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Analysis of Aromatic Aldehydes in Brandy and Wine by High-Performance Capillary Electrophoresis

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A new method of analysis of vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde in brandy and wine by high-performance capillary electrophoresis is described. Electrophoretic mobility of these compounds is achieved by a borate buffer at pH 9.3. At this pH, the sensitivity of UV detection of these phenolic aldehydes also increases. UV absorptions at 348, 362, 404, and 422 nm were selected for monitoring vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde, respectively. This procedure was performed simultaneously during one run using a diode array detector. Samples of brandy or wine were analyzed directly without concentration, extraction, or any other preliminary treatment of the test sample. The limits of detection were found to be 0.275, 0.1425, 0.1475, and 0.1975 ppm for syringaldehyde, coniferaldehyde, sinapaldehyde, and vanillin, respectively, which is acceptable for analysis of both brandy and wine aged in oak barrels. The method has been shown to be linear in a range from 0.3 to 57 mg/L. Recoveries ranged between 99.9% and 107.7% for all of the compounds tested. Repeatability and reproducibility of the method were high. The relative standard deviation was consequently ~3% and also between 4.47% and 6.89% for all tested compounds. The method is useful for the identification of counterfeit brandy, which is easy to recognize by the absence of sinapaldehyde, syringaldehyde, and coniferaldehyde, which are not detectable in false brandy

The aromatic phenolic aldehydes, vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde are formed as lignin degradation products in oak barrels during coopering and are subsequently extracted into wines or spirits during barrel aging. It is widely assumed that these aldehydes contribute to the flavor of barrel-aged alcoholic beverages[1−7] and can be used for the identification of spirits on the basis of raw material and aging time in casks.[1−3] Another practical implication of these oak wood extractive compounds is that they may be considered as markers to distinguish counterfeit alcoholic beverages from genuine brandy.

The analysis of phenolic components with a low molecular mass in wine and brandy was usually carried out by RP-HPLC with UV detection.[1−3,7−16] (Also, a spectrofluorometer may be necessary for monitoring coumarins.[1]

To increase the sensitivity of the method and to eliminate most of the ethanol when brandy is to be analyzed, the samples are subjected to a process of vacuum distillation and subsequent reconstitution of the residue in the mobile phase. If most of the ethanol is not eliminated from the brandy, the elution strength of the sample is greater than that of the mobile phase, and this leads to a split in the chromatographic peaks with a lower capacity factor, as in the case of gallic acid.[2]

Due to the diversity of phenolic compounds in must and wine, different methods, based on either conventional (discontinuous) or differential (continuous) extraction using different pH values, have been proposed for their preconcentration and extraction by ethyl ether and ethyl acetate (non-flavonoid phenols) or isoamyl alcohol (phenolic compounds with some degree of polymerization)).[16−19] Solid-phase extraction of phenolic compounds using Sep-Pak C_{18} cartridges has also been described for prepurification

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and concentration of the phenolic compounds of the wines.[7,20,21] All of these multistep pretreatments of analytical samples are time-consuming and reduce the accuracy, precision, and recovery of the analytical method. To avoid possible retention by the Sep-Pak cartridge, HPLC direct injection of wine was also reported;[7] however, such an approach dramatically reduces the lifetime of HPLC columns due to the irreversible absorption of polyphenolic compounds and the plugging of the column after several runs.

While vanillin, in aqueous alcohol extracts of oak wood, could be determined with acceptable accuracy by GC/MS, similar analysis in red and white wines usually gave anomalously low results, due to acetal formation with wine glycols during the extraction of wines with organic solvents and during subsequent concentration of the organic extracts.[4] Stable isotope dilution assays have been applied successfully for the accurate determination of vanillin.[4,22] The advantage of such assays is that the internal standard is virtually identical chemically to the substance being analyzed, and therefore, the accuracy of the analysis is not reduced by inefficiency in isolation or by analyte decomposition; however, the practical implication of this method is limited, since [$^2H_3$]vanillin is not commercially available, as well as other deuterated phenolic compounds.

In this paper, we describe a new direct method of analysis of aromatic phenolic aldehydes in brandy and wine by high-performance capillary electrophoresis. The main advantages of this method are that no pretreatment of the analytical sample is required, no organic solvents are used, and the direct injection of brandy or wine into the capillary, followed by a 10-min run time, gives the results about the content of each target compound in the brandy or wine.

