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Determination of anthocyanins in red wine using a newly developed method based on Fourier transform infrared spectroscopy

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Abstract

The feasibility of Fourier transform infrared (FTIR) spectroscopy for determination of anthocyanins in red wines was studied. The FTIR spectra were gathered using a WineScan FT 120 instrument. A process based on HPLC was used as reference method to determine the 3-monoglucosides of cyanidin, peonidin, delphinidin, petunidin and malvidin, as well as its acetic acid esters and *p*-coumaric acid esters. The calibration set was constituted by 350 samples of young red wines from different Spanish Denominations of Origin and the validation set by 40 representative samples. Partial least squares regression (PLS) was the multivariate method that carried out calibrations. Prediction error SEC was between 0.15 and 23.79 mg/L. Validation equations developed to correlate reference and FTIR methods disclosed a systematic error in the determination of certain anythocyanins, however, this error could be overcome by application of a correction factor. The results suggest that the WineScan FT 120 analyzer is suitable for routine laboratory measurement of anthocyanins and provides additional information regarding red wine colour.

Keywords: FTIR spectroscopy; WineScan; Anthocyanins; Red wine

1. Introduction

Knowledge of the composition of wine at every stage of production enables total control of the production process, thus ensuring optimal product characteristics. The wine-production sector requires analytical methods allowing simultaneous measurement of a large number of analytes. Measurement needs to be automatic, rapid, accurate and precise, requiring only low input of chemical reagents and little or no sample preparation. Techniques enabling online analysis provide immediate control over raw materials, over the production process itself and over the characteristics of the finished product, thus ensuring that the wine produced meets the expectations of increasingly demanding customers. A number of techniques have been used for this purpose: near infrared reflectance spectroscopy (NIRS) (Gishen et al., 1999; Urbano-Cuadrado, Luque de Castro, Pérez-Juan, García-Olmo, & Gómez-Nieto, 2004), Fourier transform infrared (FTIR) spectroscopy (Dubernet & Dubernet, 2000; Gishen & Holdstock, 2000; Kupina & Shrikhande, 2003; Patz, David, Thente, Kürbel, & Dietrich, 1999; Soriano, González, Delgado, & Sánchez-Migallón, 2002) and even flow injection analysis (FIA) or sequential injection analysis (SIA) coupled with FTIR-detectors (Luque de Castro, González-Rodríguez, & Pérez-Juan, 2005; Ruzicka & Marshall, 1990; Ruzicka, Marshall, & Christian, 1990; Schindler, Vonach, Lendl, & Kellner, 1998).

The use of vibrational spectroscopy for routine analysis of wine began with NIRS being the preferred method in the early years. Recently, however, focus has moved towards FTIR technology in the middle infrared region, since it offers a more accurate determination of more constituents and properties than the NIR method (Dubernet & Dubernet, 2000; Patz et al., 1999). The first purpose-built wine

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analyser of this type was marketed in 1998, the WineScan FT 120 (Foss Electric, Denmark). FTIR technology is based on the measurement of the frequencies of chemical bonds in functional groups such as C–C, C–H, O–H, C=O and N–H, upon absorption of radiation in the mid infrared region, which is usually defined as ranging from 4000 to 400 cm^{-1}, or in other terms, from 2500 to 25,000 nm (Smith, 1999). The WineScan uses FTIR spectroscopy together with multivariate statistical procedures to correlate the spectral response of a sample with compositional data as determined by reference laboratory methods. Chemometric techniques more used are principal component analysis (PCA), principal component regression (PCR) and partial least squares (PLS) regression.

The use of a FTIR instrument with ready-to-use calibration models for different products is an advantage for unskilled users and for routine analysis. However, different varieties or types of wine not included in the calibration set may introduce interference mechanisms that will affect the accuracy of the results for these types of samples. Consequently, extending the database to include real life samples, the widest possible range and scale of values for the parameter analysed, and modify the calibration is necessary for each laboratory to develop a robust calibration. However, the more robustness is gained, the more analytical precision is weakened; therefore, a compromise must be made between robustness and analytical accuracy in order to be able to deal with maximum number of wine types using the same calibration, at the same time, being sufficiently accurate for the requests of enological analysis.

When grape, must or wine is analysed for payment or quality control, analysis time, accuracy and precision are key parameters. FTIR analysis is recognised by its ability to provide very good repeatability and reproducibility. In many cases, FTIR methods are superior to the classic routine methods and often even the reference methods. Overall, the precision of FTIR methods depends directly on the quality of the calibrations being used. Normally analysis times in the range of 6–30 s are required, accuracy must be better than 1% relative and precision better than 0.5% relative (Kjaer, Waaben, & Villemoes, 2002).

