 with formic acid. Upon sample extraction, 12 mL of HPLC grade methanol was used to remove the sample from the cartridge and the resulting extract was concentrated to dryness using the Büchi rotavapor operating at the aforementioned conditions. Finally, the resulting extract was reconstituted with 1 mL HPLC grade methanol and injected into the HPLC system.

### 2.4. Chromatographic conditions

Development and validation of the chromatographic method was performed on a Varian HPLC system (Varian Inc., USA) equipped with a Varian ProStar 335 UV–vis photodiode array detector and a Varian ProStar 230 ternary LC pump. Separation of the tequila samples and standard solutions was performed at 25 °C on a 5  $\mu\text{m}$  Spherisorb ODS column (Waters, USA) of 250 mm  $\times$  4.6 mm, protected with a precolumn of the same material and using an injection volume of 10  $\mu\text{L}$ . Two solvents were used: mobile phase A consisting of 10:90 (v:v) water:methanol and mobile phase B consisting of 2:1:1 (v:v:v) water:methanol:acetonitrile, both acidified with 2 mM formic acid. The optimized elution gradient was the following: at 0 min, 90% A, 10% B, flow = 0.4 mL/min; at 7 min, 90% A, 10% B, flow = 0.6 mL/min; at 10 min, 90% A, 10% B, flow = 0.8 mL/min; at 30 min, 85% A, 15% B, flow = 0.8 mL/min; at 40 min, 70% A, 30% B, flow = 1.0 mL/min; at 55 min, 80% A, 20% B, flow = 1.0 mL/min; at 60 min, 90% A, 10% B, flow = 0.4 mL/min.

### 2.5. Identification and quantification of compounds

Identification of compounds was performed by comparing retention times and UV spectra with those of reference standards when available. Quantification of the identified compounds was then performed using the external standard method. In this way calibration curves were established for gallic acid, 5-(hydroxymethyl)-furfural, protocatechuic acid, vanillin, syringaldehyde, sinapinaldehyde, coniferaldehyde, vanillic acid, syringic acid, ferulic acid, and scopoletin. Each calibration curve contained five levels as prepared by serial dilution of working standards with HPLC grade methanol, the mid-point of which corresponding to the average concentration of the compounds in the sample extract. The detection wavelength for the 5-(hydroxymethyl)-furfural and phenolic acids was 280 nm, while for phenolic aldehydes and scopoletin was 320 nm. The absorbance spectra from 200 to 400 nm were also recorded using the diode array detector in order to confirm the identity of each compound in the sample matrix. In the sample matrix, peak purity values greater than 97% were obtained for each compound and a minimum value of 95% was obtained when comparing the spectra of sample peaks to those of the reference standards.

### 2.6. Statistical analysis

A discriminant analysis and an ANOVA test were performed in order to differentiate among the diverse aging states of tequila based on their oak related compounds content. This analysis utilized the results of the HPLC–DAD experiments and the statistics software Statgraphics Centurion XV (StatPoint Incorporated, USA). Discriminant analysis is a multidimensional statistical technique

that can be used to analyze differences among groups and/or provide a means to assign any particular case into the group which it most closely resembles [16]

