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Comparison of shikimic acid determination by capillary zone electrophoresis with direct and indirect detection with liquid chromatography for varietal differentiation of red wines

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Abstract

Two capillary zone electrophoretic (CZE) methods for determination of shikimic acid in Chilean red wine were developed and compared with a HPLC method. Both electrophoretic methods were carried out by using a reversed electroosmotic flow induced by trimethyl(tetradecyl)ammoniumbromide (TTAB) with indirect detection at 260 nm using *p*-aminobenzoic acid as a UV-absorbing co-ion or by direct detection at 213 nm. In both cases, the separation was carried out in a 50 μm I.D. uncoated capillary with an effective length of 48 cm, a negative power supply of 30 kV, using a buffer based on bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane (Bis-Tris), pH 7.0 or 7.5 and hydrodynamic injection. The chromatographic separations were carried out on a C-18 reversed phase column followed by a sulfonyl-styrene-divinylbenzene (S-DVB) ion exclusion column at 70 °C with H_2SO_4 0.02 M as isocratic mobile phase and a flow rate of 0.5 mL min^{-1}. The three methods allowed the quantification of shikimic acid with quantification limits between 1.0 and 12.0 mg L^{-1} and precision between 7.3 and 10.1%, however, only the concentrations obtained by CZE with direct detection were statistically similar to those of HPLC. This parameter was evaluated as analytical tool to verify varietal authenticity of red wines. In all cases, the Cabernet Sauvignon wines presented higher concentrations of shikimic acid, compared with Merlot or Carmenère wines.

Keywords: Shikimic acid; Red wine; HPLC; Reserved phase chromatography; Ion exclusion chromatography; CZE with direct detection; CZE with indirect detection

1. Introduction

Red wine contains organic acids, which are product of sugar oxidation (tartaric, citric and malic acid) or of alcoholic fermentation during the winemaking process (succinic and lactic acid) [1,2]. Shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) is another carboxylic acid that comes from grape skin and is always present in musts and wines [3]. It is an intermediate molecule produced in the shikimate pathway, the biosynthetic pathway of benzoic and cinnamic acid, aromatic amino acids (phenylalanine, tyrosine) and anthocyanidins, flavonoids, tannins and other compounds present in the grape, which are transferred to wine during the winemaking process [4,5].

Organic acid determination in wine is normally performed to monitor fermentation processes, product stability and organoleptic properties [6–8]. Shikimic acid does not have an important organoleptic effect in wine, and due to its low concentration in comparison with another acids, quantification of this compound has been limited in this matrix [3,5,9]. However, considering the participation of shikimic acid in the biosynthesis of antocyanines, Holbach et al. [5] proposed its determination as a tool for to differentiate between

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different red wine varieties. On the other hand, the profile of anthocyanines, specifically the ratio between acetylated and coumarylated anthocyanines (Ac/Cum), is actually used by official organisms in Germany to differentiate red wine varieties [10,11] and the concentration of shikimic acid has been proposed as an alternative in this context [5].

Ion exclusion chromatography is frequently employed to separate organic and aromatic acids in wine and others matrices [5,12–14]. The chromatographic methodology described by Holbach et al. correspond to this mode. They used a RP-18 column in series with a sulfonyl-styrene-divinylbenzene (S-DVB) for separation of shikimic acid in wine. This method was recently accepted by the International Organization of Vine and Wine (OIV) as method for determination of shikimic acid in wine [15], including it in the compendium of international methods of analysis of wines and musts.

Capillary zone electrophoresis (CZE) has emerged as one of the most efficient methods for charged compound separation. Organic acid separation acids in grapes, wine and other beverages has been described [9,16–21]; however, only Klampfl et al. proposed the separation of organic acids, including shikimic acid, in white wine by CZE and indirect UV detection [9].

The aim of this work was to present two electrophoretic methods for determination of shikimic acid in red wine and to compare these results with those obtained by application of the HPLC method developed by Holbach et al. [5]. Also, the evaluation of these methodologies as analytical tool to verify the varietal authenticity of red wine, is applied to Cabernet Sauvignon, Merlot and Carmenère wines produced in Chile.

2. Experimental

2.1. Chemicals

All solutions were prepared in 18 MΩ deionized water from a Millipore Milli-Q water purification system. HPLC grade acetonitrile and methanol were purchased form J.T. Baker (Phillipsburg, USA). *p*-aminobenzoic acid (PABA) >99%, $CaCl_2$, LiCl, LiOH, sulfuric, formic, tartaric, citric, succinic, lactic, fumaric and acetic acid were obtained from Merck (Darmstadt, Germany). Shikimic acid, malic acid, bis (2-hydroxyethyl)imino-tris(hydroxymethyl)methane (Bis-Tris) 98%; and trimethyl(tetradecyl)ammonium bromide (TTAB) 99% were obtained from Sigma (St. Louis, MO, USA). Stock standard solutions containing between 0.5 and 10 g L^{-1} of each organic acid were prepared in purified water and stored in a refrigerator at 4 °C for one week. Separation buffer for CZE methods were prepared by appropriate dilution of Bis-Tris 115 mM, PABA 25 mM, TTAB 10 mM, LiCl 600 mM and $CaCl_2$ 110 mM stock solutions and pH was adjusted with 0.1 M of LiOH or 0.1 M of HCl. HPLC mobile phase and CE buffer were prepared daily, filtered and degassed trough a 0.45 μm membrane filter.

