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UNIVERSITÀ
DEGLI STUDI
FIRENZE

DIPARTIMENTO
DI CHIMICA
"UGO SCHIFF"

Evaluation of the correlation between the results obtained with CDR WineLab® and with the official methods

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1 INTRODUCTION

1.1 Purpose

Evaluation of the correlation between the results obtained with CDR WineLab® and the official methods provided by the OIV (International Organisation of Vine and Wine) on the following parameters:

- Acetic acid
- Total acidity
- L-Malic Acid
- L-Lactic Acid
- Alcohol
- Total SO_2
- Free SO_2
- Sugars
- IPT (Total Polyphenols Index)

The Pearson correlation coefficient was used to verify the presence of a correlation between the data (R^2).

1.2 CDR WineLab® analysis system

The CDR WineLab® analysis system consists of an analyser based on photometric technology, a dedicated pipette and **disposable pre-filled reagents**, specially developed by the CDR research laboratories.

CDR WineLab® is part of the CDR FoodLab® line of analysis systems which is used to determine numerous chemical parameters in a variety of food products.

1.2.1 Analyser

The analyser is equipped with reading cells including incubation systems thermostated at 37°C and fixed wavelength LED emitters with a power that makes it possible to read absorbances up to 6 O.D.

The instrument is used to perform several analyses simultaneously on the same sample or to analyse the same parameter on 16 different samples in parallel. The analyser provided pre-calibrated does not require further calibration.

Characteristics of the system used for this study:

- CDR WineLab®: no. 671
- Production year: 2019

1.2.2 Pipette

Together with the CDR WineLab® analysis system, a 10-100 µL pipette and a 50 µL pipette are supplied with which it is possible to extract the volumes required in all the analyses performed.

1.2.3 Reagents

The reagents used in this study are supplied by CDR in ready-to-use 10-test kits.
Inside each kit, specific for a particular analysis, there are 10 disposable tubes containing the **pre-filled reagent and any reagents to be added to the tube for the specific application.**

1.3 Samples

The comparison of the results obtained with the CDR WineLab® analysis system and the reference method was performed on 22 samples of commercial wine from various origins supplied by the company CDR.
The sample white, red and rosé wines analysed were chosen in order to represent the variety of vines grown in Italy.

	Type of wine	Origin	Colour
Sample 1	Chardonnay	Sicily	White
Sample 2	Viognier	Sicily	White
Sample 3	Pinot Grigio	South Tyrol	White
Sample 4	Müller Thurgau	Trentino	White
Sample 5	Pinot Bianco	Italy	White
Sample 6	Tavernello	Italy	White
Sample 7	Vermentino	Sardinia	White
Sample 8	Trebbiano del Rubicone	Emilia Romagna	White
Sample 9	Lagrein	South Tyrol	Rosé
Sample 10	Negroamaro	Salento	Rosé
Sample 11	Tavernello	Italy	Red
Sample 12	Montecucco	Tuscany	Red
Sample 13	Cannonau	Sardinia	Red
Sample 14	Chianti	Tuscany	Red
Sample 15	Merlot	Sicily	Red
Sample 16	Dolcetto D'Alba	Piedmont	Red
Sample 17	Cabernet	Veneto	Red
Sample 18	Syrah	Sicily	Red
Sample 19	BIO V132	Tuscany	Red
Sample 20	BIO S21	Tuscany	Red
Sample 21	Vermentino	Tuscany	White
Sample 22	Trebbiano	Tuscany	White

Table 1.1: Characteristics of the wine samples analysed.

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2 DETERMINATION OF ACETIC ACID

2.1. Acetic acid in wine

The quantity of acetic acid present in the wine, expressed in g/L, is defined volatile acidity and constitutes a chemical parameter to be carefully monitored throughout the wine-making process as its concentration indicates the health of the grape, how the fermentation is proceeding and the state of preservation of the product. Its content is therefore linked to the quality of the wine.

An excess of volatile acidity, detected at tasting, is enough to make a wine judge negatively and is therefore a parameter that is subject to the maximum limits established by law. In particular, the limits set by Reg. EU 1493/99, unless in the case of exceptions for some products (for wines subjected to long ageing in barrels and for liqueur wines from botrytised grapes), are 1.08 g/L of acetic acid for white and rosé wines and 1, 2 g/L for red wines.

