Simultaneous Determination of the Major Organic Acids, Sugars, Glycerol, and Ethanol by HPLC in Grape Musts and White Wines

E. Falqué López* and **E. Fernández Gómez**

Facultad de Ciencias de Ourense, Universidad de Vigo, Laboratorio de Química Analítica, As Lagoas s/n 32004 Ourense, Spain

Abstract

A method to simultaneously identify and quantitate the major carboxylic acids, sugars, glycerol, and ethanol in wines and grape musts is proposed. The technique involves isocratic separation, an ion-exchange column, and refractive index and UV detection (at 214 nm) without sample preparation. Statistical evaluation shows that the high-performance liquid chromatographic (HPLC) method is highly reproducible and reliable. The results of the HPLC analyses and the data obtained by standard methods are compared.

Introduction

Rapid identification and quantitation of organic acids and sugars in wines are important because of the influence of these components on organoleptic properties (1,2). In addition, it is important to quantitate both organic acids and sugars because they are substrates in a variety of enzymatic transformations (3). The determination of sugar content is also necessary to obtain the endpoint of the fermentation process as well as to remain within the regulations for making and marketing the wine. Associations such as the Association of Official Analytical Chemists or Office International du Vin use classic colorimetric and enzymatic analytical procedures for their determination (4–8).

However, during recent years, chromatography has been shown to be a useful technique. Determination of carbohydrates by chromatography can be done in several ways (9), including gas–liquid chromatography, supercritical fluid chromatography, thin-layer chromatography, and high-performance liquid chromatography (HPLC). Several researchers have used gas chromatography to detect the methyl (10), silyl (11–13), and acetyl (14) derivatives and oximes (15), but HPLC probably gives the best results for the individual determination of the major acids, alcohols, and sugars (9). A variety of methods has been used to determine carboxylic acids in wine, musts, and many other food systems (16). The use of reversed-phase columns (17) usually involves either pretreating samples by separating interferences with an ion-exchange resin (18–21) or preparing derivatives such as *p*-nitrobenzyl (22–24), phenacyl (25,26), *p*-nitrophenyl (27), or naphthacyl esters (28) for ultraviolet detection.

Strong acid ion-exchange resins, in combination with refractive index (RI) or ultraviolet (UV) detectors, can be used for the simultaneous determination of acids, sugars, and some alcohols (29). The columns use ion-exchange, exclusion, and partition processes that, combined with an optimum acidic pH of the mobile phase (18), allow fractional separation (30,31) of carbohydrates and fermentation-derived chemicals.

The purpose of this research was to optimize a rapid HPLC method for the determination of sugars, organic acids, and alcohols in grape musts and wines without previous sample preparation.

Materials and Methods

HPLC

Ten microliters of the sample (filtered through 0.45-µm cellulose acetate membranes) (Millipore; Milford, MA) or standard

Table I. Retention Times of Different Acids, Sugars and Alcohols

No.	Component	Detector	Retention time (min)
1	Citric acid	RI*	17.49
2	Tartaric acid	RI	18.39
3	Glucose	RI	19.55
4	Malic acid	RI	19.99
4	Malic acid	UV	19.80
5	Fructose	RI	21.06
5	Fructose	UV	20.97
6	Succinic acid	RI	23.20
7	Lactic acid	RI	25.32
8	Glycerol	RI	26.60
9	Acetic acid	RI	28.90
10	Ethanol	RI	41.10

* RI, retention index.

* Author to whom correspondence should be addressed.

solution filtrate was analyzed using a Waters Associates chromatographic system (Millipore) equipped with a gradient controller (Model 680), a pump (Model 510), an automatic injector (Model 712), and two Aminex HPX-87H columns (300 × 7.8 mm) (Bio-Rad Labs; Richmond, CA) connected in series. The columns were operated at 75°C. The samples were eluted with 0.65mM sulfuric acid at a flow rate of 0.7 mL/min. The mobile phase was not used for more than 2 days.

The eluting compounds were detected by a UV detector (Model 481) at 214 nm and 0.2 AUFS. This detector was connected in series to an RI detector (Model 410), and the sample and reference cells were maintained at 50°C.