Red wine colour and changes in colour are a major current concern in the wine sector. Anthocyanins are largely responsible for red wine colour, which changes progressively during its lifetime, due to the replacement of grape anthocyanins by other pigments whose structures, occurrence in wine, and mechanisms of formation are still not wholly understood. The concentration and distribution of anthocyanins in grapes depend on the grape variety and on the degree of ripeness, and are also affected by climate and soil conditions (Cacho, Fernández, Ferreira, & Castells, 1992; González-San José, Barron, & Díez, 1990; Yokotsuka, Nagao, Nakazawa, & Sato, 1999). The monomeric anthocyanins are 3-monoglucosides of cyanidin, peonidin, delphinidin, petunidin and malvidin, which could be acylated with acids, including acetic acid and *p*-coumaric acid. Simultaneous analysis of anthocyanins using the WineScan FT 120 analyzer provides a fast, objective, accurate and precise indication of wine colour; this method also allows colour changes to be charted over time, and enables prediction of colour behavior during post-barrel processing. The method is therefore of considerable economic interest to the wine producer, and is also a valuable aid for researchers.

The objective of this work was to evaluate a method based on FTIR for accurate determination of 3-glucosides of delphinidin, cyanidin, petunidin, peonidin, malvidin, as well as its acetic acid esters and *p*-coumaric acid esters.

2. Materials and methods

2.1. Wine samples

The sample set consisted of young red wines (vintage of 2004) which had not undergone clarification or stabilization; wines were made from either a single variety (Cencibel, Cabernet Sauvignon, Garnacha Tintorera, Syrah, Merlot, Bobal and Monastrell) or from a blend of these. Denominations of Origin were La Mancha, Manchuela, Utiel-Requena, Almansa, Jumilla and Alicante. A total of 350 wine samples were used in the calibration of analytical methods. Methods for measuring anthocyanin concentrations were validated using a further 40 wine samples not used for calibration, covering the range of concentrations previously used for calibration purposes. Table 1 shows the number of samples and the anthocyanin concentrations used for calibration and validation of analytical methods. Levels of acetates, *p*-coumarates and total anthocyanins were also calculated, since they are of value in differentiating wine types.

2.2. Reference HPLC method for determination of anthocyanins

The analysis of anthocyanins content was performed following the method described by Lamuela-Raventós and Waterhouse (1994) and modified by Vaadia (1997). The fractionation was carried out using a Waters equipment, model 2690, equipped with a 996 diode array UV–vis detector managed by Millenium software (Waters). A Spherisorb ODS2 C18 column, 4.6 × 250 mm, 3-μm particle size, from Symta (Madrid, Spain), was maintained at 40 °C with a flow rate of 0.6 ml/min during analysis. The solvents used were solvent A = 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B = 20% A with 80% acetonitrile; solvent C = 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5. Solvent gradient conditions were the same described by Lamuela-Raventós and Waterhouse (1994). Ten microliters of sample, previously filtered (0.45 μm, Millipore, Bedford, MA, USA), was injected. The identification of the peaks using authentic standards for anthocyanins was not possible, because they were not available commercially; thus the identification was done by deter-

Table 1
Number of samples and anthocyanins concentration (mg/L) using to develop calibration and validation of analytical methods

	Calibration set			Validation set		
	No. of samples	Mean	Standard deviation	No. of samples	Mean	Standard deviation
Delphinidin-3-glucoside	332	15.51	10.37	40	10.01	3.98
Cyanidin-3-glucoside	331	1.58	1.79	40	1.75	1.52
Petunidin-3-glucoside	320	17.70	9.53	40	12.46	3.93
Peonidin-3-glucoside	331	18.94	24.79	40	15.26	11.6
Malvidin-3-glucoside	336	128.09	59.38	40	102.13	35.52
Delphinidin-3-glucoside-acetate	336	1.96	1.18	40	0.76	0.50
Cyanidin-3-glucoside-acetate	350	1.01	0.95	40	0.78	0.50
Petunidin-3-glucoside-acetate	346	1.56	1.15	40	1.01	0.90
Peonidin-3-glucoside-acetate	341	2.61	2.34	40	2.21	1.60
Malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate	334	21.78	21.21	40	17.00	9.23
Total acetates	339	29.09	19.91	40	22.35	12.23
Cyanidin-3-glucoside-*p*-coumarate	351	1.39	1.01	40	1.06	0.40
Petunidin-3-glucoside-*p*-coumarate	344	0.75	0.55	40	0.37	0.64
Peonidin-3-glucoside-*p*-coumarate	333	3.81	4.53	40	2.96	2.09
Malvidin-3-glucoside-*p*-coumarate	337	14.83	7.57	40	9.27	2.90
Total *p*-coumarates	342	20.44	10.63	40	14.86	5.38
Total anthocyanins	323	222.13	96.51	40	179.28	66.38

mining the spectra in ultraviolet, which vary from one compound to another, and from the elution order (Cantos, Espín, & Tomás-Barberán, 2002; Cheynier, Remi, & Fulcrand, 2000).

Monomeric anthocyanin concentrations were measured using an external malvidin-3-glucoside-chloride standard (Symta, Madrid, Spain). A suitable standard solution was made with 5 mg of chromatographically pure malvidin-3-glucoside-chloride dissolved in 10 ml of 0.1 M HCl; successive dilutions of this solution were used to obtain points for the generation of calibration curves.

