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A Rapid Sample Screening Method for Authenticity Control of Whiskey Using Capillary Electrophoresis with Online Preconcentration

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ABSTRACT: The present study aimed to develop a methodology using capillary electrophoresis for the determination of sinapaldehyde, syringaldehyde, coniferaldehyde, and vanillin in whiskey samples. The main objective was to obtain a screening method to differentiate authentic samples from seized samples suspected of being false using the phenolic aldehydes as chemical markers. The optimized background electrolyte was composed of 20 mmol L^{-1} sodium tetraborate with 10% MeOH at pH 9.3. The study examined two kinds of sample stacking, using a long-end injection mode: normal sample stacking (NSM) and sample stacking with matrix removal (SWMR). In SWMR, the optimized injection time of the samples was 42 s (SWMR42); at this time, no matrix effects were observed. Values of r were >0.99 for the both methods. The LOD and LOQ were better than 100 and 330 mg mL^{-1} for NSM and better than 22 and 73 mg L^{-1} for SWMR. The CE-UV reliability in the aldehyde analysis in the real sample was compared statistically with LC-MS/MS methodology, and no significant differences were found, with a 95% confidence interval between the methodologies.

KEYWORDS: screening method, capillary electrophoresis, stacking, whiskey analysis, sinapaldehyde, syringaldehyde, coniferaldehyde, vanillin

INTRODUCTION

Scotch is a major export commodity of the United Kingdom, with a value of $4 billion in 2008, according to the Scotch Whisky Association.[1] Apart from Scotland, other countries also produce the beverage (spelled "whisky" in Scotland, and "whiskey" outside of that country) and export their products, such as Ireland, the United States and Canada.[2] In the market for alcoholic drinks, whiskey can be considered a luxury item, featuring high added value, and is thus prone to tampering. The characteristics of each whiskey depend on various factors such as the raw material used in manufacturing and also the distillation. Over the years, many studies of the chemical composition of whiskey have been made, especially during the stage of maturation, because in this final stage of production, the drink acquires color, aroma, and flavor characteristics.[3−5] Aging is done through contact of the beverage with the surface of a wooden barrel, so during this time of contact, a variety of chemical compounds are released into the beverage that modify its organoleptic properties.[3−7] The chemical composition of whiskey at the end of the aging process depends on several factors such as the type of wood, the thermal treatment applied to the wood, and aging time.[7,8] Oak is the main wood used for aging distillates due to its durability, strength, and flexibility in making the barrels, among other qualities.[9−11] The aging time required for the production of whiskey varies from country to country. In Scotland, Ireland and Canada, to be classified as an aged whiskey, the beverage must be stored for at least 3 years in a special wooden barrel,[12] whereas in the United States and Brazil, this period of storage should be at least 2 years[13] and 1 year,[14] respectively. It is a fact that a longer aging time allows the best organoleptic properties to aggregate into the whiskey, which increases drinkers' appreciation and consequently also the whiskey's market value.[6] Some chemicals incorporated into the whiskey during the aging of the distillate can be highlighted: phenolic acids, phenolic aldehydes, tannins, and other phenolic compounds of low molecular weight. Phenolic aldehydes such as vanillin and syringaldehyde are predominant compounds in aged spirits and can therefore be used as indicators or markers of an aged drink.[7,15−17] Thus, the determination of chemical markers in samples of whiskey can be used to study the process to certify the quality and to verify the authenticity of the drink, because it is expected that adulterated and/or falsified products will differ significantly in composition compared to authentic samples.[15,18] The authenticity of food products is an important factor in quality control for consumer protection in a globalized world with increasing importance for the beverages market, especially Scotch whiskey, in terms of monitoring the adulterated whiskey.[19]

Different analytical techniques have been employed for the determination of chemical compounds present in samples of distillates, such as the determination of phenolic constituents, furans, and total antioxidants by means of high-performance liquid chromatography with an ultraviolet detector (HPLC-UV) in various types of spirits;[16] determination of different