2.2. Equipment

An Agilent capillary electrophoresis instrument (Palo Alto, CA, USA) equipped with a diode array detector set at 260 or 213 nm and a Hewlett-Packard Chemstation data processing station (Rev. A.09.01[1206]) were used to perform the electrophoretic analyses. The HPLC analyses were carried out on a Shimadzu HPLC system (Kyoto, Japan) equipped with a quaternary LC-10AD*VP* pump, FCV-10AL*VP* elution unit and DGU-14A degasser unit. The column temperature was maintained at 70 °C in a CTO-10A*VP* oven. A SPD-M10A*VP* UV/VIS spectrophotometric detector was used and data was processed with a CLASS-*VP* Shimadzu Chromatography Data System.

2.3. Electrophoretic separation conditions

Electrophoretic separation for the indirect detection method was performed on a fused-silica capillary of 56 cm (length to detector, 48 cm) × 50 μm I.D. from Bio-Rad (CA, USA). Separation was carried out by using a power supply of −30 kV (current of −7 μA) at a temperature of 22 °C and indirect detection at 260 nm. The separation buffer consisted of 10.5 mM Bis-Tris; 7.5 mM PABA; 0.2 mM TTAB; 0.53 mM $CaCl_2$, pH 7.5. The capillary was conditioned by passing 0.1 M NaOH for 30 min, water for 15 min and the run buffer for 15 min. The injection protocol began with 3 min of water, 4 min of NaOH and 5 min of the separation buffer, followed by hydrodynamic injection of the standards and samples at 25 mbar during 12 s.

The direct detection mode was performed on a capillary with the same characteristics by using a power supply of −30 kV (current of −80 μA) at 18 °C and 213 nm for detection. The separation buffer consisted of 200 mM Bis-Tris; 1.1 mM TTAB; 16 mM LiCl, pH 7.0. The capillary was conditioned by passing methanol for 30 min, 1.0 M NaOH for 30 min, water for 30 min and the run buffer for 30 min. The injection protocol began with 5 min of buffer with 2.0 mM TTAB followed by 10 min of running buffer. The injection was hydrodynamic at 50 mbar for 4 s. Analyte peaks were assigned by comparison of their retention times with those of reference compounds. Also, a co-injection of each standard with the sample was made in all cases.

2.4. Chromatographic separation conditions

HPLC separation was carried out on a C-18 of 15 cm × 4 mm I.D. and 5 μm particle size as pre-column (Institut Heidger, Kesten, Germany) and a sulfonyl-styrene-divinylbenzene (S-DVB) 30 cm × 4 mm I.D. column (Institut Heidger, Kesten, Germany) as main column. The mobile phase was isocratic 0.02 M H_2SO_4 with 0.1 mL min^{-1} as flow rate in the preconditioning phase and 0.5 mL min^{-1} in the working phase. The detection was at 225 nm and the injection volume 6 μL. Analyte peaks were assigned by comparison of their retention times with those of reference compounds.

Also, a co-injection of each standard with the sample was made in all cases.

2.5. Calibration curves

Working standard solutions spanning the concentration range from 0.1 mg L^{-1} to 5.6 g L^{-1} for HPLC analysis, from 1 to 10 mg L^{-1} for indirect CZE analysis, and from 5 to 100 mg L^{-1} for direct CZE method were prepared by appropriate dilution of stock standard solutions in purified water. For all proposed methods, the limits of quantification (LOQ) were calculated as 10 times the blank standard deviation, which were obtained from calibration curves. Repeatability of the injection of shikimic acid were obtained by eight consecutive sample introductions of Cabernet Sauvignon wine and these were measured as the RSD (%) of their respective areas at two concentration levels, the precision was determined inter and intra-daily. Accuracy was calculated for a red wine sample spiked with different concentrations of shikimic acid. The error was determined as difference between added and found concentration.

2.6. Wine samples

Chilean red wines were previously filtered through a 0.22 μm membrane filter and than injected directly into the HPLC or CE system; however, due to the matrix interferences, samples were diluted 10 times for the indirect CE method.

3. Results and discussion

3.1. Performance of chromatographic method (method 1)

The separation power of the RP-18 column, S-DVB column alone and the RP-18 in series with the S-DVB column was evaluated in order to determinate their functionality in the separation of shikimic acid in wine. Fig. 1 shows the chromatograms for a Cabernet Sauvignon wine obtained by using the different columns under the same chromatographic conditions. The RP-18 column allowed only a poor resolution between shikimic and lactic acid (R_s = 0.7). In this case, the separation mode is the reversed-phase chromatography of protonated acids. The poor separation between shikimic and lactic acid is mainly due to a low selectivity of the RP-18 column for those analytes (separation factor of 1.05) and the quantification of peak under this conditions was not suitable (Fig. 1A).