Limits of detection and quantification for each anthocyanin were calculated following the IUPAC recommendations, accordingly 10 sample blanks were analyzed. Detection limit was estimated as $3s_b$ and quantification limit as $10s_b$, where s_b was the standard deviation of blank measurements. Repeatability for each anthocyanin was estimated as relative standard deviation of 10 replicates of the same sample analysed on the same day. Intralaboratory reproducibility, also recently called intermediate precision, was calculated as relative standard deviation of 10 replicates of the same sample analysed in consecutive days. Anthocyanin concentrations in the samples were used to calculate precision lay in the central portion of the range of concentrations obtained for calibration samples.

On the other hand, the standard error laboratory (SEL) was measured from all samples duplicates of calibration set and calculated as

$$\mathrm{SEL} = \sqrt{\frac{\sum_{i=1}^{N}(y_{i1} - y_{i2})^2}{N}}$$

where y_{i1} and y_{i2} are the results of duplicate determinations of sample i, and N is the number of samples.

2.3. FTIR spectral measurement

A WineScan FT 120 instrument (Foss Electric, Denmark) that employs a Michelson interferometer was used to obtain the FTIR spectra. The instrument was equipped with a model 5027 autosampler (64 tray, 40 ml-cups). A sample volume of 7 ml (standard setting) was pumped through the cuvette (optical path length 37 μm), which is located at the heater unit of the instrument. The temperature of samples was set to 40 °C. Analysis time took 30 s/sample. Cleaning was automatically programmed to occur every 5 min. The instrument was zeroed before any set of analyses with the zeroing solution (S-6060, Foss Electric). The instrument was standardized before the initial calibration with FTIR equalizer solution (537811, Foss Electric) and repeated at least one a month. Samples were scanned from 926 to 5012 cm^{-1} at 4 cm^{-1} intervals, which includes a small section of the near-IR region. The numbers of scans generated per sample, the selection of wavenumbers, and the processing of spectra have been fixed by the manufacturer and are not accessible to change by the user.

2.4. Multivariate data analysis

Multivariate calibration techniques are used to perform quantitative measurements based on IR spectra. These types of spectral data contain overlapping bands from all constituents. The problem of obtaining a result independent of interfering signals can only be solved in a mathematical way. Partial Least Squares regression (PLS), available within the software package of the WineScan FT 120, was the multivariate method that carried out calibrations. Statistical data analysis has been set by the manufacturer and is not accessible to change by the user. The original spectrum variables were used as the basis for defining so-called "filters", which are wavenumbers or small groups of wavenumbers. If all wavenumbers were used in the calibration there would be a risk of "diluting" the useful regions of the spectrum, making the major spectral peaks less visible to the PLS algorithm, as well as incorporating noise into the calibration model. The PLS calibration is based on the assumption that only a combined use of all selected filters can give the true concentration value. These filters are compressed into "factors". Cross validation was automatically done by the software and involved keeping out successive groups of samples from the calibration set (25% of the total number of calibration samples at a time), and using these subsets or segments for prediction on the basis of the rest of the samples, until all samples have been kept out. The lowest possible number of factors were manually selected based on the lowest cross validation error (SECV) value (all outliers were removed). This procedure provides a good estimate of how accurately the calibration may be expected to work with an independent sample set. Root Mean Squared Error of Cross Validation was estimated as follows:

$$\mathrm{RMSECV} = \sqrt{\frac{\sum_{s=1}^{S}\sum_{i=1}^{n}(y_{is} - x_{is})^2}{N}}$$

where S is the number of segments, n is the number of samples in a given segment, N is the number of samples, y_{is} is the reference value for sample i and segment s, and x_{is} is the predicted value for sample i and segment s.

Bias gives an indication of a systematic error in the predictive values, and it was calculated as the average of the residuals (difference between the reference values and the predicted values)

$$\mathrm{Bias} = \frac{\sum_{i=1}^{N}(x_i - y_i)}{N}$$

where N is the number of samples, x_i is the predicted value for sample i, andy_i is the reference value for sample i.

Once each basic calibration was generated, a fine-tuning of it was carried out by a slope and intercept adjustment of the initial calibration. A full slope and intercept, or solely a slope or an intercept adjustment, was performed following the recommendation of the software of the Wine Scan FT 120 according to the range of reference data. The objective was to achieve the lowest standard error of calibration after adjustment (SEC), which indicates the accuracy with which the reference value can be predicted for the calibration sample using the proposed adjustment. SEC was calculated as

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{N}(y_i - x_i)^2}{N}}$$

where N is the number of samples, y_i is the reference value for sample i, and x_i is the predicted value for sample i.