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alcohols using gas chromatography coupled to a mass spectrometer (GC-MS) for the authentication and differentiation of whiskey;[20] analysis of volatile compounds by a solid phase microextraction gas chromatography coupled with quadrupole mass spectrometry (SPME-GC-MS) for samples of malt whiskey;[21] determination of sugars, disaccharides, and phenols using electrospray ionization mass spectrometry (ESI-MS) for verification of authenticity in samples of whiskey;[2] determination of phenolic compounds of low molecular weight by HPLC-UV in aged spirits;[17] and determination of the carbon isotope ratio mass using flow injection analysis−isotope ratio mass spectrometry (FIA-IRMS) in different samples of alcoholic beverages.[19] However, many of the methods mentioned are laborious and/or require relatively expensive equipment and are associated with high maintenance and operation costs that are inappropriate for a screening method. From an analytical point of view, the term "screening" refers to methods that indicate the presence of the analytes in a given sample at a level above or below a certain limit and allow rapid semiquantitative data acquisition about the components of a sample. The characteristics that a screening method must provide are more qualitative than quantitative analysis, little or no sample treatment, and the possibility to quickly generate a response for decision-making, although the response often requires confirmation using more sophisticated methods. In addition, screening methods are designed to avoid the need to process a large number of samples to make timely decisions or to obtain global measures of toxics or polluants; to minimize the effort that goes into the operation of conventional analytical processes, which are typically lengthy, laborious, and sources of systematic errors; and to minimize the need for permanent use of expensive instruments that incur high purchase and maintenance costs, instead using such equipment only for samples with positive results.[22] In this sense, capillary electrophoresis (CE) appears to be an interesting alternative. In beverage analysis, CE offers attractive advantages over established techniques, including low consumption of chemical reagents and samples, good resolution, reduced residue generation, low cost of operation, and compatibility with various types of detectors. Moreover, the technique often allows the use of preconcentration online methods, which promote increased sensitivity when this condition is required. There are different modes of online preconcentration such as field-enhanced sample stacking, transient isotachophoresis, dynamic pH junction, sweeping, and the combination of different preconcentration techniques. Each mode has unique characteristics and can obtain enrichment factors that typically range from 10 to 5000 times.[23−29]

The present study aimed to develop a rapid analytical methodology using CE for the determination of the aromatic aldehydes vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde in whiskey samples and to monitor the concentration of these compounds in authentic samples and seized samples suspected of being fake. Online preconcentration strategies (stacking) were used to increase the sensitivity of the method. The field-enhanced sample stacking methods, as well as normal sample stacking (NSM) and sample stacking with matrix removal (SWMR), were tested.

MATERIALS AND METHODS

Instrumentation. All experiments were performed on an Agilent Technologies HP[3D]CE Instrument (Palo Alto, CA), equipped with a diode array detector set at 360 nm (vanillin and syringaldehyde) and at 410 nm (coniferaldehyde and sinapaldehyde), respectively. The measurements were performed at 25 °C in an uncoated fused-silica capillary (48.5 cm × 75 μm i.d. × 365 μm o.d.) obtained from Microtube (Sao Paulo, SP, Brazil). Daily, the capillary was conditioned by a pressure flush of 1.0 mol L^{-1} NaOH solution (5 min), deionized water (5 min), and electrolyte solution (5 min). Between runs, the capillary was rinsed for 1 min with a running buffer. Standard solutions and samples were introduced from the inlet capillary extremity and injected hydrodynamically at 50 mbar (50 mbar = 4996.2 Pa). The applied separation voltage was 25 kV, with positive polarity on the injection side. Data acquisition and treatment were performed with HP Chemstation software.

Table 1. Parameters[a] of Mass Spectrometer

analyte	parent ion (m/z)	quantitative ion	DP	EP	CEP	CE	CXP
syringaldehyde	183.17	123	26	4.5	10	15	4
vanillin	153.30	110	31	3.5	10	13	4
sinapaldehyde	209.10	145	31	10.5	16	15	4
coniferaldehyde	179.18	119	31	4.0	12	17	4

[a] DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential.