Table II. High-Performance Liquid Chromatographic Standard Component Concentration

Component	Detector	Range (g/L)	r*	LOD*
Citric acid	RI*	0.03 – 0.60	0.9990	0.033
Tartaric acid	RI	0.11 – 2.20	0.9992	0.044
Glucose	RI	0.05 – 1.00	0.9995	0.041
Malic acid	UV	0.051 – 3.400	0.9998	0.115
Fructose	UV	0.30 – 6.00	0.9999	0.148
Succinic acid	RI	0.10 – 2.00	0.9997	0.058
Lactic acid	RI	0.0907 – 1.81	0.9990	0.102
Glycerol	RI	0.44 – 8.82	0.9992	0.162
Acetic acid	RI	$5.25e^{-3}$ – 0.1050	0.9999	$1.84e^{-3}$
Ethanol†	RI	0.50 – 10	0.9997	0.332

* Abbreviations: *r*, correlation coefficient of linearity of response; LOD, limit of detection; RI, retention index.
† Expressed in % v/v.

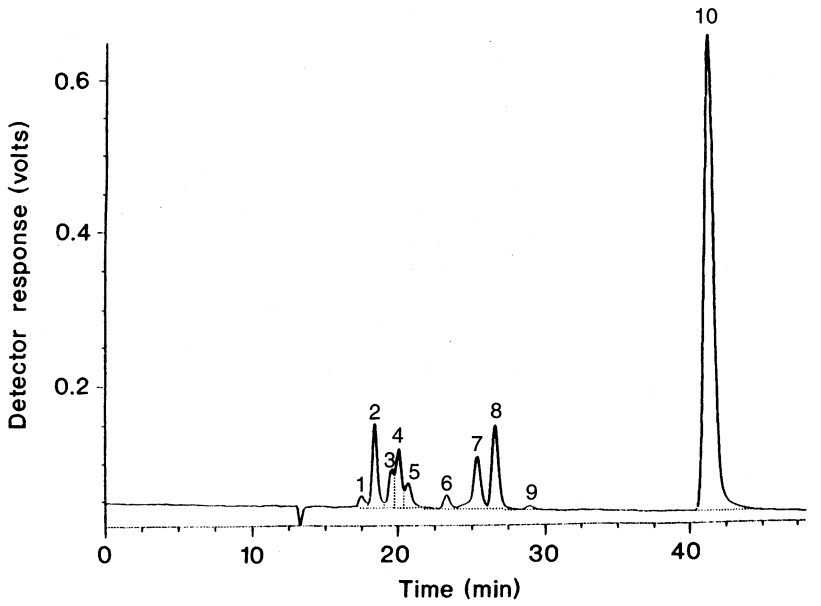

Figure 1. Chromatogram of a standard mixture. Ten microliters of each compound in concentrations typical of a range of wines and grape musts were injected through two Aminex HPX-87H (300 mm × 7.8 mm) columns. Conditions: mobile phase, 0.65mM sulfuric acid; flow rate, 0.7 mL/min; column temperature, 75°C; and detection, refractive index. Peaks: 1, citric acid; 2, tartaric acid; 3, glucose; 4, malic acid; 5, fructose; 6, lactic acid; 7, succinic acid; 8, glycerol; 9, acetic acid; and 10, ethanol.

A Model 820 Workstation was used to integrate peak areas using calibration by external standard solution.

Standard solutions

Standard solutions were prepared individually in double distilled and filtered (0.45-µm) water from analytical reagent grade chemicals (E. Merck; Darmstadt, Germany). The solutions, which contained organic acids, sugars, glycerol, and ethanol, were chromatographed individually to determine the retention time for each compound (Table I) in concentrations typical of a range of wines and grape musts (Table II).

Must and wines

The white grape must and wine Treixadura samples were supplied by the Estación de Viticultura y Enología de Galicia (Spain). The method does not require further extraction of the samples, as filtration through a 0.45-µm Millipore filter is enough.

Results and Discussion

The best optimizations of the chromatographic conditions were as follows: mobile phase, 0.65mM sulfuric acid; flow rate, 0.7 mL/min; and column temperature, 75°C. We studied the following ranges for each condition: mobile phase concentrations between 0.48 and 0.95mM in sulfuric acid; column temperatures between 65°C and 80°C; and flow rates between 0.5 and 1 mL/min.

Two detectors were used in series to quantitate L-malic acid and the sugars. In samples with high sugar levels, the L-malic acid peak was found to be completely masked by the high concentrations of glucose and fructose (Figure 1) when RI detection was used. However, the same sample was detected by UV at 214 nm. Glucose does not absorb and, thus, gives no response; fructose and L-malic acid absorb strongly, and they are shown as two separate peaks (Figure 2). For this reason, we quantitated citric, tartaric, succinic, L-lactic, and acetic acids, and glucose, glycerol, and ethanol by RI detection and L-malic acid and fructose by UV detection.