2.5. Validation of the FTIR method for determination of anthocyanins

To test the predictive accuracy of the calibration models, the validation of the methods was carried out using an independent validation sample set. The correlation between the HPLC reference and FTIR methods was studied following the Resolution OENO 6/99 (1999) proposed by the Office International de la Vigne et du Vin (OIV). The slope (b), intercept, regression coefficient (r^2) and bias of data obtained by reference and FTIR methods were calculated. It is clear that if each sample yields an identical result with both analytical methods the regression line will have a 0 intercept, and a slope and a regression coefficient of 1. Deviation from the "ideal" situation was evaluated by analyzing slope and mean bias for the values obtained by the two methods. Thus, the slope was checked for significant difference from 1 ($\alpha = 0.05$) by studying confidence intervals

$$b - \left(t_{1-\alpha/2} \times s_b\right) < 1 < b + \left(t_{1-\alpha/2} \times s_b\right)$$

where s_b is the standard deviation of the slope.

At the same time, the bias of data obtained by reference and FTIR methods was checked for significant difference from 0 ($\alpha = 0.05$) by studying confidence intervals

$$\mathrm{bias} - \left(t_{1-\alpha/2} \times \frac{s_d}{\sqrt{q}}\right) < 0 < \mathrm{bias} + \left(t_{1-\alpha/2} \times \frac{s_d}{\sqrt{q}}\right)$$

where s_d is the standard deviation of difference between the two results and q the number of samples.

When slope or bias was differing significantly from 1 or 0, respectively, it indicated a systematic error.

Finally, in order to provide a graphical representation of systematic error, anthocyanin concentration values obtained by FTIR were plotted against those calculated from the calibration equation. The same graph shows the ideal situation, where the results for a single sample analysed by each of the two methods are exactly the same ($y = x$).

3. Results and discussion

The HPLC chromatogram (520 nm) of a Cencibel wine is shown in Fig. 1. Peaks 1–5 were identified as 3-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin.

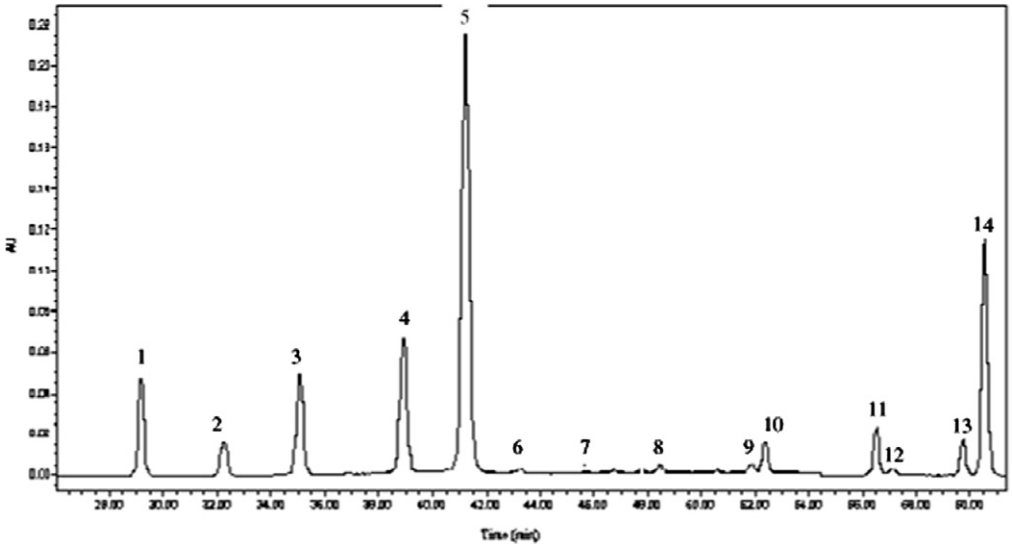

Fig. 1. HPLC chromatogram at 520 nm of cencibel wine. 1: delphinidin-3-glucoside; 2: cyanidin-3-glucoside; 3: petunidin-3-glucoside; 4: peonidin-3-glucoside; 5: malvidin-3-glucoside; 6: delphinidin-3-glucoside-acetate; 7: cyanidin-3-glucoside-acetate; 8: petunidin-3-glucoside-acetate; 9: peonidin-3-glucoside-acetate; 10: malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate; 11: cyanidin-3-glucoside-*p*-coumarate; 12: petunidin-3-glucoside-*p*-coumarate; 13: peonidin-3-glucoside-*p*-coumarate; 14: malvidin-3-glucoside-*p*-coumarate.

Peaks 6–9 corresponded to acetic acid esters of 3-glucosides of delphinidin, cyanidin, petunidin and peonidin. A further peak 10 included malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate. Peaks 11–14 were identified as *p*-coumaric acid esters of 3-glucosides of cyanidin, petunidin, peonidin and malvidin. The most abundant anthocyanin in all samples was malvidin-3-glucoside (peak 5), and concentrations of unesterified anthocyanins were in all cases higher than their acylated derivatives.