EXPERIMENTAL SECTION

A technique for the simultaneous determination of vanillin, sinapaldehyde, syringaldehyde, and coniferaldehyde in brandy was developed by use of high-performance 3D capillary electrophoresis. Quantifying was performed by an external standard method.

Reagents and Materials. Coniferaldehyde, vanillin, sinapaldehyde, and syringaldehyde were purchased from Extrasynthese. Water for HPCE (Part No. 5062-8578), 50 mM borate buffer pH 9.3 for HPCE (Part No. 5062-8573), and 0.1 N sodium hydroxide solution for HPCE (Part No. 5062-8575) were purchased from Agilent Technology.

Instrumentation. A Hewlett-Packard capillary electrophoresis system, consisting of HP^{3D}CE, HP KAYAK XA, and a HP laser jet 4000 printer, was used for sample analysis under the conditions given in Table I.

Procedure. (1) Standard Solution (SS). Weigh accurately 11.8 mg of sinapaldehyde, 15.8 mg of coniferaldehyde, 11.4 mg of syringaldehyde, and 22.0 mg of vanillin into a 100-mL volumetric flask. Dissolve and dilute to volume with 40% ethanol to obtain the stock solution. Dilute the stock solution to obtain solutions used for the preparation of a calibration curve.

(2) Analyte Solution (AS). Use brandy or wine in original form for analysis by CE. Dilute when necessary with 40% ethanol.

System Suitability Test. (A) Verify that the response and standard concentrations of aldehydes exhibit a linear relationship. The correlation should not be less than 0.999. (B) The areas of aldehydes peaks should be reproducible to within 4.0% (RSD, n = 6). (C) At regular intervals (at least every tenth sample injection), inject the standard solution and compare the response to the previously established calibration graph. (D) Perform assay using new mobile phase each tenth injection.

Validation of the Method. (1) Precision. (A) Intraday Repeatability of the Method. The repeatability is characterized

Table 1. Experimental Conditions

data collection	HP ChemStation for CE systems
HPCE mode	CE
statistical analysis	GraphPad Prism software, version 2.0.1996, GraphPad Software Inc.
mobile phase	borate buffer pH 9.3 (50 mM)
preconditioning	0.1 N NaOH flush for 1.0 min, rinse water vial to remove residual NaOH, flush with buffer for 3 min
capillary	(HP G 1600−61232), total length 53.5 cm, effective length 45 cm, i.d. 50 μm, optical path length 150 μm
injection	50 mbar/4 s
temperature	20 °C
voltage	30 kV (current ~105 μA)
polarity	positive
detection	target signals −348/25 nm, ref 500/50 nm −362/25 nm, ref 500/50 nm −404/30 nm, ref 550/50 nm −422/30 nm, ref 550/50 nm
response time	0.3 s
peak width	>0.03
prerun autobalance	on
postrun autobalance	off
stop time	10 min (adjust as needed)
post time	off

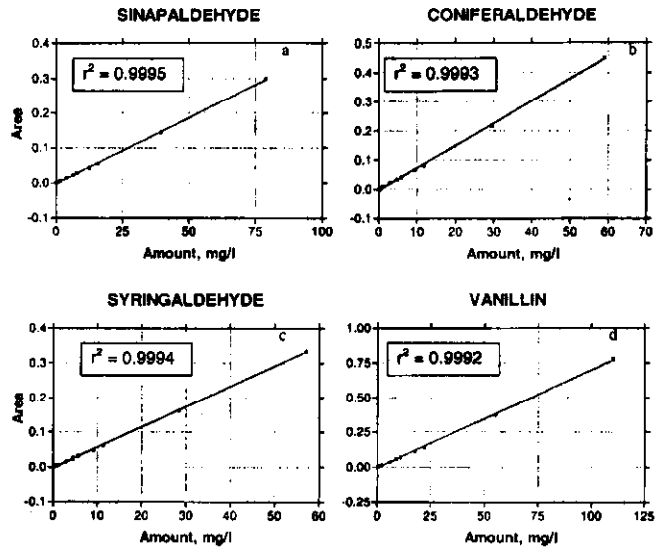

Figure 1. Linear relationship of detector response (peak area) vs concentration (mg/L) of sinapaldehyde (a), coniferaldehyde (b), syringaldehyde, (c) and vanillin (d) and goodness-of-fit values (r^2) of regression lines.