### 3. Results and discussion

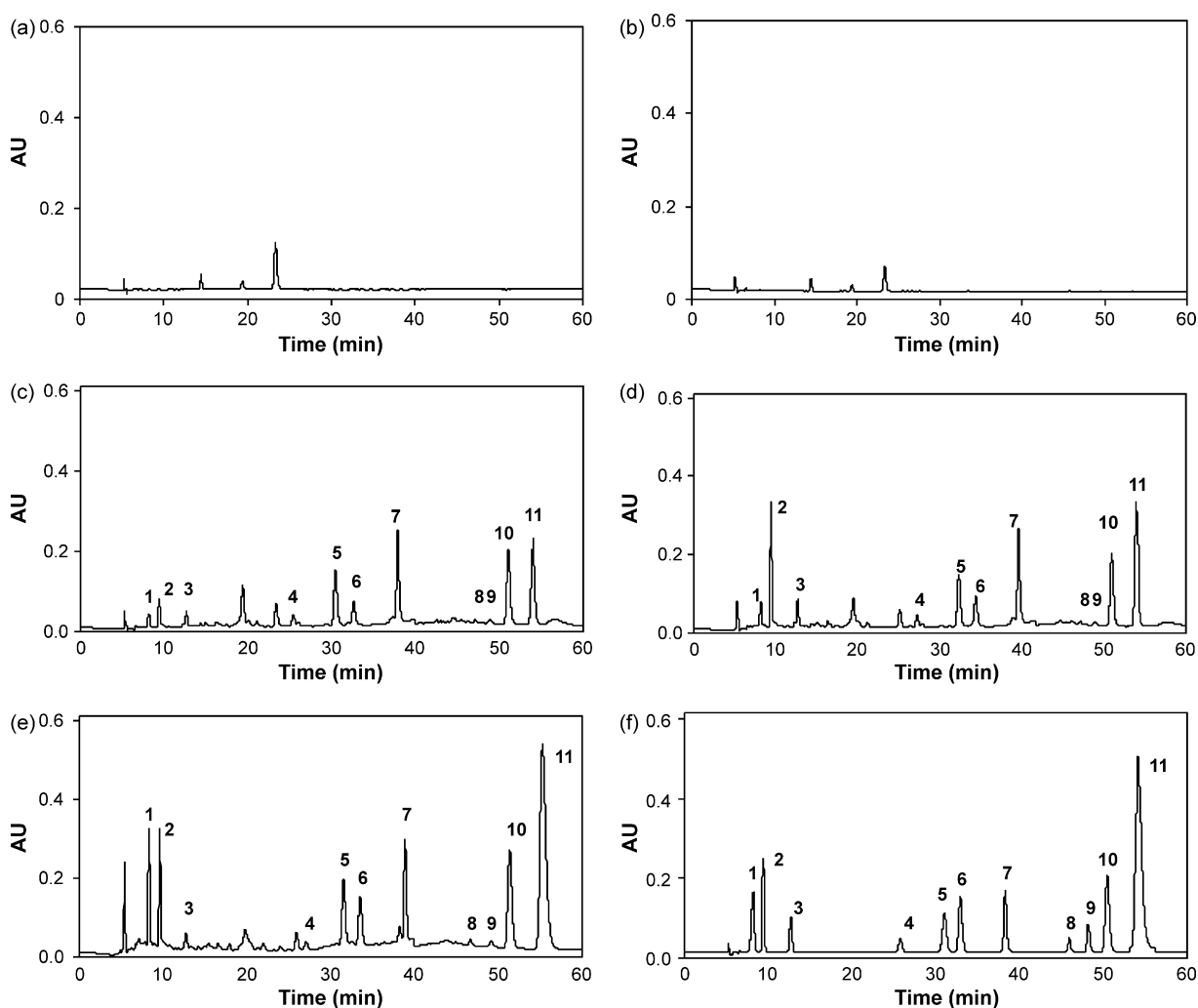
#### 3.1. Optimization of the chromatographic method

Chromatographic conditions such as mobile phase gradient, flow rate, column temperature and injection volume were optimized in order to limit run time while obtaining the best possible peak symmetry and resolution. Though several methods have been described for detecting this group of compounds [9,17–19], the present method was adapted to account for the unique matrix of tequila. In initial experiments, the mobile phase composition consisted of methanol and acidified water, however, a shorter analysis time and a better resolution between syringic acid and vanillin were observed when a mixture of water:methanol:acetonitrile was implemented. Likewise, a gradient flow rate and gradient mobile phase composition were used to optimize resolution and run time. The final elution program, as described in Section 2.4, allowed for a well-resolved chromatogram of the 11 standards in less than 60 min (Fig. 1).