Table 2 shows the limits of detection and quantification, repeatability and intralaboratory reproducibility for determinations of anthocyanins by HPLC. The lowest detection and quantification limits were recorded for the 3-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin and their acetic acid esters (< 0.01 and < 0.05 mg/L, respectively). Although detection limits for *p*-coumaric acid esters were slightly higher, they were in all cases below 0.03 mg/L, while quantification limits did not exceed 0.10 mg/L. With regard to repeatability, RSD values for all anthocyanins were below 6.6%, indicating good repeatability, with the exception of cyanidin-3-glucoside (14.6%). In addition, cyanidin-3-glucoside displayed a high SEL value considering its mean concentration in the calibration set samples, 1.58 mg/L (Table 1). Reproducibility was considered good, with RSD values of below 10.1% in all cases, and slightly higher than those obtained for repeatability, as was to be expected.

Table 3 shows statistical results for the initial calibration and for the calibration obtained after bias and/or slope adjustment to better fit the data. The number of samples for initial calibration after removal of outliers was between 320 and 350. The lowest number of factors selected during PLS calibration ranged from 13 to 18, and the lowest SECV values ranged from 0.33 mg/L (petunidin-3-glucoside-*p*-coumarate) to 34.31 mg/L (total anthocyanins). SECV values reflected the concentration of each anthocyanin in the calibration set (Table 1), i.e. the greatest concentration of anthocyanins displayed the highest SECV values, and the least concentration displayed the lowest values. SECV confirmed the accurate predictive ability of the calibration model relative to reference data. Once basic calibrations for each anthocyanin had been generated, slope and/or intercept adjustments of these were made in order to achieve the lowest SEC, indicating the accuracy with which the reference value can be predicted for the calibration sample using the proposed adjustment. Calibration adjustments were made with roughly 20% of the initial samples. As expected, calibration error was lower post-adjustment in all cases, with a decrease ranging from 4% for malvidin- and peonidin-3-glucoside-*p*-coumarate and total *p*-coumarates to 54% for delphinidin-3-glucoside-acetate. Values for r^2 ranged between 0.706 and 0.931, while slope values ranged from 0.86 to 1.45, except for delphinidin-3-glucoside-acetate (0.5). It was not possible to compare statistical calibration parameters with the results obtained by other authors, since there are no previous published studies of anthocyanin determination using FTIR spectroscopy. However, for anthocyanin-related analytes such as total polyphenols, expressed in terms of the Folin–Ciocalteu index or total polyphenol index, conflicting results are reported. Patz et al. (1999) measured total phenols using the Folin-Ciocalteu reagent as reference method, and found calibration for total phenol to be unacceptable in terms of bias, slope and intercept. By contrast, Urbano-Cuadrado et al. (2004) obtained a good degree of precision in the calibration of the total phenol index. Versari, Boulton, and Thorngate (2004) attained an acceptable regression and validation for total free anthocyanins, copigmented anthocyanins, the polymeric pigment fraction and total color at pH 3.6 using 20 young red wines. Equally

Table 2
Limits of detection and quantification, precision and standard error laboratory (SEL) of the HPLC method for anthocyanins determinations

	Detection limit (mg/L)	Quantification limit (mg/L)	Repeatability (RSD, %)	Intralaboratory reproducibility (RSD, %)	SEL (mg/L)
Delphinidin-3-glucoside	0.01	0.05	0.52	2.69	0.98
Canidin-3-glucoside	0.01	0.03	14.57	3.99	1.01
Petunidin-3-glucoside	0.01	0.04	1.32	3.97	1.32
Peonidin-3-glucoside	0.01	0.02	4.37	7.13	1.74
Malvidin-3-glucoside	0.01	0.04	0.54	1.28	5.45
Delphinidin-3-glucoside-acetate	0.01	0.03	2.60	7.85	0.19
Canidin-3-glucoside-acetate	0.01	0.03	6.59	10.07	0.22
Petunidin-3-glucoside-acetate	0.01	0.03	1.27	4.71	0.17
Peonidin-3-glucoside-acetate	0.01	0.03	1.77	4.89	0.49
Malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate	0.01	0.04	2.02	1.19	1.47
Cyanidin-3-glucoside-*p*-coumarate	0.02	0.07	0.52	4.56	0.16
Petunidin-3-glucoside-*p*-coumarate	0.03	0.10	2.22	5.99	0.07
Peonidin-3-glucoside-*p*-coumarate	0.01	0.04	3.32	2.83	1.03
Malvidin-3-glucoside-*p*-coumarate	0.02	0.06	0.89	0.96	0.97

RSD: relative standard deviation.

Table 3
Summary of statistical characteristic of the initial calibration and after slope and bias correction