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Table 2. Method Repeatability and Reproducibility

	amount, mg/L			
	sinapaldehyde	coniferaldehyde	syringaldehyde	vanillin
	Intraday Repeatability			
mean	1.0980	1.1520	9.8460	3.7690
standard deviation	0.0339	0.03672	0.2452	0.1125
standard error	0.01384	0.01499	0.1001	0.04593
relative standard deviation (%)	3.09	3.19	2.49	2.98
confidence interval of the mean	1.062−1.133	1.114−1.191	9.589−10.100	3.651−3.887
	Interday Reproducibility			
mean	1.1110	1.1840	10.290	3.8190
standard deviation	0.07651	0.07714	0.5957	0.1706
standard error	0.03123	0.03149	0.2432	0.06965
relative standard deviation (%)	6.89	6.52	5.79	4.47
confidence interval of the mean	1.031−1.191	1.103−1.265	9.668−10.92	3.640−3.998

Table 3. Accuracy of the Method

	recovery, $R = A \times 100/S$ (%)			
	sinapaldehyde	coniferaldehyde	syringaldehyde	vanillin
mean	99.910	102.90	107.70	106.40
standard error	1.870	2.434	4.052	3.152
relative standard deviation (%)	4.18	5.29	8.41	6.62
confidence interval of the mean	94.72−105.10	96.13−109.6	96.45−118.9	97.7−115.2

Table 4. LOD and LOQ of Phenolic Aldehydes and Their Content in Brandy

			concentration, mg/L			
N	sample	country of origin[a]	sinapaldehyde	coniferaldehyde	syringaldehyde	vanillin
1	3	AM	0.511	0.497	2.320	1.129
2	Entir	AM	0.615	0.626	3.702	1.742
3	Akhthamar	AM	1.040	0.830	5.580	2.427
4	Nairi	AM	1.169	1.179	10.483	3.788
5	Erebuni	AM	1.493	1.763	19.289	7.244
6	Nojan Tapan	AM	1.618	2.544	34.202	12.834
7	Remy Martin	FR	2.005	1.490	5.139	2.413
8	Virdziya	GR	nd[b]	0.909	5.064	2.893
9	Kizlyar	RU	nd	1.085	6.547	3.780
10	Tisa	UR	nd	0.870	4.315	2.567
11	Manas	KZ	0.815	0.753	3.789	2.038
	LOQ		0.343	0.317	2.560	1.130
	LOD		0.147	0.142	0.275	0.197

[a] AM, Armenia; FR, France; GR, Georgia; RU, Russia; UR, Ukraine; KZ, Kirgiztan. [b] nd, not detected.

by the mean, the standard deviation, the relative standard deviation, and the error of the mean calculated on a series of $n = 6$ injections of the same measured sample of brandy (Table 2).

(B) Interday Reproducibility of the Method. The repeatability is characterized by the mean, the standard deviation, the relative standard deviation, and the error of the mean calculated on a series of $n = 6$ injections of the same measured sample of brandy in a 3-day period by two analysts (Table 3).

(2) Accuracy. The accuracy of the method is characterized by the mean, the standard deviation, the relative standard deviation, and the error of the mean by analysis of samples ($n = 5$) obtained by the addition of known amounts of aldehydes to the brandy and measured under the conditions prescribed in the method (Table 4).

(3) Linearity. The linearity of the method is performed by correlation between the amount and corresponding peak area determined on electropherograms (Figure 1).