After attempting several direct injections of the tequila samples, as has been reported for both wine and brandy [8,10,18], it was decided to use a preconcentration step in order to increase detector signal. Preconcentration with SPE was chosen over liquid–liquid extraction due to its advantages with these compounds. The optimized SPE procedure is described in Section 2.3.

The representative chromatograms of 100% agave tequila *blanco* and mixed tequila *blanco* are shown in Fig. 1a and b, respectively. Here, three peaks can be observed with retention times between 14 and 26 min. These peaks correspond to furfural, 2-acetyl furan and 5-(methyl)-furfural, and belong to the group of furanic compounds which are generated by the Maillard reaction during agave cooking [20]. These compounds, however, were not considered in the present study to be oak aging markers due to their presence in unaged tequila samples, even though their concentrations may in fact increase during aging time as a result of oak extraction [7,17].

The representative chromatograms of the tequilas that have been aged in oak barrels are shown in Fig. 1c, d and e, presenting the 11 age-marking compounds identified in this study. It must be noted here that several of the compounds identified in tequilas that have been aged in oak barrels, in particular, vanillin, 5-(hydroxymethyl)-furfural, and syringaldehyde, have also been identified in cooked agave juice before fermentation [20,21]. Sev-



**Fig. 1.** Chromatograms of certified tequilas: (a) 100% agave *blanco*; (b) mixed *blanco*; (c) 100% agave *reposado*; (d) mixed *reposado*; (e) 100% agave *añejo*; and (f) reference standard solution. Compound identification: (1) gallic acid; (2) 5-(hydroxymethyl)-furfural; (3) protocatechuic acid; (4) vanillic acid; (5) syringic acid; (6) vanillin; (7) syringaldehyde; (8) scopoletin; (9) ferulic acid; (10) coniferaldehyde; and (11) sinapinaldehyde.

**Table 1**  
Compound identification, retention time (RT), regression equation including the determination coefficient ( $R^2$ ) and range of calibration.

Compound	Name	RT media (RSD)	Regression equation	$R^2$	Calibration range ( $\mu\text{g/mL}$ )
1	Gallic acid	8.33 (0.24)	$y = 0.63x - 0.97$	0.9998	10–200
2	5-(Hydroxymethyl)-furfural	9.60 (0.38)	$y = 1.40x - 1.71$	0.9997	10–100
3	Protocatechuic acid	12.75 (0.45)	$y = 0.32x - 0.68$	0.9995	10–150
4	Vanillic acid	25.95 (0.34)	$y = 0.34x - 0.30$	0.9999	5–100
5	Syringic acid	31.55 (1.02)	$y = 0.58x - 1.88$	0.9990	20–200
6	Vanillin	33.52 (0.93)	$y = 0.70x - 0.68$	0.9998	10–200
7	Syringaldehyde	38.88 (0.77)	$y = 0.30x - 0.52$	0.9999	20–400
8	Scopoletin	46.72 (0.49)	$y = 0.53x - 0.09$	0.9998	2.5–40
9	Ferulic acid	49.68 (0.57)	$y = 0.57x - 0.80$	0.9999	5–100
10	Coniferaldehyde	51.37 (1.29)	$y = 1.14x - 0.92$	0.9997	5–250
11	Sinapinaldehyde	55.28 (0.91)	$y = 0.90x - 0.49$	0.9993	10–500

eral studies, including the present, however, have not been able to detect these compounds in tequila blanco [22,23]. This may in fact be due to the loss of the relatively low volatile compounds during the distillation phase of tequila production. Similar results have been reported for cider brandy where it was found that 5-(hydroxymethyl)-furfural originated during thermal treatment of the apple juice by the Maillard reaction, however was lost during distillation [17]. The reappearance of these compounds during aging, however, suggests extraction from the oak barrels as has been reported for other alcoholic beverages [7,9,17–19]. As a result, these compounds are considered as age-marking compounds for the purposes of this study.