The comparative method, using the LC-MS/MS analysis, was performed on chromatographic equipment consisting of a high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany). Separation was performed on a Shim-pack XR-ODS C18 column (30 mm, 2.0 mm i.d., 2.2 μm particle size) Shimadzu. A multistep isocratic and linear gradient of solvent A (H_2O + 0.1% formic acid) and B (95:5 acetonitrile/H_2O + 0.1% formic acid) was applied. The runs were performed using a mobile phase as follows: 0−11 min, 95% solvent A (isocratic mode); 11−19 min, 5% solvent A (linear gradient mode); 19−30 min, 5% solvent A (isocratic mode). The flow rate was set at 0.2 mL/min. In all instances, the injection volume was 5 μL. The column temperature was set to 30 °C. The LC system was coupled to a mass spectrometry system consisting of a hybrid triple-quadrupole/linear ion trap mass spectrometer Q Trap 3200 (Applied Biosystems/MDS Sciex, Concord, Canada). Analyst version 1.5.1 was used for the LC-MS/MS system control and data analysis. The mass spectrometer was tuned in the negative and positive modes by infusion of polypropylene glycol solution. The experiments were performed using the TurboIonSpray source (electrospray-ESI) in positive ion mode. The capillary needle was maintained at 5500 V. MS/MS parameters: curtain gas, 10 psi; temperature, 400 °C; gas 1, 45 psi; gas 2, 45 psi; CAD gas, medium. Other parameters for the cone and collision energy are listed in Table 1. Aldehyde residues were monitored and quantified using multiple reaction monitoring (MRM). Optimization of the mass spectrometer was performed by the direct infusion of an aqueous solution containing the four analytes investigated here.

Reagents and Solutions. All chemicals used in the experiments were of analytical reagent grade. Sodium tetraborate for borate buffer preparation was obtained from Merck (Rio de Janeiro, RJ, Brazil), and methanol (MeOH) was purchased from Tedia Brazil (Rio de Janeiro, RJ, Brazil). The standard compounds (vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde) were obtained from Sigma-Aldrich (Sao Paulo, SP, Brazil). Deionized water (Milli-Q deionizer, Millipore, Bedford, MA) was used to prepare the solutions. A standard stock solution (20 mg L^{-1}) of the aldehydes was prepared in deionized water, containing 40% (v/v) ethanol (EtOH). A stock solution of borate buffer at 100 mmol L^{-1} was used to prepare the background electrolyte.

Samples. For the study, 32 samples of a Scotch whiskey blend were used. Of the total samples, 31 were kindly donated by Policia Cientifica of Sao Paulo and 1 was purchased in the local market. Whiskey samples were transferred directly into autosampler vials for injection into the equipment.

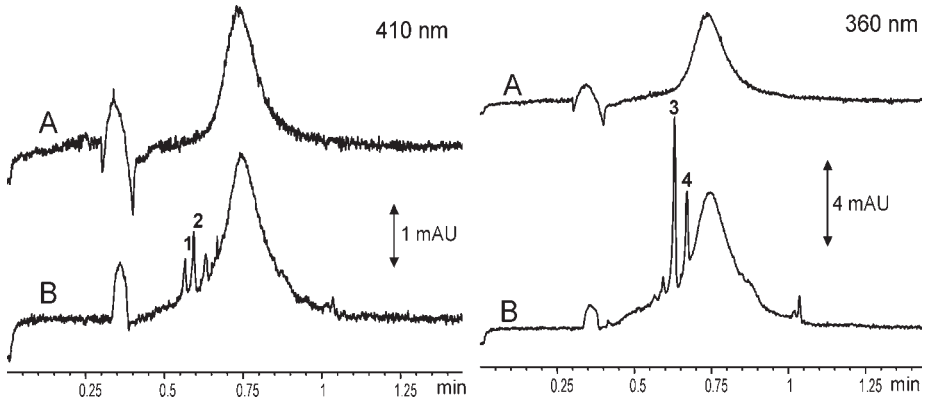

Figure 1. Electropherograms of a seized sample (A) and an authentic whiskey sample (B), 360 and 410 nm, using short-end injection mode. Peaks: 1, sinapaldehyde; 2, coniferaldehyde; 3, syringaldehyde; 4, vanillin. Experimental conditions: fused silica capillary (L_{total} = 48.5 cm; L_{det} = 8.5 cm, i.d. = 75 μm); voltage 25 kV (positive polarity in the injection side); cassette temperature, 25 °C; hydrodynamic injection, 50 mbar/3 s. Optimized running electrolyte: 20 mmol L^{-1} borate buffer and 10% MeOH (pH 9.3).