Linearity

Peak areas at seven concentration levels of standard components (Table II) were used to determine the linearity of a detector response. The correlation coefficients were all between 0.9990 and 0.9999.

Precision

Repeatability was evaluated using 10 replicate analyses of a white wine sample. The reproducibility study was carried out for a period of 10 working days to determine the time variation in the values of the

standard components. The repeatability yielded relative standard deviations from 0.90 to 2.80%, and the reproducibility yielded relative standard deviations between 0.73 and 2.97% (Table III).

Recovery efficiency

The samples of Treixadura wine were spiked with a known amount of each compound, and the results of their analysis are shown in Table IV. A good recovery (R) was obtained ($97.9 < \%R < 108.7$).

Comparison with other methods

The major carboxylic acids, sugars, and alcohols in wines can be analyzed by standard methods (2) such as those based on enzymatic reactions for citric, malic, succinic, and lactic acids and glycerol (Boehringer Mannheim). Tartaric acid can be determined by the Rebelein method (5); acetic acid is often evaluated by acid–base titration of the acid fraction, which is obtained by steam distillation of wine (5). Fructose and glucose are measured as reducing sugars (Fehling); and ethanol has been determined by the ebulliometer technique (5).

Table V shows the results obtained by the HPLC method and by standard methods. The calculated content of carboxylic acids, sugars, and alcohols are in very good agreement with the values obtained by other standard methods.

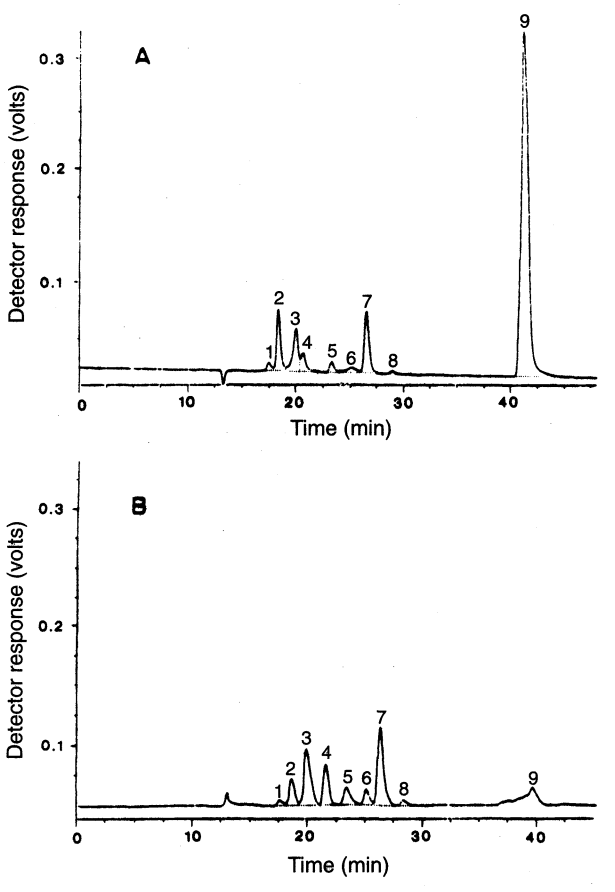

Figure 2. Chromatograms of Treixadura wine according to the described procedure by (A) refractive index and (B) UV (at 214 nm). Quantitation of each compound is reported in Table III. Conditions: mobile phase, 0.65mM sulfuric acid; flow rate, 0.7 mL/min; column temperature, 75°C. Peaks: 1, citric acid; 2, tartaric acid; 3, malic acid; 4, fructose; 5, lactic acid; 6, succinic acid; 7, glycerol; 8, acetic acid; and 9, ethanol.

Conclusion

When this method is used, the major carboxylic acids, sugars, glycerol, and ethanol of grape musts and wines were separated and quantitated in less than 45 min and with minimal sample preparation (filtration through a 0.45-µm membrane only). The proposed procedure is a rapid method for determination of these compounds and can be used for quality control in the wine industry.