Limits of Detection and Quantitation. Limits of detection (LOD) were found directly (Table 4) by analysis of the method's peak-to-peak baseline noise, limits of quantitation (LOQ) via the precision of replicate blank analyses (10 times the RSD of the replicates).[23]

RESULTS AND DISCUSSION

In the first step of our study, we found an optimal condition for the separation of phenolic aldehydes I−IV. Thus, borate buffer and pH 9.3 were found to be good enough both for the separation and for the UV detection of these aromatic compounds. In an alkaline solution, a bathochrome shift of UV absorption bands corresponding to n → p* electron transition of conjugated carbonyl group is accompanied by an increase of their coefficient of extinction (Figure 2a-A). Consequently, λ_{max} at 348, 362, 404, and

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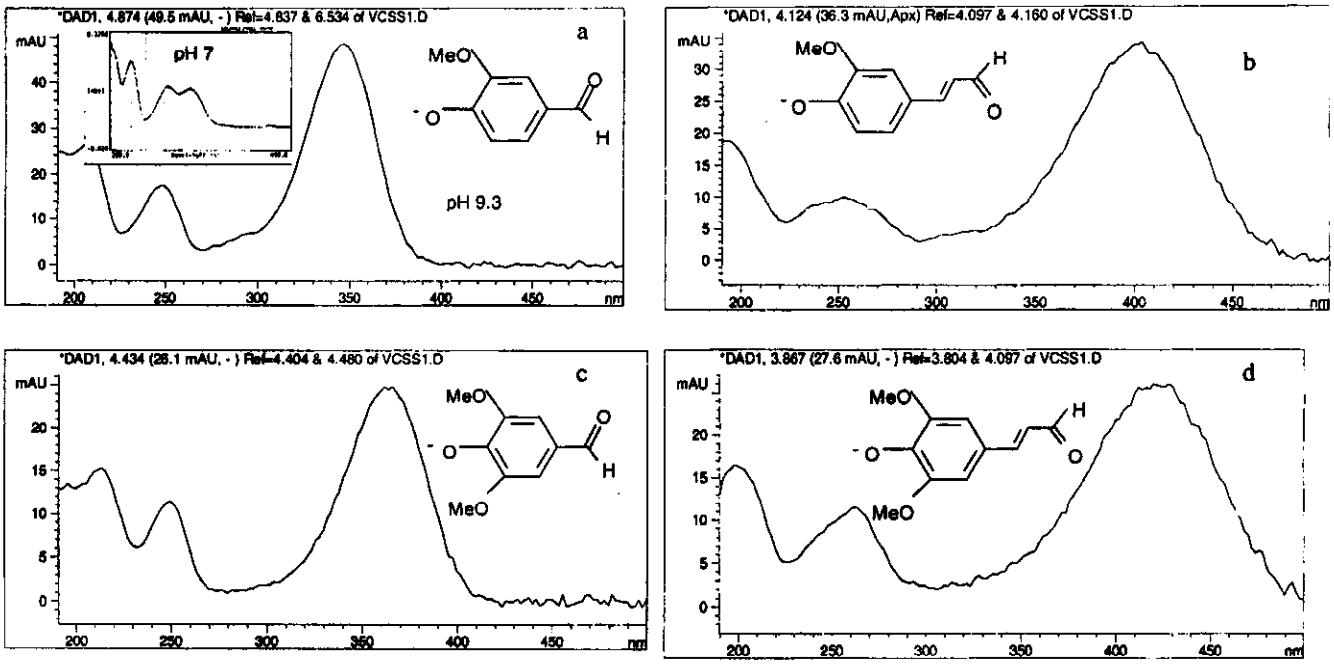

Figure 2. On-line UV spectra of vanillin (a), coniferaldehyde (b), syringaldehyde (c), and sinapaldehyde (d) in borate buffer, pH 9.3; inset in (a) UV spectrum of vanillin in 40% ethanol (a-A).