	Initial calibration				Calibration after adjustment				
	No. of samples	Factors	SECV (mg/L)	Bias (mg/L)	No. of samples	Slope	Intercept	r^2	SEC (mg/L)
Delphinidin-3-glucoside	332	17	5.12	−2.83	71	1.42	−4.02	0.834	4.27
Cyanidin-3-glucoside	331	13	0.66	−0.20	68	0.93	0.28	0.768	0.34
Petunidin-3-glucoside	320	13	5.18	−1.99	80	1.45	−7.70	0.884	4.81
Peonidin-3-glucoside	331	18	9.32	−1.18	76	1.09	−0.90	0.931	7.41
Malvidin-3-glucoside	336	17	22.28	1.16	73	1.10	−16.51	0.816	19.72
Delphinidin-3-glucoside-acetate	336	13	0.81	0.21	58	0.50	1.04	0.844	0.37
Cyanidin-3-glucoside-acetate	350	16	0.53	−0.04	80	1.24	−0.18	0.903	0.30
Petunidin-3-glucoside-acetate	346	18	0.80	0.09	65	1.06	−0.21	0.725	0.51
Peonidin-3-glucoside-acetate	341	11	1.12	0.03	67	1.24	−0.65	0.865	0.75
Malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate	334	19	8.25	0.19	82	0.93	1.03	0.834	4.98
Total acetates	339	17	9.79	−0.81	81	1.09	−1.57	0.863	6.89
Cyanidin-3-glucoside-*p*-coumarate	351	17	0.62	−0.02	68	1.13	−0.19	0.840	0.39
Petunidin-3-glucoside-*p*-coumarate	344	14	0.33	−0.08	56	0.86	0.19	0.812	0.15
Peonidin-3-glucoside-*p*-coumarate	333	15	1.50	0.36	77	1.04	−0.53	0.860	1.44
Malvidin-3-glucoside-*p*-coumarate	337	17	3.40	−0.06	77	0.89	1.94	0.706	3.26
Total *p*-coumarates	342	18	4.47	0.56	76	1.09	−2.77	0.805	4.29
Total anthocyanins	323	15	34.31	8.55	70	1.38	−10.87	0.929	23.79

satisfactory results have been reported for the calibration of other compounds found in higher concentrations in wine: alcoholic degree, total acidity, pH, volatile acidity, glycerol, reducing sugars, fructose, glucose, lactic acid, malic acid, tartaric acid, gluconic acid and citric acid (Dubernet & Dubernet, 2000; Gishen & Holdstock, 2000; Kupina & Shrikhande, 2003; Nieuwoudt, Prior, Pretorius, Manley, & Bauer, 2004; Patz et al., 1999; Ruzicka & Marshall, 1990; Schindler et al., 1998).

Calibration models were tested using a validation set comprising samples not used for calibration. Table 4 shows the number of samples used for validation, the determination coefficient (r^2), slope, intercept and bias. Forty representative samples were used, with anthocyanin concentrations similar to those used for calibration. The ranges of non-significance for slope and bias are also shown in Table 4. This study was performed at a significance level of 0.05, following the criteria proposed in Resolution OENO 6/99 (1999). Slope or bias differed significantly from 1 or 0, respectively, indicating a systematic error. Delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside-acetate and total acetates yielded values within these limits, while values for the remaining anthocyanins lay beyond the limits, and are marked in the table with an asterisk. The *p*-coumaric acid esters of cyanidin, petunidin and malvidin are not shown in Table 4, since r^2 values were <0.5. Fig. 2 plots the values obtained for anthocyanin levels using the FTIR (*x*-axis) against those obtained from the validation equation (*y*-axis). The same graph shows the ideal situation, where the results analysed for a single sample by each of the two methods (HPLC and FTIR) are exactly the same ($y = x$). No systematic error was observed in the determination of delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside-acetate and total acetates; Fig. 2a shows delphinidin-3-glucoside as an example. By contrast, systematic error was observed in the determination of cyanidin-, peonidin- and malvidin-3-glucoside, delphinidin-, cyanidin-, petunidin- and peonidin-3-glucoside-acetate, peonidin-3-glucoside-*p*-coumarate, total *p*-coumarates and total anthocyanins. Fig. 2b shows values for cyanidin-3-glucoside-acetate as an example where the slope did not differ significantly from 1 but bias differed significantly from 0. Finally, Fig. 2c shows the validation plot for petunidin-3-glucoside acetate, as an example in which slope and bias differed significantly from 1 and 0, respectively. In all cases where systematic error was observed, the values predicted by the regression line based on validation equations were lower than those obtained by the initial calibration, with slope values significantly lower than 1 and bias significantly higher than 0, taking into account that bias is calculated as the difference between FTIR and HPLC values. Thus, the values were systematically higher as predicted by FTIR than by HPLC. This systematic error can therefore be corrected in the agrifood laboratory by applying a correction factor, consequently values for anthocyanin concentrations could be calculated from calibration curves taking into account the correction factor.