### 3.2. Method validation

The proposed method was validated for linearity, precision, accuracy, limit of detection, and limit of quantification, as well as stability of both the sample extract and stock solutions. The linearity study was performed by triplicate injection of standard solutions corresponding to each point in the calibration curve. The regression equations and the determination coefficient ( $R^2$ ) are shown in Table 1. It can be observed that the  $R^2$  is greater to 0.999 for each of the compounds at the calibration range studied.

Intra- and interday repeatability studies were carried out by injecting the same tequila sample extract five times ( $n=5$ ) during three consecutive days ( $n=3$ ). It can be noted that the RSD of the detector response is less than 1.6% for both intra- and interday repeatability, and the RSD of the retention times is lower than 1.3% for each compound (Tables 1 and 2).

Accuracy was confirmed by spiking a control sample of tequila blanco at three different concentrations (80, 100, and 120% of the mid-point concentration of the calibration curve) of the standard compounds. Triplicate extraction and analysis was performed at

each level, as prepared according to Section 2.3. The recovery percentage was calculated using the following equation:

$$\text{Recovery(\%)} = \frac{\text{Measured concentration}}{\text{Expected concentration}} \times 100$$

Accuracy experiments showed a recovery between 84.2% (5-(hydroxymethyl)-furfural, low spike) and 108.5% (vanillic acid; high spike) for all analyzed compounds with the exception of gallic acid, where recovery ranged from 64.5 to 67.3%. The relatively low recovery of gallic acid may be explained by its high water solubility as compared to the other phenolic compounds studied. This result is similar to that obtained by Michalkiewicz [24], who obtained low gallic acid recoveries (approximately 80%) in SPE extracts from aqueous samples of honey.

Evaluation of the limits of detection and quantification was performed according to ICH guidelines [25]. To summarize, each of the five points of the calibration curve was injected in triplicate. The standard deviation of the y-intercept ( $S_a$ ) of the regression line was then calculated for each compound. The  $S_a$  parameter was used to calculate the limits of detection and quantification with the following equations:

$$\text{LOD} = 3.3 \times \frac{S_a}{b}$$

$$\text{LOQ} = 10 \times \frac{S_a}{b}$$

where  $b$  is the slope of the calibration curve.

The limits of detection and quantification determined in this study ranged from 0.62 to 4.09  $\mu\text{g/mL}$  and 1.9–12.4  $\mu\text{g/mL}$ , respectively (Table 2). A solution containing each phenolic and furanic standard at the calculated limit of detection was then injected to confirm detection at this level (data not shown). The phenolic

**Table 2**  
Recovery percentages (high, medium and low spike), intra- and interday precision and limit of detection (LOD) and quantification (LOQ) of the tequila assay.

Compound	(Low) rec1 <sup>a</sup>	(Medium) rec2 <sup>a</sup>	(High) rec3 <sup>a</sup>	LOD <sup>b</sup>	LOQ <sup>b</sup>	Day 1 <sup>b</sup>	Intraday ( $n=5$ ) Day 2 <sup>b</sup>	Day 3 <sup>b</sup>	Interday ( $n=3$ ) <sup>b</sup>
1	64.5	65.5	67.3	2.71	8.20	49.4 (0.17)	49.7 (0.79)	49.0 (0.61)	49.4 (0.70)
2	84.2	85.0	101.6	1.74	5.26	25.5 (0.37)	25.4 (0.35)	25.7 (0.60)	25.5 (0.63)
3	90.1	96.5	102.6	3.66	11.1	48.3 (0.28)	49.1 (1.06)	49.2 (0.40)	48.9 (1.00)
4	94.4	98.8	108.5	0.84	2.55	23.9 (0.89)	24.1 (0.45)	24.4 (0.37)	24.1 (1.21)
5	91.5	94.7	101.5	3.83	11.6	53.8 (0.46)	54.6 (0.55)	53.2 (1.03)	53.9 (1.31)
6	89.0	94.1	96.5	2.43	7.38	44.1 (0.25)	44.5 (0.43)	43.5 (0.80)	44.0 (1.22)
7	86.9	91.0	99.7	4.09	12.4	92.8 (0.75)	93.6 (0.59)	93.6 (0.34)	93.4 (0.51)
8	105.5	106.4	105.9	0.71	2.17	9.93 (0.99)	10.1 (1.02)	10.2 (0.66)	10.1 (1.54)
9	88.2	89.3	92.3	1.19	3.61	21.1 (0.68)	21.3 (1.04)	21.3 (0.75)	21.2 (0.76)
10	87.5	94.1	96.8	0.62	1.90	24.6 (0.57)	24.8 (0.71)	25.1 (0.56)	24.8 (0.85)
11	96.9	97.6	106.4	1.90	5.75	18.0 (0.22)	18.0 (0.34)	18.0 (1.02)	18.0 (0.21)