When the S-DVB column was used, the separation mechanism was ion exclusion, where separation is accomplished by differences in pK_a, size and hydrophobicity of the organic acid [22,23]. The H_2SO_4 facilitated the protonation of weak organic acids for their separation by this mode. Fig. 1B shows the chromatogram obtained when S-DVB column was used alone. Although separation between shikimic and lactic acid was possible (R_s = 1.8), both eluted on the tail of a very big peak present in the matrix of red wine, which is not retained in the S-DVB column. Fig. 1C shows the chromatogram obtained when both (RP-18 and S-DVB) columns were used in series. In this case the RP-18 column remove this interfering peak, which was detected at 225 nm, while the S-DVB column give the required selectivity for separation. Under this conditions, the determination of shikimic acid was accomplished in less than 20 min with a resolution of 1.9 between shikimic and lactic acid. The UV spectra of standards and wine samples, obtained by diode array detection showed no significant differences between analyte peaks in standards and samples.

Table 1 shows that the response of all organic acids is linear at 225 nm throughout the evaluated concentration range when both columns together were used. Due to the presence of a cyclohexene as a chromophore group in shikimic acid, its absorptivity was higher than for the other carboxylic acids present in wine. This property allow the quantification of low concentrations of shikimic acid in presence of high concentrations of other acids present in the wine. The relevant elements of the regression equation and other characteristic parameters of the method for shikimic acid are presented and compared in Table 2. The intra-day precision for 90 mg L^{-1} of shikimic acid was 1.7%, while the inter-day precision was 3.1%. Accuracy of the method, obtained at two concentration levels and expressed as error, was −4.8% and 4.0% for 30 and 60 mg L^{-1}, respectively. The proposed method allows the quantification of shikimic acid between 1 and 90 mg L^{-1} in red wine. Its trueness was also confirmed recently by other authors by intercomparison of results with GC–FID and GC–MS [15].

3.2. Performance of electrophoretic method with indirect detection (method 2)

The electrophoretic separation was carried out by using a reversed electroosmotic flow induced by a cationic surfactant, TTAB, with a concentration below its critical micelle concentration, which dynamically coated the negative charged inner surface of the silica capillary with a positively charged layer [24]. In this way, anionic species such as shikimate and other carboxylic acids were forced to migrate in the same direction as the EOF and to reach the detector before the EOF marker (co-electroosmotic principle) [24,25]. For this system type, the pH of the electrolyte buffer was higher than pK_a of carboxylic acids. An electropherogram of a standard mixture of acids, obtained by using a selective buffer consisting in 10.5 mM Bis-Tris, 7.5 mM of PABA and 0.2 mM of TTAB as electroosmotic modifier at pH 7.5, is presented in Fig. 2A. The separation of shikimic acid was possible in less than 4 min with a total resolution for shikimic acid. However, when a real red wine sample was analyzed under these conditions (Fig. 2B), a low resolution between shikimic acid and a compound present in the red wine matrix