Acetic acid can be formed in small quantities, as a by-product of alcoholic and malolactic fermentation, but in these cases the volatile acidity remains below 0.7 g/L, a concentration that does not interfere with the taste.

Instead a greater increase in acetic acid than wine is often the work of bacteria, *Acetobacter, which cause the so-called "acetic spike"*. The formation of volatile acidity occurs in an oxidising environment in which, through the use of atmospheric oxygen, these aerobic acetic bacteria transform ethyl alcohol into acetic acid and water. The starting point is the initial phase of the acescence, a very serious disease that makes the wine unsuitable for consumption.

Acetic bacteria are present everywhere: on grapes, in wineries and cellars, on walls, in the soil and inside the wood of empty containers. Even limiting contamination as much as possible, wine, especially if it is not sulphited, contains a certain number. It is therefore essential to place the wines in such conditions that the development of bacteria is minimised

*T*he use of unselected yeasts also increases the possibility of considerable percentages of volatile acidity, especially if combined with grapes with low levels of yeasts and nitrogenous substances. In fact, the combination of these conditions increases the risk of non-starts or stops of fermentation which could cause an increase in volatile acidity. *During the* ***fermentation stops****, whenever the yeast activity ceases before the total consumption of sugars, the anaerobic lactic bacteria are activated and can use these sugars in their metabolism to produce acetic acid (lactic acid).*

Acetic acid can also be found in the must if the grapes are not *in an optimal state of health. The presence of lactic bacteria and yeasts is favoured by particular conditions such as acid rot and by attacks of parasites which, tearing the grape skin, promote their development. Due to these alterations, the unwanted beginning of the fermentation of sugars can occur with the formation of acetic acid.*

For all these reasons the determination of acetic acid is frequently carried out in the winery: before racking, before each transfer and before bottling.

The volatile acidity is determined on a wine distillate obtained by steam distillation. This distillate is then titrated with 0.1 N NaOH using phenolphthalein as an indicator as required by the OIV-MA-AS313-02 method.

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Alternatively HPLC can be used in the simultaneous determination of organic acids according to the method OIV-MA-AS313-04 and also allows the quantification of acetic acid.

2.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of the analyses of 22 wine samples (*Table 1.1*) obtained with CDR WineLab® and those obtained with the HPLC (High Performance Liquid Chromatography) method as required by the reference method OIV-MA-AS313-04.

	Acetic Acid (g/L)	
	CDR WineLab ®	Reference
Sample 1	0.29	0.22
Sample 2	0.23	0.16
Sample 3	0.28	0.24
Sample 4	0.15	0.16
Sample 5	0.4	0.38
Sample 6	0.15	0.17
Sample 7	0.45	0.47
Sample 8	0.20	0.16
Sample 9	0.22	0.20
Sample 10	0.28	0.26
Sample 11	0.49	0.42
Sample 12	0.49	0.48
Sample 13	0.38	0.39
Sample 14	0.39	0.37
Sample 15	0.71	0.72
Sample 16	0.46	0.43
Sample 17	0.44	0.41
Sample 18	0.68	0.69
Sample 19	0.39	0.36
Sample 20	0.33	0.28
Sample 21	0.24	0.25
Sample 22	0.15	0.15

Table 2.1: Acetic acid results obtained with CDR WineLab® and with the reference method.