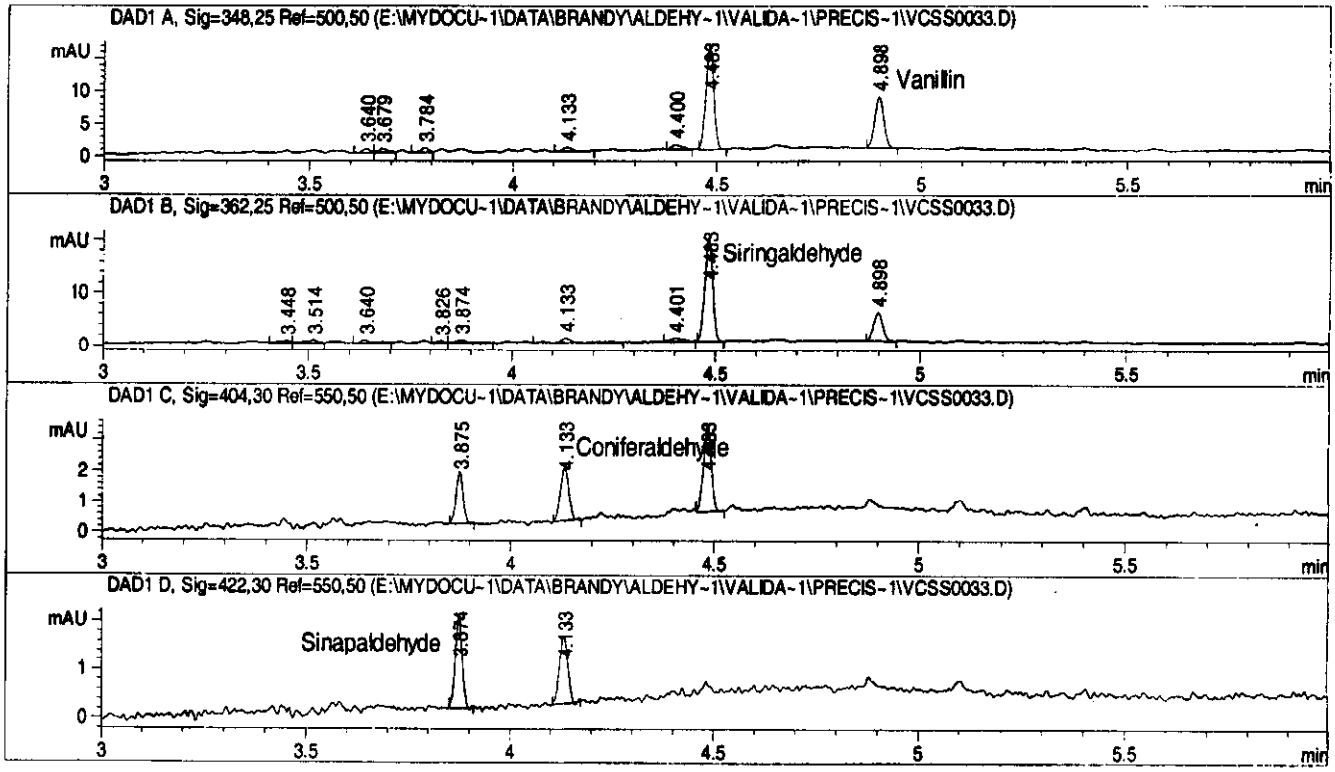

Figure 3. Electrophoregrams of a brandy.

422 (Figure 1) were selected for monitoring vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde correspondingly. This procedure was performed simultaneously during one run using a diode array detector (Figure 3). Samples of brandy or wine were injected directly without concentration, extraction, or any other preliminary treatment of the test sample. The limits of detection were found to be 0.275, 0.1425, 0.1475, and 0.1975 ppm for syringaldehyde, coniferaldehyde, sinapaldehyde, and vanillin, respectively, which is acceptable for analysis of both brandy (Table 4) and wine aged in oak barrels.[16]

The method has been shown to be linear in the range from 0.3 to 57 mg/L (Figure 1), which is relevant to brandy analysis (Table 4). The regression analysis shown that the correlation coefficient was determined to be $r = 0.9981-0.995$ and the factor

of the curvature to be $n = 1.00$ ($Y = a + bX^n$), showing a good linear fit within the range tested. The regression lines are shown in Figure 1.

The concentrations of phenolic aldehydes were calculated by calibration to the peak areas of the external standards. To demonstrate the accuracy of the analytical method, known amounts of analytes (five spiking levels) were added to brandy samples, and the resulting spiked samples were subjected to the entire analytical protocol. The contents of the tested compounds are shown in Tables 3 and 4.

As we can see, recoveries ranged between 99.9% and 107.7% for all the compounds tested (Table 3). Repeatability and reproducibility of the method were high; relative standard deviations were consequently about 3% and between 4.47% and 6.89% (Table 2) for all tested compounds.

The method is useful for the identification of counterfeit brandy, which is easy to recognize by the absence of sinapaldehyde, syringaldehyde, and coniferaldehyde, which are not detectable in false brandy.

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