RESULTS AND DISCUSSION

Background Electrolyte Optimization. The capillary zone electrophoresis (CZE) optimization method used a standard solution with aromatic aldehydes at 10 mg L^{-1} (vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde) prepared in EtOH/water (40:60, v/v) to mimic the whiskey matrix and an authentic whiskey sample. Because the pK_a of these aldehydes ranged from 7.5 to 8.5,[30] a borate buffer at pH 9.3 was chosen as one of the background electrolyte (BGE) components. At this pH, the analytes are in anionic form and are almost fully dissociated. Thus, the analysis was carried out in a counter-electroosmotic flow. Another BGE component employed to maximize the resolution of the analytes was MeOH, used as an organic modifier. The borate buffer concentration in BGE ranged from 10 to 30 mmol L^{-1}, and the percentage of MeOH changed from 0 to 20%. The optimized BGE was composed of 20 mmol L^{-1} borate buffer with 10% MeOH. This BGE presented satisfactory results in relation to the analysis time, peak shape, resolution, and electric current, suitable for the separation.

CZE Method Using Short-End Injection Mode. To obtain a rapid separation method, one of the characteristics of a screening method, was opted for hydrodynamic injection at the end of the capillary nearest the detector (short-end injection). The injection of samples by this method allowed to differentiate samples of whiskey suspected of being tampered with from authentic samples with separation times of <1 min. As can be seen in Figure 1, in the sample of authentic whiskey it is possible to identify the peaks of the phenolic aldehydes used as markers (electropherogram B) and their absence in the seized sample (electropherogram A).

Because the signal/noise relationship of coniferaldehyde (peak 2) and sinapaldehyde (peak 1) was <10, quantifying the samples was impossible. To try to increase the detectability of aldehydes using this method, the injection time was increased to 6 and 9 s. The strategy employed did not allow quantification of all analytes in the sample, because the increased injection time decreased the resolution between peaks, causing comigration of the analytes. Although the method developed using the short-end injection mode has been shown to be effective in screening samples of whiskey, it is not suitable to quantify the aldehydes. Then, it was chosen to inject samples and standards at the farthest end from the detector (long-end injection) and to test different modes of online preconcentration. With an increase of the length of the capillary from injector to detector (L_{det}), the amount of sample injected can be increased and the highest amount injected associated with preconcentration process can result in an increase in the signal without resolution difficulties.

Normal Stacking Method (NSM) Using Long-End Injection. Using the optimized analytical conditions, the injection time ranged from 3 to 15 s in the NSM, and the standard solution prepared in EtOH/water (40:60, v/v) was analyzed to mimic the whiskey matrix. The time of injection for which a major signal was obtained without a loss of efficiency of the peaks was 9 s at 50 mbar (around 3.7% of capillary volume). Figure 2 shows the electropherogram was obtained using the optimized NSM strategy. It can be observed that a good resolution was achieved for the analytes in a relatively short analysis time. For the determination of vanillin and syringaldehyde, the wavelength was set at 360 nm, and for coniferaldehyde and sinapaldehyde it was set at 410 nm to maximize the analytical signal (UV spectra insert in Figure 2) and its selectivity.

Electropherograms A and C of Figure 3 were obtained from a reference whiskey (360 nm) and electropherograms B and D from a seized whiskey sample (410 nm), using the developed method. As can be observed, there is a considerable difference in the electrophoretic profile of the two samples and the absence of peaks relating to the analytes studied for the seized sample, suggesting that it is false.

Stacking with Matrix Removal Method (SWMR). At the pH of the optimized electrolyte, there is a strong positive electroosmotic flow. Under normal electroosmotic flow, the flow direction is toward the cathode. Thus, the online strategies investigated for sensitivity enhancement were based on preconcentration during sample injection, called SWMR. First, a large volume of the authentic whiskey sample was hydrodynamically injected into the capillary (10−180 s/50 mbar; data not shown). A negative voltage (−15 kV) was then applied to remove the sample matrix, and the polarity became positive (+25 kV) when the current reached 95% of the actual current (−37 μA when negative polarity was applied). The results of these experiments showed that the maximum signal was obtained with 120 s (SWMR120) of hydrodynamic injection (at around 50% of the capillary volume). To evaluate the matrix effect of the SWMR120