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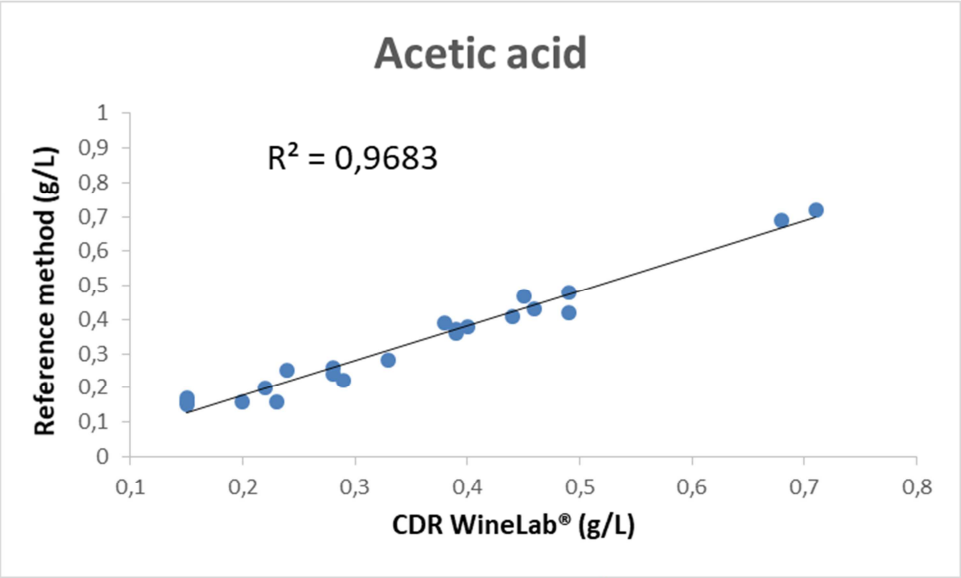

Figure 2.1: Correlation between CDR WineLab® and reference method

The two methods are correlated (R^2= 0.9683).

2.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the CDR WineLab® method were evaluated by analysing two different TITRIVIN solutions, certified reference solutions for oenology laboratories.
In particular, TITRIVIN AA1 (batch number A 03171222 1) were chosen whose acetic acid value declared by the manufacturer is 0.28±0.04 g/L (the uncertainty is expressed with a coverage factor k=2) and TITRIVIN AA4 (batch number A 03171222 4) whose acetic acid value is 0.72±0.05 g/L. The choice of the two standards was made in such a way as to test the repeatability of the method at both low and high values of acetic acid. For each standard, 5 consecutive analyses were performed, repeating the test for 5 different days.
Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	0.32	0.31	0.32	0.32	0.31
	0.33	0.30	0.32	0.33	0.33
	0.32	0.32	0.30	0.33	0.30
	0.31	0.32	0.32	0.32	0.30
	0.31	0.31	0.32	0.31	0.31
Average	0.32	0.31	0.32	0.32	0.31
Standard deviation	0.01	0.01	0.01	0.01	0.01

Table 2.2: Acetic acid results obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.30	0.33	0.32	0.01

Table 2.3: Reproducibility of the acetic acid measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVIN AA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	0.73	0.75	0.73	0.73	0.73
	0.72	0.75	0.75	0.75	0.75
	0.74	0.74	0.74	0.74	0.70
	0.72	0.73	0.76	0.75	0.73
	0.72	0.74	0.72	0.72	0.70
Average	0.73	0.74	0.74	0.74	0.72
Standard deviation	0.01	0.01	0.02	0.01	0.02

Table 2.4: Acetic acid results obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.70	0.76	0.73	0.02

Table 2.5: Reproducibility of the acetic acid measurement obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

The average acetic acid value measured in TITRIVIN AA1 is 0.32 g/L±0.02 g/L and 0.73 g/L ±0.04 g/L in TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed as uncertainty extended to a 95% confidence range with coverage factor k=2. The CDR WineLab® system provides a value in accordance with that of the standard and demonstrates good repeatability and reproducibility in the determination of acetic acid.

3 DETERMINATION OF TOTAL ACIDITY

3.1 Total acidity in win

Total acidity includes all the fixed acids (tartaric, malic, succinic, lactic, citric) and volatile acids (acetic acid but also minimal quantities of propionic, butyric, formic acids) present in the musts or wines; acidities derived from CO_2 and SO_2 are not included.
Acid substances are naturally formed during the ripening of the grapes and during the fermentation processes. In the correct proportions they are essential to give the wine a distinct character in terms of taste, but also to guarantee its preservation over time as acidity influences microbiological and oxidative stability.
The acidity gives freshness to the wine, influences its colour, aroma and, in balance with the sweet and dry flavours of the other components, contributes to the taste of the final product; overly high acidity makes the wine sour, if too low it makes it flat and tasteless.
The total acid content varies over time due to the state of natural instability of the wine and there is

no link between the acidity of the must and the acidity of the wine as in the step from must to wine different acids will be consumed and produced due to the microbiological activity of yeast and bacteria.
The analysis of total acidity is therefore useful for checking the correct progress of fermentation, preventing and/or verifying the onset of alterations and evaluating the correct functioning of all stages of wine production in order to make any adjustments.
For these reasons, in the production process of a wine, total acidity is one of the most important and frequent oenological analyses.
As tartaric acid is generally present to a greater extent, total acidity is conventionally expressed in g/L of tartaric acid and is usually determined by manual titration, with a strong base (NaOH 1 N) using bromothymol blue as an indicator.