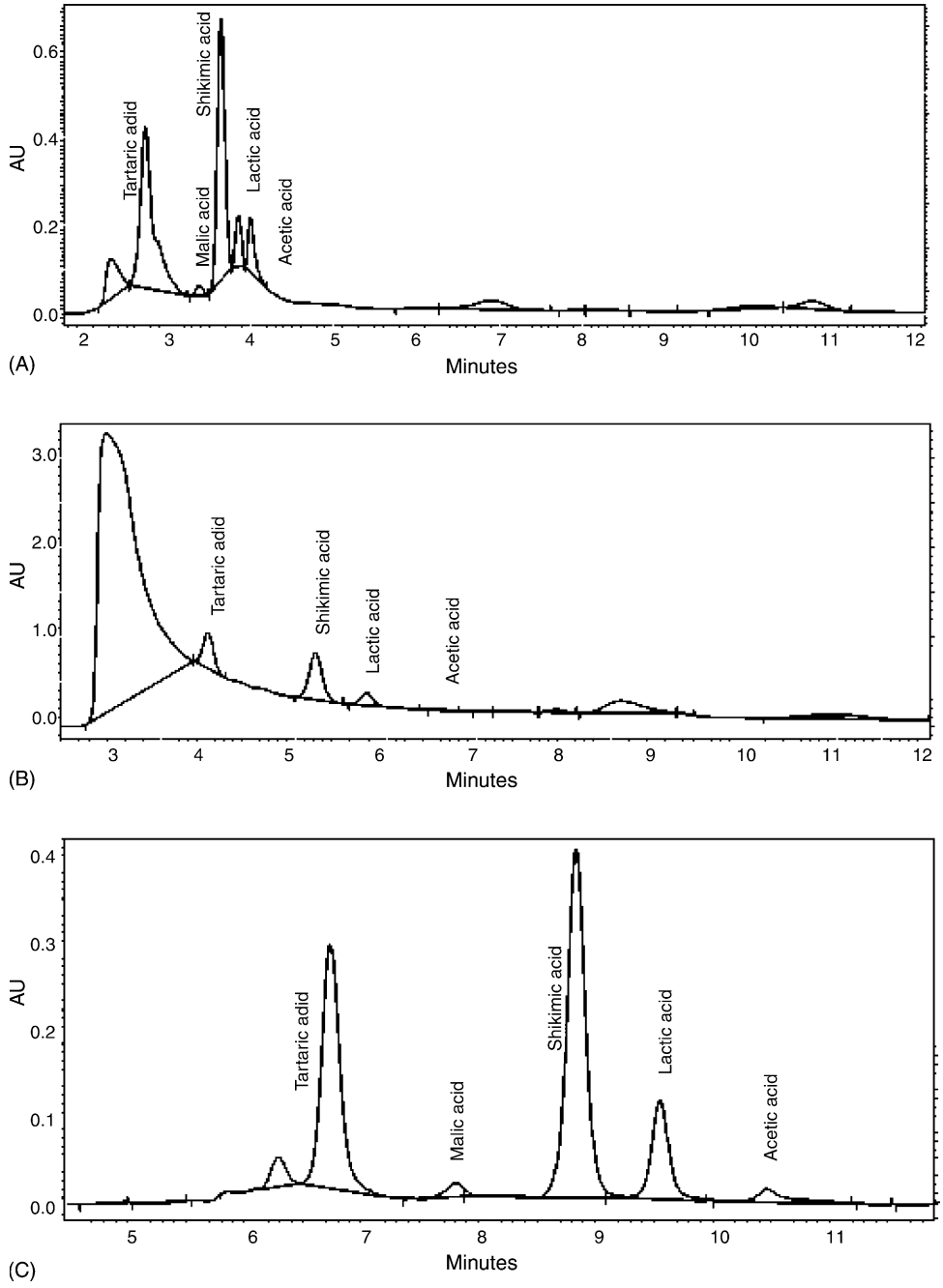

Fig. 1. HPLC separation of shikimic and other organic acids in a Cabernet Sauvignon wine. (A) By using a column RP-18 (15 cm, 4 mm, 5 μm), (B) By using a column S-DVB (30 cm, 4 mm) and (C) by using a RP-18 column followed by a S-DVB column. The mobile phase was isocratic of 0.02 M of H_2SO_4 with 0.1 ml min^{-1} as flow in the preconditioning phase and 0.5 mL min^{-1} in the working phase. The detection was at 225 nm and the injection volume 6 μL.

was observed. Due to this, the carrier buffer was modified with 0.5 mM $CaCl_2$, which decreased the electroosmotic flow and the electrophoretic mobility of acidic compounds (Fig. 2C). The separation between shikimic acid and the interfering matrix was improved ($R_s = 1.4$); however, this modification decreased the separation efficiency of the method. The figures of the regression equation and other characteristic parameters obtained under these conditions are shown in Table 2. The proposed method allows the quantification of shikimic acid between 1 and 10 mg L^{-1} in red wine. In this case, a previous dilution was necessary to reduce the interference of other organic acids present in wine.

3.3. Performance of electrophoretic method with direct detection (method 3)

The separation was accomplished by using a run buffer based on the previously described buffer for indirect detection with several modifications: the incorporation of PABA as a UV-absorbing co-ion was not necessary for direct detection;

Table 1
Analytical parameters of optimized HPLC method for organic acid

Acid	Linear range (mg L^{-1})	Equation	R^2 (%)	RSD (%)	LOD (mg L^{-1})
Tartaric	10.9–5600	$y = 549231x + 39753$	99.96	7.8	3.3
Malic	16.5–3600	$y = 257490x + 37237$	98.83	7.8	4.9
Shikimic	1.0–90	$y = 2e7x - 1961$	99.00	1.7–7.9[a]	0.3
Lactic	37.1–5000	$y = 148876x + 1602$	99.01	8.0	11.1
Acetic	8.2–3000	$y = 124288x + 6921$	99.64	8.6	2.5
Citric	12.8–1500	$y = 352267x + 712$	99.97	8.1	3.8
Succinic	48.2–3500	$y = 150535x - 2807$	99.55	9.1	14.4
Fumaric	2.3–160	$y = 4e7x + 46357$	99.71	7.8	0.7

R^2: coefficient of determination (obtained by ANOVA for validation of the model), RSD (%): reproducibility of injections. ([a]obtained for 90 and 30 mg L^{-1} respectively), LOD: limit of detection, y: mAU, x: mg L^{-1}.

however, high concentrations of Bis-Tris were required in order to have a better buffering capacity. The UV absorption of shikimic acid allows its detection at 213 nm; however, a low sensitivity was observed due to its low absorption coefficient at this wavelength. This fact determined that wine samples were directly injected into CE equipment without any dilution, which explains why a high buffering capacity was required. The UV spectra of standards and peak samples, obtained by diode array detection showed no significant differences between analyte peaks in standards and samples.