Compound identification: (1) gallic acid; (2) 5-(hydroxymethyl)-furfural; (3) protocatechuic acid; (4) vanillic acid; (5) syringic acid; (6) vanillin; (7) syringaldehyde; (8) scopoletin; (9) ferulic acid; (10) coniferaldehyde; (11) sinapinaldehyde.

<sup>a</sup> Values of the recovery study are expressed in %.

<sup>b</sup> Values of the LOD, LOQ and precision studies are expressed in  $\mu\text{g/mL}$  sample solution; relative standard deviations are in parentheses.

**Table 3**  
Means of the quantities obtained by the HPLC-DAD analysis of certified tequila samples.

Compound	Tequila <i>Añejo</i> 100% agave	Tequila <i>Reposado</i> 100% agave	Tequila <i>Reposado</i> mixed	Tequila <i>Blanco</i> 100% agave	Tequila <i>Blanco</i> mixed
1	0.19 ± 0.05	0.07 ± 0.05	0.04 ± 0.02	nd	nd
2	0.29 ± 0.36	0.05 ± 0.04	0.11 ± 0.13	nd	nd
3	0.05 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	nd	nd
4	0.12 ± 0.02	0.06 ± 0.03	0.03 ± 0.02	nd	nd
5	0.31 ± 0.08	0.13 ± 0.14	0.07 ± 0.03	nd	nd
6	0.23 ± 0.02	0.09 ± 0.07	0.05 ± 0.04	nd	nd
7	0.69 ± 0.14	0.22 ± 0.18	0.16 ± 0.13	nd	nd
8	0.07 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	nd	nd
9	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	nd	nd
10	0.19 ± 0.11	0.05 ± 0.03	0.09 ± 0.02	nd	nd
11	0.40 ± 0.42	0.08 ± 0.05	0.21 ± 0.08	nd	nd

Compound identification: (1) gallic acid; (2) 5-(hydroxymethyl)-furfural; (3) protocatechuic acid; (4) vanillic acid; (5) syringic acid; (6) vanillin; (7) syringaldehyde; (8) scopoletin; (9) ferulic acid; (10) coniferaldehyde; (11) sinapinaldehyde. Mean ± SD. Values in mg/100 mL of ethanol. nd = not detected.

extract and the standard solutions were stable for at least 30 days if stored at 4 °C under dark conditions (confirmed by reanalyzing solutions).