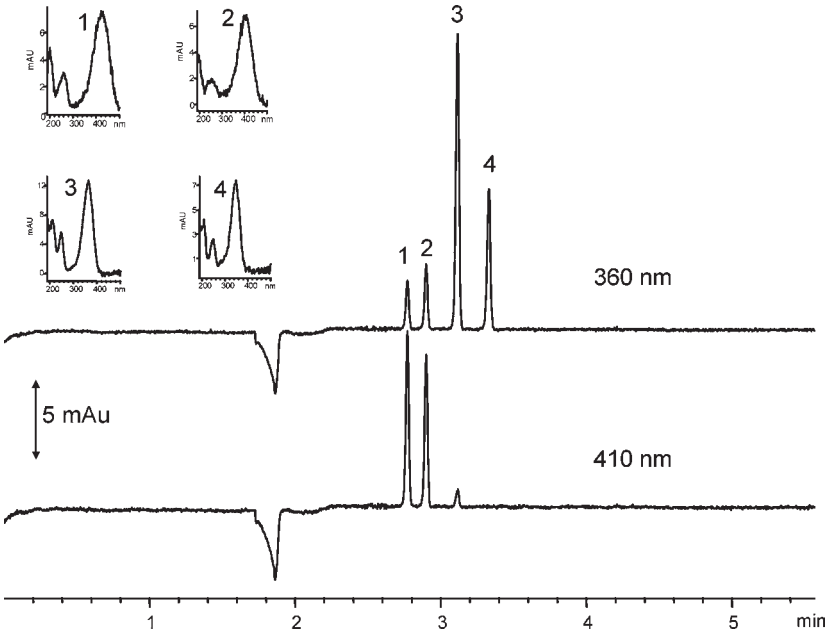

Figure 2. Electropherograms of a standard solution (aldehydes at 2 mg L^{-1}) using NSM method. Peaks: 1, sinapaldehyde; 2, coniferaldehyde; 3, syringaldehyde; 4, vanillin. Experimental conditions: fused silica capillary (L_{total} = 48.5 cm; L_{det} = 40 cm, i.d. = 75 μm); hydrodynamic injection, 50 mbar/9 s. For other conditions see Figure 1.

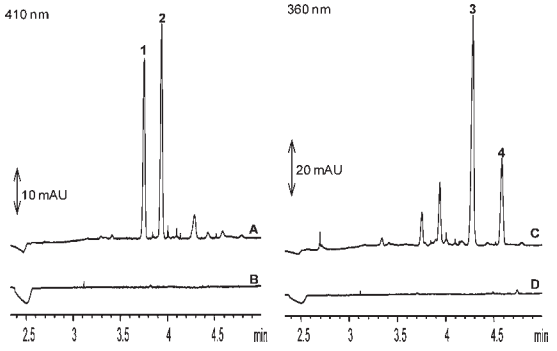

Figure 3. Electropherograms of a reference authentic whiskey sample (A, C) and a seized sample (B, D) using NSM method. Peaks: 1, sinapaldehyde; 2, coniferaldehyde; 3, syringaldehyde; 4, vanillin. For experimental conditions see Figure 2.

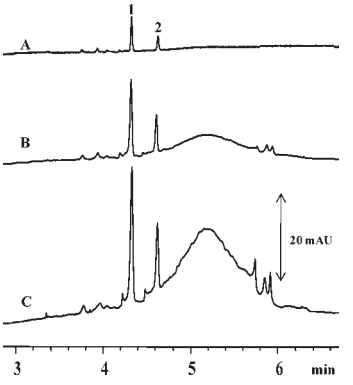

Figure 4. Electropherograms of the authentic whiskey sample analyzed using the three methodologies developed, (A) NSM, (B) SWMR42, and (C) SWMR120, at 360 nm. Peaks: 1, syringaldehyde; 2, vanillin. Experimental conditions: fused silica capillary (L_{total} = 48.5 cm; L_{det} = 40.0 cm, i.d. = 75 μm); voltage, 25 kV (positive polarity in the injection side); cassette temperature, 25 °C. Optimized running electrolyte: 20 mmol L^{-1} borate buffer and 10% MeOH (pH 9.3). Hydrodynamic injection at 50 mbar during 9 s (NSM), 42 s (SWMR42), and 120 s (SWMR120).