To determine the optimal concentration of TTAB, the EOF behavior at different concentrations of TTAB was determined. The pH was kept constant at 7.5. No reverse EOF was observed when TTAB concentration was less than 0.4 mM. Higher concentrations of TTAB increased the reverse EOF. This is in agreement with Cocke et al. [26]. However, poor reproducibility and long migration times for shikimic acid were observed when TTAB concentration was below 0.8 mM. Better reproducibility was observed when the TTAB concentration was 1.1 mM. In this context, the protocol to precondition the capillary was essential for an acceptable reproducibility of migration times. The introduction of a washing step with 2.0 mM of TTAB in the buffer was necessary to assure EOF reproducibility. Without this washing step, poor reproducibility and efficiency were observed. An initial washing with methanol before each experiment was also necessary to assure the absolute TTAB extraction from the inner wall of the capillary and to improve the reproducibility of migration times. Fig. 3 presents an electropherogram of a Cabernet Sauvignon wine obtained under the optimal separation conditions. The characteristic analytical parameters of the methods, determined as described by Miller and Miller [27], are summarized in Table 2. The reproducibility of injection, obtained for standard solutions at two concentration levels, were 7.8% and 5.7% for 20 and 60 mg L^{-1}, respectively. The intra-day precision for 60 mg L^{-1} of shikimic acid was 5.7%, while the inter-day precision 6.6%. Accuracy of the method, obtained at two concentration levels and expressed as % of error, was 10% and −7% for 20 and 30 mg L^{-1}, respectively. The method allows the determination of shikimic acid between 12 and 100 mg L^{-1}.

3.4. Analytical applications

The results obtained in wine samples by the three methodologies were compared in order to test their comparability and to identify any significant differences between them. The optimized HPLC method (method 1) was considered a reference method and both electrophoretic methods were compared with this method. Twenty-two wine samples were analyzed by the electrophoretic method with indirect detection (method 2) and by HPLC for comparison, whereas 42 were analyzed by electrophoretic method with direct detection (method 3) and by HPLC for their comparison.

The significance test (F-test) was applied to determine if there were statistical differences between variances of methods. In both cases, no statistical differences were found. The comparison of methods 1 and 2 had an experimental F_{cal} of 1.73 and F_{tab} of 3.84 ($\alpha = 0.05$). For methods 1 and 3, F_{cal} was 1.54 and F_{tab} was 3.69 ($\alpha = 0.05$). Consequently, the precision of both electrophoretic methods presents no statistical differences with the HPLC method.

To compare the exactitude of electrophoretic methods with HPLC, a significance test (t-test) for pair wise of samples was applied. The null hypothesis was a zero difference between the concentrations of shikimic acid in wine samples obtained by the different methods ($u_d = 0$). The experimental t value

Table 2
Comparison of analytical parameters of described methodologies

	Linear range (mg L^{-1})	Equation	R^2 (%)	RSD (%)	LOQ (mg L^{-1})
HPLC	1–90	$y = 2e7x - 1961$	99.00	7.3	1
CE indirect detection	1–10	$y = 2.5x + 0.7$	97.91	10.1	1
CE direct detection	12–100	$y = 0.31x + 0.02$	98.90	9.4	12

R^2: coefficient of determination (obtained by ANOVA for validation of the model), RSD (%): reproducibility of injections (obtained for a wine Cabernet Sauvignon), LOQ: limit of quantification, y: mAU, x: mg L^{-1}.