Table 4
Summary of statistical characteristic of the validation of methods for determination of anthocyanins

	No. of samples	r^2	Intercept	Slope	Non-significance range of slope	Bias	Non-significance range of bias
Delphinidin-3-glucoside	40	0.858	0.81	0.933	0.746–1.120	−0.178	−0.877–0.521
Cyanidin-3-glucoside	40	0.653	−0.29	0.821	0.271–1.372	0.538[a]	0.352–0.724
Petunidin-3-glucoside	40	0.762	1.61	0.859	0.622–1.096	0.076	−0.847–0.999
Peonidin-3-glucoside	40	0.889	−16.83	0.842[a]	0.695–0.989	22.750[a]	20.715–24.785
Malvidin-3-glucoside	40	0.880	−27.79	1.007	0.822–1.192	26.972[a]	21.291–32.653
Delphinidin-3-glucoside-acetate	40	0.643	−0.88	0.917	0.406–1.427	1.032[a]	0.876–1.188
Cyanidin-3-glucoside-acetate	40	0.920	−0.22	0.978	0.835–1.121	0.240[a]	0.176–0.304
Petunidin-3-glucoside-acetate	40	0.613	0.34	0.524[a]	0.306–0.742	0.266[a]	0.038–0.494
Peonidin-3-glucoside-acetate	40	0.923	−1.17	0.991	0.850–1.132	1.195[a]	0.994–1.396
Malvidin-3-glucoside-acetate and delphinidin-3-glucoside-*p*-coumarate	40	0.940	0.04	0.975	0.853–1.097	0.229	−0.800–1.258
Total acetates	40	0.936	1.63	0.894	0.778–1.010	−0.196	−1.733–1.341
Peonidin-3-glucoside-*p*-coumarate	40	0.741	−1.26	0.933	0.746–1.120	1.51[a]	1.044–1.984
Total *p*-coumarates	40	0.829	0.15	0.785[a]	0.615–0.955	4.326[a]	3.114–5.538
Total anthocyanins	40	0.920	−12.50	0.789[a]	0.673–0.905	61.005[a]	49.049–72.961

[a] Slope or bias inside the range of non-significance.

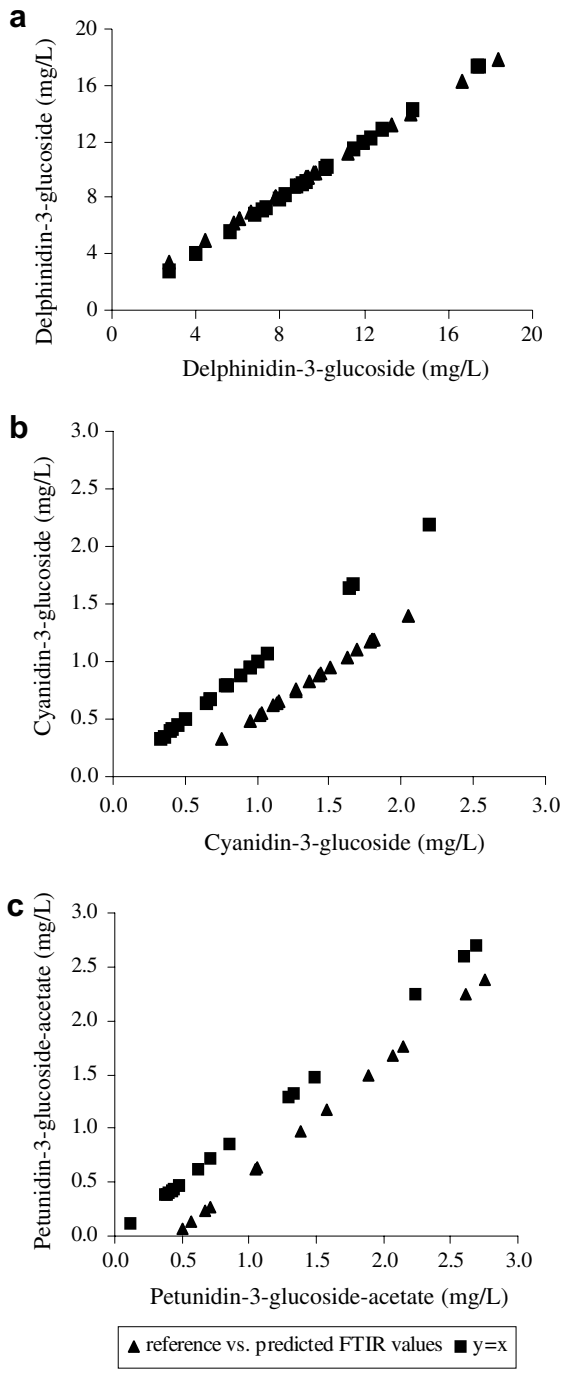

Fig. 2. Values for three anthocyanins concentrations of validation set and the "ideal" situation, where values obtained by HPLC exactly match those obtained by FTIR ($y = x$).

4. Conclusions

The WineScan FT 120 analyzer is suitable for measuring anthocyanin levels in wine. However, and as expected, the statistical parameters obtained in the calibration of anthocyanins are less good than those reported for components present at greater concentrations. In any event, the results obtained suggest that values for statistical parameters could shortly be enhanced by including new wine samples. It should be stressed that this method provides additional information on wine colour as well as other wine-making parameters routinely determined in the agrifood laboratory.

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References

Cacho, J., Fernández, P., Ferreira, V., & Castells, J. E. (1992). Evolution of five anthocyanidin-3-glucosides in the skin of Tempranillo, Moristel, and Garnacha grape varieties and influence of climatological variables. *American Journal of Enology and Viticulture, 43*, 244–248.