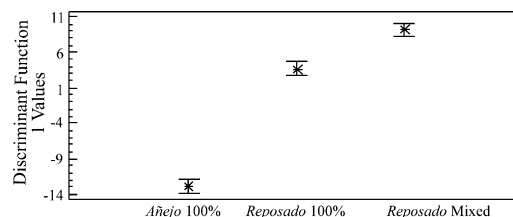
### 3.3. Statistical analysis

Table 3 presents the mean quantities of the age-marking compounds found in the tequila samples analyzed in this study. Here, it can be observed that phenolic and furanic content increases with aging time; none of the age-marking compounds were found in either of the tequila *blanco* types studied, and the quantity of the age-marking compounds was greater in *añejo* tequilas than in *reposado* tequilas. The increase in the content of oak related compounds during aging may be due to a variety of chemical processes. For instance, the increase in the concentration of gallic acid may be due to the hydrolysis of wood tannins as has been reported for cider brandy [17]. An increase in concentration during aging time in aldehydes such as vanillin, syringaldehyde, coniferaldehyde and sinapinaldehyde that may be derived from the lignin hydrolysis, as has previously been reported as well [7,17,26]. Likewise, the quantities of vanillic and syringic acid, which originate from the oxidation of their corresponding aldehydes, as well as 5-(hydroxymethyl) furfural, which originates from the charring of the oak casks, have been shown to increase with oak aging [17].

A discriminant analysis was performed in order to further differentiate the tequilas based on age classification. In this analysis, the concentrations values of the tequilas that have been aged in oak barrels presented in Table 3 were imputed as variables with aging time as the classification factor. A backward stepwise selection procedure [27] was then applied with the purpose of identifying those variables that are statistically significant discriminators among the three different classifications of aged tequilas (100% *reposado*, mixed *reposado* and 100% *añejo*). The variables selected by this analysis were: gallic acid, 5-(hydroxymethyl)-furfural, syringic acid, vanillic acid and scopoletin. In this way, the first discriminant function took the form:

$$D_1 = -18.09 * \text{gallic acid} + 11.26 * \text{5-(hydroxymethyl)-furfural} \\ - 11.17 * \text{vanillic acid} + 14.38 * \text{syringic acid} \\ + 16.89 * \text{scopoletin}$$

where the coefficient values of the compounds are standardized. This function describes 99.5% of the total data variability. For this reason, this function may be used to discriminate the three classifications of tequilas that have been aged in oak barrels studied. To support this assessment, an ANOVA test was performed using the values of the  $D_1$  function as a response variable and aging time as a factor, giving a  $p$ -value of 0.0000. A mean comparison with



**Fig. 2.** Means and 95% LSD intervals for tequilas 100% agave *reposado*, mixed *reposado*, and 100% agave *añejo*.

the Least Significant Difference (LSD) test is shown in Fig. 2. In Fig. 2, a statistically significant difference at the 95% confidence level is observed between the three tequila types: 100% *reposado*, mixed *reposado* and 100% *añejo*. The observed difference between the *reposado* and *añejo* age classifications suggests the accumulation of the age-marking compounds by oak extraction. Of note as well is the differentiation among the 100% *reposado* and mixed *reposado* tequilas. The difference in extraction kinetics among different matrix types coincides with the results obtained by García Moreno and García Barroso [28] with wine, where matrix effects of various wines caused different quantities of phenolic compounds to be extracted from oak casks during aging. Further study is necessary, however, to properly investigate the relationship between extraction kinetics and matrix type in mixed and 100% *reposado* tequilas.

The results of the presented work suggest that the proposed methodology can be used to not only detect and quantify age-marking compounds in tequila, but to use them to differentiate among the different age classifications (*blanco*, *reposado* and *añejo*). These results show that the content of oak related compounds in tequilas that have been aged in oak barrels varies based on both aging time and matrix, as made evident by using an ANOVA test combined with discriminant analysis. It must be pointed out that given the limited number of samples analyzed in this study, the identified compounds may in fact be markers of the particular tequila companies studied and not representative of all aged tequilas produced.

## 4. Conclusion

This work presents a methodology for the simultaneous determination by HPLC-DAD of 11 representative compounds that act as aging markers in tequila beverages. The developed method was validated and applied to discriminate among tequila types according to aging time. Using an ANOVA test combined with discriminant analysis, a correct classification among tequilas that have been aged

in oak barrels was obtained. These results present an HPLC methodology, which may be applied as a quality control tool for industrial laboratories to distinguish among tequila types according to aging time.

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