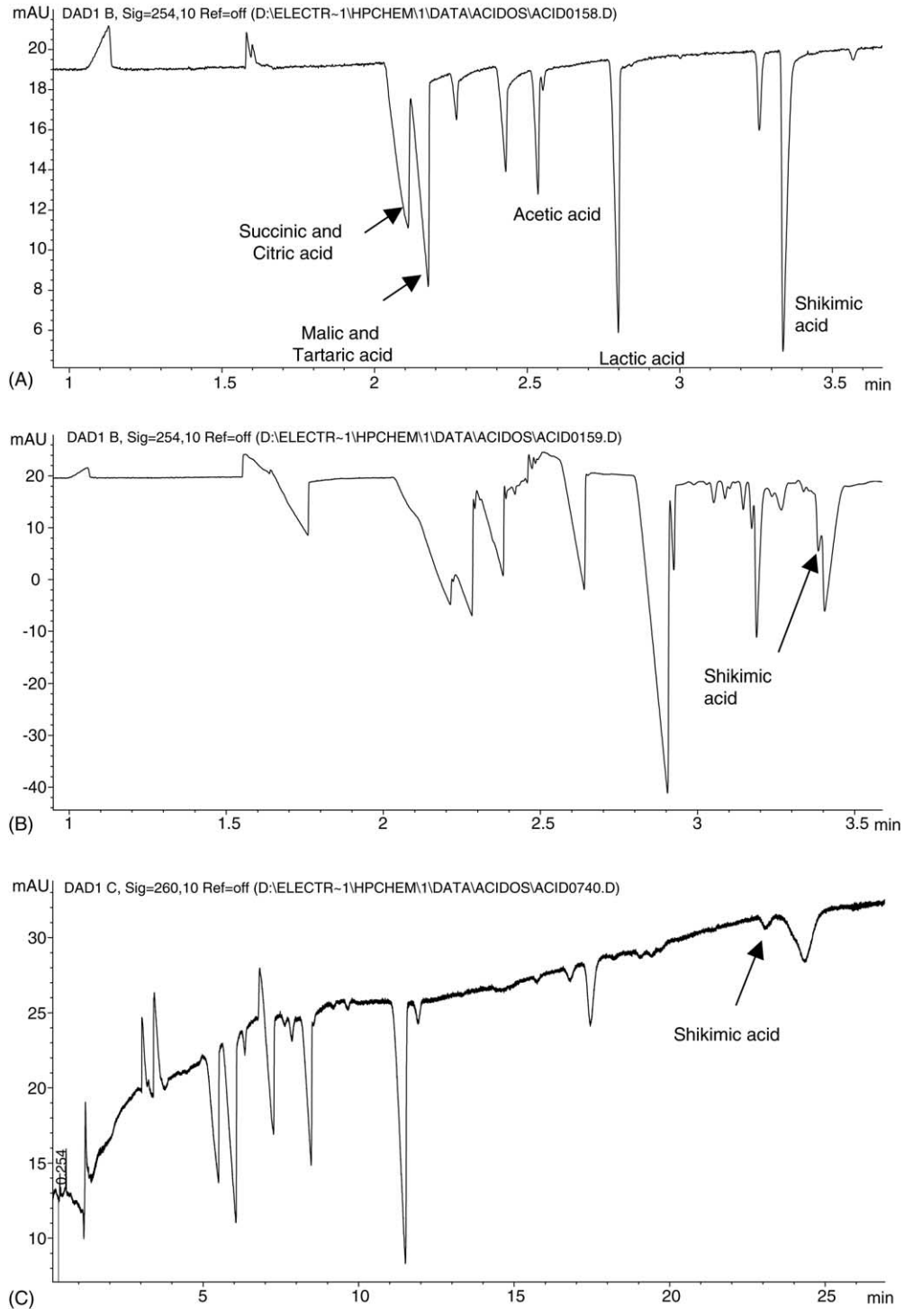

Fig. 2. Electrophoretic separation with indirect detection. The separation buffer consisted of 10.5 mM Bis-Tris; 7.5 mM PABA; 0.2 mM TTAB, pH 7.5. (A) Electropherogram of standard mixture of 25 mg L^{-1} of organic acids without $CaCl_2$ in the buffer. (B) Electropherogram of Cabernet Sauvignon wine (dilution, 1:10), without $CaCl_2$ in the buffer. (C) Electropherogram of Cabernet Sauvignon wine (dilution, 1:10) with 0.53 mM of $CaCl_2$ in the buffer.

for two tails was 2.58, whereas the critical *t* value was 1.71. Comparing methods 1 and 2, the null hypothesis cannot be accepted, and the lack of differences between concentrations found by CE with indirect detection and HPLC can be assumed. On the other hand, the comparison between methods 1 and 3 shows no statistical difference between concentrations obtained by CE with direct detection and by HPLC. In this case, the experimental *t* value for two tails was 0.67, while the critical value was 2.02 when 46 samples were analyzed by both methodologies.

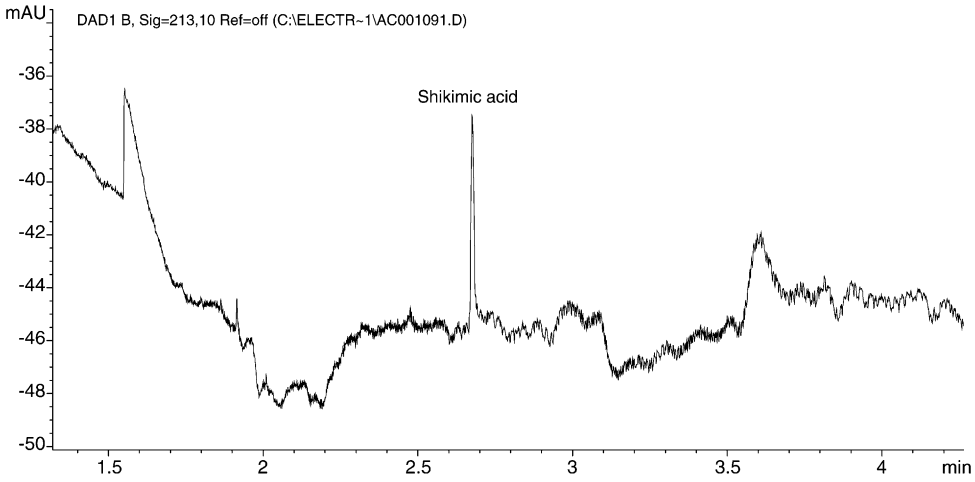

Fig. 3. Capillary electrophoretic separation with direct detection. Electropherogram of Cabernet Sauvignon wine. The separation buffer consisted of 200 mM Bis-Tris; 1.1 mM TTAB; 16 mM LiCl, pH 7.0.