Cantos, E., Espín, J. C., & Tomás-Barberán, F. A. (2002). Varietals differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD–MS–MS. *Journal of Agricultural and Food Chemistry, 50*, 5691–5696.

Cheynier, V., Remi, S., & Fulcrand, H. (2000). Mechanisms of anthocyanin and tannin changes during winemaking and aging. *In Proceedings of the ASEV 50th anniversary annual meeting* (pp. 337–344). Seattle, Washington.

Dubernet, M., & Dubernet, M. (2000). Utilisation de l'analyse infrarouge a transformee de Fourier pour l'analyse œnologique de routine. *Revue Française d'Œnologie, 181*, 10–13.

Gishen, M., Dambergs, R.G., Kambouris, A., Kwiatkowski, M., Cynkar, W.U., Høj, P.B., and Francis, I.L. (1999). Application of near infrared spectroscopy for quality assessment of grapes, wine and spirits. *In Proceedings of 9th International Conference on Near Infrared Spectroscopy* (pp. 917–920), Verona, Italy.

Gishen, M., & Holdstock, M. (2000). Preliminary evaluation of the performance of the Foss WineScan FT 120 instrument for the simultaneous determination of several wine analyses. *Australian Grapegrower Winemaker, Annual Technical Issue*, 75–81.

González-San José, M. L., Barron, L., & Díez, C. (1990). Evolution of anthocyanins during maturation of tempranillo grape variety (*Vitis vinifera*) using polynomial regression models. *Journal of the Science and Food Agriculture, 51*, 337–343.

Kjaer, S., Waaben, P., & Villemoes, H. (2002). Vibrational Spectroscopy in the analysis of dairy products and wine. In J. M. Chalmes & P. R. Griffiths (Eds.), *Handbook of vibrational spectroscopy* (pp. 1–10). Chichester: Willey and Sons.

Kupina, A. K., & Shrikhande, A. (2003). Evaluation of a Fourier transform infrared instrument for rapid quality-control wine analyses. *American Journal of Enology and Viticulture, 54*, 131–134.

Lamuela-Raventós, R. M., & Waterhouse, A. L. (1994). A direct HPLC separation of wine phenolics. *American Journal of Enology and Viticulture, 45*, 1–5.

Luque de Castro, M. D., González-Rodríguez, J., & Pérez-Juan, P. (2005). Analytical methods in wineries: Is it time to change?. *Food Reviews International 21*, 231–265.

Nieuwoudt, H. H., Prior, B. A., Pretorius, I. S., Manley, M., & Bauer, F. F. (2004). Principal component analysis applied to Fourier transform infrared spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier simples. *Journal of Agricultural and Food Chemistry, 52*, 3726–3735.

Patz, C. D., David, A., Thente, K., Kürbel, P., & Dietrich, H. (1999). Wine analysis with FTIR spectrometry. *Viticultural and Enological Sciences, 54*, 80–87.

Resolution OENO 6/99. Validation protocol for a typical analytical method compared to the OIV reference method. Office International de la Vigne te du Vin. http://www.oiv.int/database/images/client/oeno699.uk.doc.

Ruzicka, J., & Marshall, G. D. (1990). Sequential injection: A new concept for chemical sensors, process analysis and laboratory assays. *Analytica Chimica Acta, 237*, 329–343.

Ruzicka, J., Marshall, G. D., & Christian, G. D. (1990). Variable flow rates and sinusoidal flow pump for flow injection analysis. *Analytical Chemistry, 62*, 1861–1866.

Schindler, R., Vonach, R., Lendl, B., & Kellner, R. (1998). A rapid automated method for wine analysis based upon sequential injection (SI)-FTIR spectrometry. *Fresenius' Journal of Analytical Chemistry, 362*, 130–136.

Smith, B. (1999). *Infrared spectral interpretation: A systematic approach* (1st ed.). Boca Raton: CRC Press LLC.

Soriano, A., González, J. M., Delgado, J. A., & Sánchez-Migallón, V. (2002). Método rápido para el análisis simultáneo de los compuestos volátiles mayoritarios y del ácido cítrico del vino mediante espectroscopia infrarroja. *Enólogos, 19*, 30–33.

Urbano-Cuadrado, M., Luque de Castro, M. D., Pérez-Juan, P. M., García-Olmo, J., & Gómez-Nieto, M. A. (2004). Near infrared reflectance spectroscopy and multivariate analysis in enology. Determination or screening of fifteen parameters in different types of wines. *Analytica Chimica Acta, 527*, 81–88.

Vaadia, M. (1997). Copigmentation in varietal wines from Napa Valley. *Master of Science Thesis*. University of California, Davis.

Versari, A., Boulton, R., & Thorngate, J. (2004). The prediction of the color components of red wines using FTIR, wine analyses, and the method of partial least squares. *ACS Symposium Series, 886*, 53–67.

Yokotsuka, K., Nagao, A., Nakazawa, K., & Sato, M. (1999). Changes in anthocyanins in berry skins of merlot and Cabernet Sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *American Journal of Enology and Viticulture, 50*, 1–12.