method, calibration curves were constructed using a standard addition calibration method (with an authentic whiskey sample) and matrix-matched calibration method (standards in EtOH/water, 40:60, v/v). The slopes obtained for two methods were statistically different. Thus, it was concluded that quantification using the SWMR120 methodology must be carried out by the standard addition method due to matrix effects. However, it was verified that for lower time of injection, injection times around 42 s, the matrix effect is not significant, and moreover, the efficiency of the peaks is suitable. In this case, the calibration curves, standard addition, and matrix-matched calibration methods did not show significant differences, indicating the possibility for the use of external calibration with this methodology. Thus, the optimized injection time was 42 s of hydrodynamic injection (SWMR42). The electropherograms obtained from the analysis of the same authentic whiskey sample, using the three different strategies of preconcentration studied in this paper, are shown in Figure 4.

Method Validation. The *r* values were >0.99 for NSM and SWMR42. The values for the precision of the proposed methods are expressed in terms of relative standard deviations (RSD). Repeatability was established through two independent sample preparations and triplicate injections. The ranges for peak area and migration time were as follows: NSM, 2.1−3.3 and 1.2−1.8%, respectively; SWMR42, 3.4−4.5 and 2.8−3.6%, respectively. The interday precision obtained for the methods using a whiskey authentic sample (peak area, n = 6) were lower than 4.5% for vanillin, 1.8% for syringaldehyde, 2.1% for coniferaldehyde, and 2.7% for sinapaldehyde. The values found were considered to be appropriate for the determination of aldehydes in whiskey samples.

Table 2. Some Figures of Merit[a] of the NSM and SWMR42 Methodologies

	NSM					SWMR42				
aldehyde	*a*	*b*	*r*	LOD[b]	LOQ[b]	*a*	*b*	*r*	LOD[b]	LOQ[b]
syringaldehyde	6.94	−2.47	0.9997	100	330	28.17	0.23	0.9996	22	73
vanillin	7.89	−1.42	0.9996	30	99	31.23	0.29	0.9990	6	22
sinapaldehyde	6.17	−1.15	0.9994	40	132	24.60	−0.21	0.9994	9	30
coniferaldehyde	7.74	−1.37	0.9972	50	165	30.98	0.14	0.9996	11	37

[a] *a*, linear coefficient; *b*, angular coefficient; *r*, correlation coefficient. [b] Concentration, μg L^{-1}.