The recovery efficiency, linear regression, reproducibility, and repeatability show that this HPLC method can effectively

Table III. Study of Between-Day Repeatability and Reproducibility for a White Wine

		Repeatability (n = 10)		Reproducibility (n = 10)	
Compound	Detector	Mean (g/L)	RSD* (%)	Mean (g/L)	RSD (%)
Citric acid	RI*	0.301	2.00	0.301	2.20
Tartaric acid	RI	1.50	1.40	1.50	1.42
Glucose	RI	ND*	–	ND	–
Malic acid	UV	2.45	1.60	2.45	1.43
Fructose	UV	0.199	1.95	0.199	1.94
Succinic acid	RI	0.360	2.04	0.360	1.52
Lactic acid	RI	0.180	2.40	0.180	1.98
Glycerol	RI	6.45	0.90	6.45	0.92
Acetic acid	RI	0.01	2.80	0.01	2.97
Ethanol†	RI	9.16	0.95	9.16	0.73

* Abbreviations: RSD, relative standard deviation; RI, retention index; ND, not detected.
† Expressed in % v/v.

Table IV. Recovery Efficiency of the High-Performance Liquid Chromatographic Method for Treixadura Wine

	Concentration (g/L)				
Compound	In wine	Added	Calculated	Found	Recovery (%)
Citric acid	0.301	0.5	0.801	0.798	99.6
Tartaric acid	1.50	0.5	2.00	2.04	102.0
Glucose	ND*	0.5	0.5	0.53	106.0
Malic acid	2.45	0.5	2.95	2.93	99.3
Fructose	0.199	0.5	0.699	0.690	98.7
Succinic acid	0.360	0.5	0.860	0.842	97.9
Lactic acid	0.180	0.5	0.680	0.670	98.5
Glycerol	6.45	0.5	6.95	6.94	99.8
Acetic acid	0.013	0.010	0.023	0.025	108.7
Ethanol†	9.16	1	10.16	10.18	100.2

* Abbreviation: ND, not detected.
† Expressed in % v/v.

Table V. Comparison of Results by HPLC and Standard Methods

Compound	Concentration (g/L)	
	HPLC method	Standard methods
Citric acid	0.310	0.32*
Tartaric acid	1.56	1.61†
Glucose–Fructose	0.2	0.24‡
Malic acid	2.40	2.38*
Succinic acid	0.32	0.30*
Lactic acid	0.21	0.21*
Glycerol	6.70	6.68*
Acetic acid	0.036	0.040§
Ethanol[II]	9.75	9.77#

* Enzymatic method.
† Titration method.
‡ Lane–Eynon method (reducing sugar).
§ Steam distillation method.
II Expressed in % v/v.
Ebulliometer technique.

separate these compounds in wine. The precision of the quantitation is comparable with traditional methods.

Acknowledgments

A fellowship from Diputación de Ourense is acknowledged (EFL), and Estación de Viticultura y Enología de Galicia are thanked for supplying the wine samples.