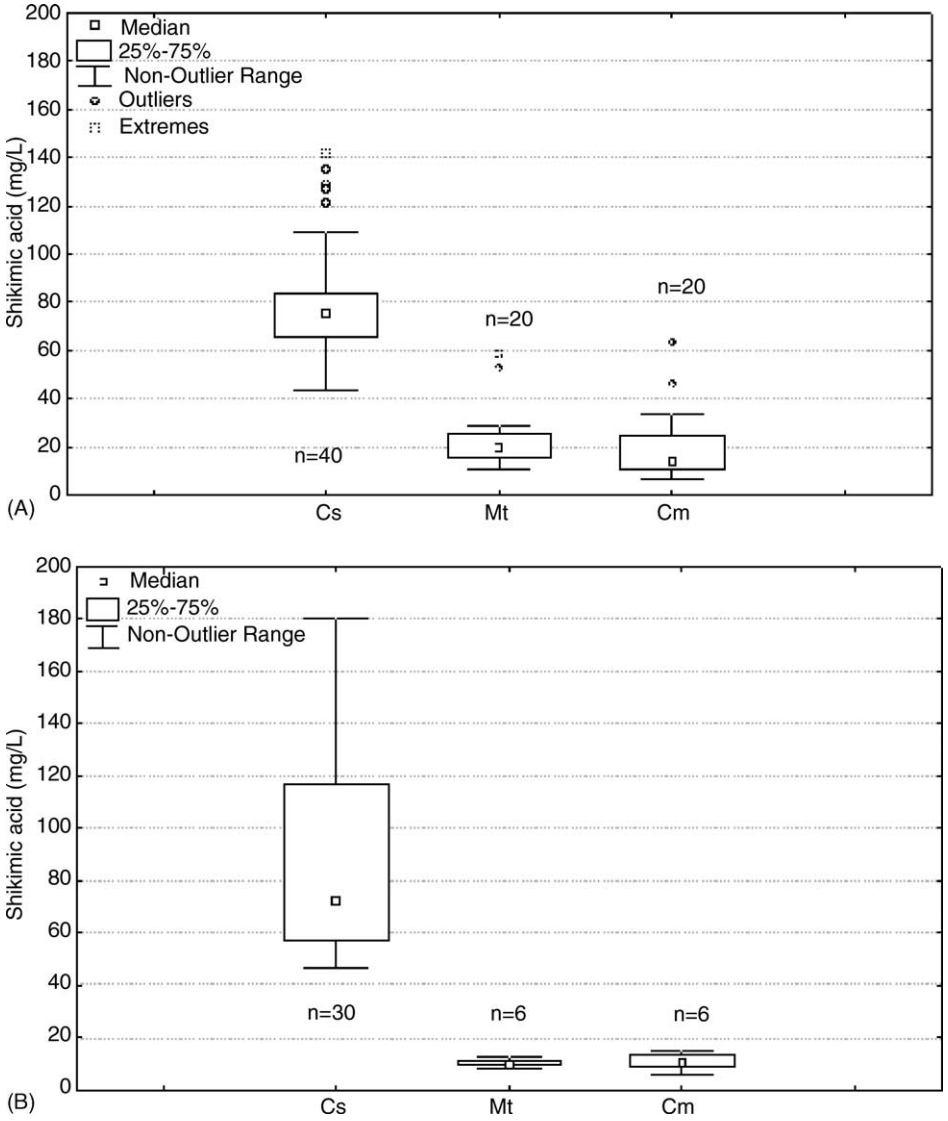

Fig. 4. Concentration of shikimic acid as a tool for varietal differentiation of wines. (A) By HPLC method ($n = 80$). (B) By CE with direct detection ($n = 42$).

The three methodologies were applied in order to evaluate if shikimic acid concentrations differ in wine Cabernet Sauvignon, Merlot and Carmenère produced in Chile. The results are summarized in Fig. 4. Shikimic acid concentrations were higher in Cabernet Sauvignon than in Merlot or Carmenère wines. By HPLC methodology, the range was between 45 and 130 mg L^{-1} for Cabernet Sauvignon, and between 11 and 28 mg L^{-1} and 8 and 35 mg L^{-1} for Merlot and Carmenère, respectively (Fig. 4A).

With the use of CE with indirect detection, the shikimic acid concentration in Cabernet Sauvignon and Merlot wines could be determined. In this case, Carmenère wine was not analyzed. Although in application of this method, the concentration for all samples was different than those obtained by using the HPLC and CE with direct detection, a higher concentration of shikimic acid was observed for Cabernet Sauvignon than Merlot wine.