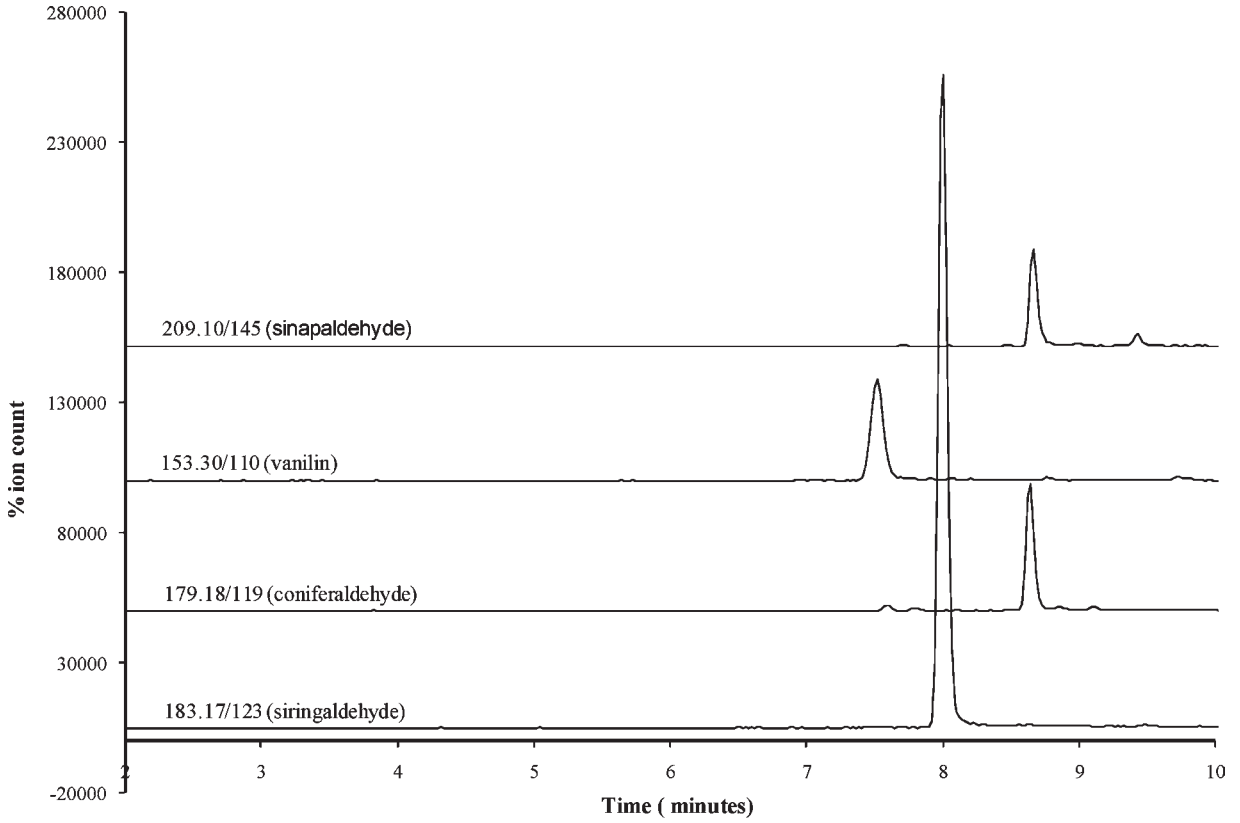

Figure 5. Chromatograms of an authentic whiskey sample analyzed by comparative method LC-MS/MS. Experimental conditions: C18 column (30 mm, 2.0 mm i.d., 2.2 μm particle size) with temperature set to 30 °C; mobile phase composed by solvent A, H_2O + 0.1% formic acid, and solvent B, 95:5 acetonitrile/H_2O + 0.1% formic acid; injection volume was 5 μL; ESI + MS/MS detection.

The data of linearity and limits of detection (LOD) and quantification (LOQ) for the NSM and SWMR42 methodologies are shown in Table 2. Signal-to-noise ratios (S/N) of 3 and 10 were considered to estimate LOD and LOQ, respectively. The LOD and LOQ obtained by the NSM method ranged between 100 and 330 μg L^{-1} for syringaldehyde, between 30 and 99 μg L^{-1} for vanillin, between 40 and 132 μg L^{-1} for sinapaldehyde, and between 50 and 165 μg L^{-1} for coniferaldehyde, respectively, whereas for the SWMR42 method, the LOD and LOQ were 22 and 73 μg L^{-1} for syringaldehyde, 6 and 22 μg L^{-1} for vanillin, 9 and 30 μg L^{-1} for sinapaldehyde, and 11 and 37 μg L^{-1} for coniferaldehyde, respectively.

LC-MS/MS Confirmation Analysis. To show the CE-UV reliability in the aldehyde analysis in a real sample, a comparison was performed using the LC-MS/MS methodology analysis (Figure 5). Thus, a paired-samples *t* test was carried out taking into account the four aldehydes present in the sample: vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde. The statistical results (for $n = 3$) were p value = 0.19 for paired-samples *t* test and p value = 0.42 for the Shapiro−Wilk normality test. As the p value was >0.05, no significant difference within the 95% confidence interval between CE-UV and LC-MS/MS methodologies was evidenced.

Sample Analysis. A total of 32 different whiskey samples were analyzed, consisting of 10 reference samples, 21 samples seized on suspicion of being false, and 1 sample (sample A3) acquired at a local market. All samples were first analyzed with the NSM method developed in this study. The reference and locally acquired samples had peaks larger than the LOQ.

Of the 21 seized samples, only 5 had an analytical signal greater than the LOD, all for vanillin. These five samples and the reference sample D1 were analyzed using the SWMR42 methodology. Table 3 gives the results for the analyzed samples.