References

1. J. Ribereau-Gayon, E. Peynaud, P. Sudraud, and P. Ribereau-Gayon. *Science et Technique du Vin*, Tome I. Dunod, Paris, France, 1976.
2. M.A. Amerine and C.S. Ough. *Methods for Analysis of Musts and Wines*. Wiley, New York, NY, 1980.
3. L.U. Tomasset. *Chimica Enologica*. AEB, Brescia, Italy 1978.
4. *Recueil del Méthodes Internationals d' Analyse des Vins*, 4th ed. Office International de la Vigne et du Vin, Paris, France, 1978.
5. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists, Arlington, VA, 1984.
6. EEC Regulations, No. 2984/78, Brussels, 1978. EEC Official Publications Department, Luxembourg.
7. EEC Regulations, No. 337, Brussels, 1979. EEC Official Publications Department, Luxembourg.
8. *Methods of Biochemical Analysis and Food Analysis*. Boehringer Mannheim GmbH, Mannheim, Germany, 1989.
9. S.C. Churms. Review: Recent developments in the chromatographic analysis of carbohydrates. *J. Chromatogr.* **500:** 555–83 (1990).
10. A. Bertrand and R. Triquet-Pissard. Dosage des acides organiques fixes dans les moûts et les vins par chromatographie en phase gazeuse. *Ann. Fals. Exp. Chim.* **73:** 623–33 (1980).
11. P. Fantozzi and J. Bergeret. Application de la chromatographie en phase gazeuse (CPG) à l'étude des acides organiques et des acides-phénols du vin. *Ind. Aliment. Agric.* **90:** 731–37 (1973).
12. P. De Smedt, P.A.P. Liddle, B. Cresto, and A. Bossard. The analysis of non-volatile constituents of wine by glass capillary gas chromatography. *J. Inst. Brew.* **17:** 349–51 (1981).
13. J.E. Marcy and D.E. Carrol. Research note: A rapid method for the simultaneous determination of major organic acids and sugar in grape must. *Am. J. Enol. Vitic.* **33:** 176–77 (1982).
14. M.C. Gomez-Cordovés and L. Hernández. Determinación rápida de polialcoholes y azúcares en vinos por CGL. *La Semana Vitivinícola* **2102:** 4673–75 (1986).
15. M.I. Al-Hazmi, K.R. Staufer, and C. Olney. A new derivate for improved GCL separation of mannose, galactose and glucose. *Chromatographia* **20:** 403–406 (1985).
16. R. Schwarzenbach. High-performance liquid chromatography of carboxylic acids. *J. Chromatogr.* **251:** 339–58 (1982).
17. R.M. Marcé, M. Calull, R.M. Manchobas, F. Borrull, and X. Rius. An optimized direct method for the determination of carboxylic acids in beverages by HPLC. *Chromatographia* **29:** 54–58 (1990).
18. A. Schneider, V. Gerbi, and M. Redoglia. A rapid HPLC method for separation and determination of major organic acids in grape musts and wines. *Am. J. Enol. Vitic.* **38:** 151–55 (1987).
19. D. Tusseau and C. Benoit. Routine high-performance liquid chromatographic determination of carboxylic acids in wines and champagne. *J. Chromatogr.* **395:** 323–33 (1987).
20. M.C. Polo, F. Barahona, and I. Cáceres. Dosage par chromatographie liquide haute performance des principaux acides organiques du vin. *Connais. Vigne Vin.* **20:** 175–87 (1986).
21. J.P. Goiffon, A. Blachere, and C. Reminiac. Dosage des acides organiques du vin par chromatographie en phase liquide. *Analusis* **13:** 218–25 (1985).
22. R. Badoud and G. Pratz. Improved high-performance liquid chromatographic analysis of some carboxylic acids in food and beverages as their *p*-nitrobenzylesters. *J. Chromatogr.* **360:** 119–36 (1986).
23. W. Steiner, E. Müller, D. Fröhlich, and R. Battaglia. HPLC-bestimmung von carbonsäuren als *p*-nitrobenzylester in fruchtsäften und wein. *Mitt. Geb. Lebensm. Hyg.* **75:** 37–50 (1984).
24. I. Roorda, C. Gonnet, and J.L. Rocca. Pre-column derivatization of short-chain carboxylic acids. UV and electrochemical detection. *Analusis* **10:** 409–12 (1982).
25. E. Mentasti, M.C. Gennaro, C. Sarzanini, C. Baiocchi, and M. Savigliano. Derivatization, identification and separation of carboxylic acids in wines and beverages by high performance liquid chromatography. *J. Chromatogr.* **322:** 177–89 (1985).
26. F. Caccamo, G. Carfagnini, A. Corcia, and R. Samperi. Improved high-performance liquid chromatographic assay for determination of organic acids in wines. *J. Chromatogr.* **362:** 47–53 (1986).
27. E. Grushka, H.D. Durst, and E.J. Kikta. Liquid chromatographic separation and detection of nanogram quantities of biologically important dicarboxylic acids. *J. Chromatogr.* **112:** 673–78 (1975).
28. M.J. Cooper and M.W. Anders. Determination of long chain fatty acids as 2-naphthacyl esters by high pressure liquid chromatography and mass spectrometry. *Anal. Chem.* **46:** 1849–52 (1974).
29. R.F. Frayne. Direct analysis of the major organic components in grape must and wine using high-performance liquid chromatography. *Am. J. Enol. Vitic.* **37:** 281–87 (1986).
30. M. Calull, R.M. Marcé, and F. Borrull. Determination of carboxylic acids, sugars, glycerol, and ethanol in wine and grape must by ion-exchange high-performance liquid chromatography with refractive index detection. *J. Chromatogr.* **590:** 215–22 (1992).
31. J.J. Hunter, J.H. Visser, and O.T. De Villiers. Preparation of grapes and extraction of sugars and organic acids for determination by high performance liquid chromatography. *Am. J. Enol. Vitic.* **42:** 237–44 (1991).

Manuscript accepted November 28, 1995.