Finally, by using the CE method with direct detection the mean concentration of shikimic acid in Cabernet Sauvignon wines was 72 mg L^{-1} (ranging between 48 and 180 mg L^{-1}) whereas for Merlot and Carmenère it was close to the quantification limit of the method (12 mg L^{-1}) (Fig. 4B). Even though the concentrations of shikimic acid in an important number of Merlot and Carmènere wine samples were below this limit, it was possible to differentiate Cabernet Sauvignon wines because in all cases the shikimic acid concentration in Merlot and Carmenère wines was less than in Cabernet Sauvignon; however, the proposed method does not permit differentiation between Merlot and Carmenère.

4. Conclusions

For the direct analysis of shikimic acid in red wine by HPLC is highly recommendable to use a RP-18 column in series with a S-DVB column. The first is to remove the interferents of wine and the latter to separate shikimic from the other organic acids in wine. Each column alone do not achieve the required resolution for quantitative applications.

Two CE methodologies for determining shikimic acid concentration in red wine were developed and compared with an optimized HPLC method. The three methodologies permitted the determination of shikimic acid concentrations in a range applicable to red wine samples without sample pre-treatment, with enough sensitivity, and a comparable precision. CE with direct detection shows to be statistically comparable with the HPLC methodology. No significant difference between these two methods was demonstrated; however, CE with indirect detection shows statistical differences with HPLC.

Shikimic acid concentrations in Cabernet Sauvignon wines were higher than in Merlot or Carmenère. This allows the use of shikimic acid as a chemical marker to differentiate between Cabernet Sauvignon and Merlot or Carmenère wines, but not between the last two varieties.

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References

[1] E. Bordeu, J. Scarpa, Análisis Químico del Vino, in: Ediciones, Universidad Católica de Chile, Santiago, 1998.
[2] M. Cocchi, P. Lambertini, D. Manzini, A. Marchetti, A. Ulrici, J. Agric. Food Chem. 50 (2002) 5255.
[3] D. Tusseau, C. Benoit, J. Chromatogr. 395 (1987) 323.
[4] P. Ribéreau-Gayon, D. Dubourdieu, B. Donèche, A. Lonvaud, Handbook of Enology, vol. 1, Wiley, New York, 2001.
[5] B. Holbach, R. Marx, M. Zimmer, Lebensmittelchemie 55 (2001) 32.
[6] A. Castiñeira, R.M. Peña, C. Herrero, S. García-Martín, J. Food Comp. Anal. 15 (2002) 319.
[7] P. Masson, J. Chromatogr. A 881 (2000) 387.
[8] B. Zoecklein, K. Fugelsang, B. Gump, F. Nury, Laboratory Procedures, Chapman & Hall, New York, 1995.
[9] C.W. Klampfl, M.U. Katzmayr, W. Buchberger, Electrophoresis 19 (1998) 2459.
[10] B. Holbach, R. Marx, M. Ackermann, Lebensmittelchemie 51 (1997) 78.
[11] Resolution OENO 22/2003, OIV, International Organisation of Vine and Wine (OIV), Paris, 2003.
[12] G. Bresnahan, F. Manthey, K. Howatt, M. Chakraborty, J. Agric. Food Chem. 51 (2003) 4004.
[13] B. Silva, P. Andrade, G. Mendes, R. Seabra, M. Ferreira, J. Agric. Food. Chem. 50 (2002) 2313.
[14] F. Chinnici, U. Spinabelli, A. Amati, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 2551.
[15] Resolution OENO 33/2004, OIV, International Organisation of Vine and Wine (OIV), Paris, 2004.
[16] C. KIampfl, W. Buchberger, P. Haddad, J. Chromatogr. A 881 (2000) 356.
[17] J. Sádecká, J. Polonský, J. Chromatogr. A 880 (2000) 243.
[18] M. Martin-Jouet, A. Hagège, M. Leroy, J. Chromatogr. A 946 (2002) 255.
[19] A. De Villiers, F. Lynen, A. Crouch, P. Sandra, Eur. Food Res. Tech. 217 (2003) 535.
[20] Y. Sing Fung, K. Man Lau, Electrophoresis 24 (2003) 3224.
[21] A. De Villiers, G. Vanhoenacker, F. Lynen, P. Sandra, Electrophoresis 24 (2004) 664.
[22] J. Weiss, Ionenchromatographie, third ed., Wiley-VCH, Weinheim, 2001.
[23] K. Ling Ng, B. Paull, P. Haddad, K. Tanaka, J. Chromatogr. A 850 (1999) 17.
[24] S. Masselter, A. Zemann, Anal. Chem. 67 (1995) 1047.
[25] C. Mardones, A. Ríos, M. Valcárcel, Anal. Chem. 72 (2000) 5736.
[26] D. Cocke, R. Schennach, Z. Yu, J. Chromatogr. Sci. 40 (2002) 187.
[27] J.M. Miller, J.C. Miller, Estadística y Quimiometría para Química Analítica, fourth ed., Pearson Prentice Hall, Madrid, 2002.