Table 3. Concentrations of the Phenolic Aldehydes in the Samples of Whiskey, Obtained by the Developed Methods Using Capillary Electrophoresis

			concentration (μg L^{-1})			
sample	origin	maturation	syringaldehyde	vanillin	sinapaldehyde	coniferaldehyde
A1[a]	authentic	8 years	1792 ± 39	805 ± 19	354 ± 10	380 ± 12
A2[a]	authentic	8 years	2171 ± 47	945 ± 23	419 ± 11	401 ± 11
B1[a]	authentic	12 years	2269 ± 64	1100 ± 34	436 ± 14	394 ± 13
C1[a]	authentic	12 years	2733 ± 58	1288 ± 38	487 ± 15	465 ± 15
E1[a]	authentic	12 years	4345 ± 143	1997 ± 63	701 ± 22	715 ± 23
F1[a]	authentic	12 years	4592 ± 147	2249 ± 73	739 ± 23	511 ± 16
F2[a]	authentic	12 years	3386 ± 112	1596 ± 47	383 ± 11	481 ± 13
G1[a]	authentic	12 years	2979 ± 91	1413 ± 44	529 ± 18	463 ± 14
H1[a]	authentic	unknown	22055 ± 551	6996 ± 178	9068 ± 268	8775 ± 255
A3[a]	purchased	8 years	1986 ± 58	795 ± 23	508 ± 16	374 ± 12
G2[b]	suspicion	12 years	<LOD	269 ± 12	<LOD	<LOD
C2[b]	suspicion	12 years	<LOD	280 ± 13	<LOD	<LOD
B2[b]	suspicion	12 years	<LOD	271 ± 11	<LOD	<LOD
H2[b]	suspicion	unknown	<LOD	367 ± 16	<LOD	<LOD
F3[b]	suspicion	12 years	<LOD	450 ± 15	<LOD	<LOD
D1[b]	authentic	1 year	1984 ± 71	802 ± 24	124 ± 5	166 ± 7

[a] Quantified by NSM method. [b] Quantified by SWMR42 method.

Although five of the seized samples contained vanillin, these differed from the reference samples in relation to the electrophoretic profile and analytical concentration of the other aromatic aldehydes, including vanillin. This suggests that these are adulterated samples because they are samples of 12-year-old whiskey and the concentrations of the compounds after aging should be high. The D1 sample showed low values for the studied substances (Table 3), probably because of the reduced aging time (1 year).

In conclusion, the results obtained demonstrate the applicability of capillary electrophoresis in the identification of fake whiskey samples through comparison with phenolic aldehydes identified in the authentic whiskey. The CZE method using the short-end injection mode showed that it is possible to differentiate authentic samples from suspected samples using separation in <1 min. The procedure of stacking with matrix removal was found to be a powerful tool, increasing the sensitivity of the method toward the phenolic compounds analyzed. Using SWMR, the sensitivity was increased almost 10-fold in relation to NSM for the four compounds used in this study, but the use of longer injection times led to matrix effects, and calibration must be carried out by standard addition. The new method can be used to study phenolic aldehydes in whiskey samples. The determination of these compounds in whiskey samples can be employed to study the processes involved in whiskey production and to verify beverage quality and authenticity.

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ABBREVIATIONS USED

HPLC-UV, high-performance liquid chromatography with an ultraviolet detector; GC-MS, gas chromatography coupled to a mass spectrometer; SPME-GC-MS, solid phase microextraction gas chromatograph coupled to a quadrupole mass spectrometer; ESI-MS, electrospray ionization mass spectrometry; FIA-IRMS, flow injection analysis—isotope ratio mass spectrometry; CE, capillary electrophoresis; NSM, normal sample stacking; SWMR, sample stacking with matrix removal; MeOH, methanol; EtOH, ethanol; CZE, capillary zone electrophoresis; BGE, background electrolyte; LOD, limit of detection; LOQ, quantification; RSD, relative standard deviation; S/N, signal-to-noise ratio; LC-MS/MS, liquid chromatography—tandem mass spectrometry; ESI, electrospray source ionization; CAD, collisionally activated dissociation; MRM, multiple reaction monitoring; DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential.

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