3.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*) obtained through the analyses carried out with CDR WineLab® and those obtained with the reference method OIV-MA-AS313-01 R2015 par 5.2.

	Total acidity (g/L of tartaric acid)	
	CDR WineLab ®	Reference
Sample 1	5.5	5.5
Sample 2	5.8	5.5
Sample 3	4.9	4.8
Sample 4	5.6	5.3
Sample 5	4.7	4.7
Sample 6	5.6	5.4
Sample 7	4.6	4.8
Sample 8	5.0	5.2
Sample 9	5.4	5.2
Sample 10	5.5	5.4
Sample 11	5.3	5.1
Sample 12	5.7	5.5
Sample 13	5.5	5.3
Sample 14	5.0	5.0
Sample 15	6.0	5.9
Sample 16	4.9	4.7
Sample 17	4.6	4.9
Sample 18	6.0	6.1
Sample 19	4.9	4.9
Sample 20	4.5	4.4
Sample 21	4.6	4.6
Sample 22	6.5	6.2

Table 3.1: Results of the total acidity measurement obtained with CDR WineLab® and with the reference method.

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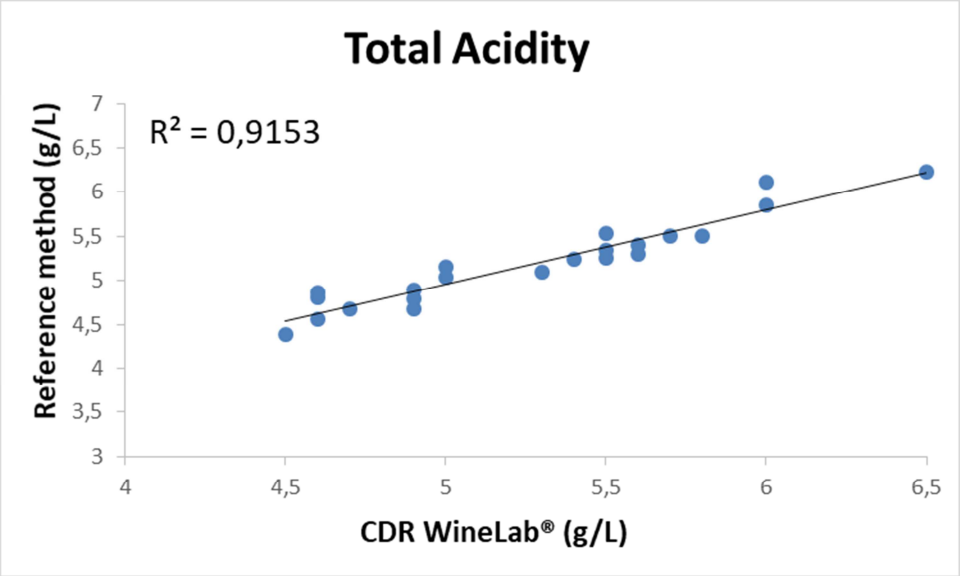

Figure 3.1: Correlation between CDR WineLab® and reference method

The two methods demonstrate a correlation coefficient R^2= 0.9153. However, this non-optimal correlation is the result of a distribution of the values measured on the 22 samples, in a narrow range (all the samples appear to have values of between 4.5 and 6.5 g/L of tartaric acid even if the equivalent content of tartaric acid in a wine is between 2 and 9 g/l.). The Pearson correlation coefficient is a not particularly reliable index whose value can change significantly based on a few extreme values and the low dispersion of the samples on the total acidity scale negatively affects the correlation estimate.

3.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the CDR WineLab® method are evaluated by analysing two different certified reference solutions: TITRIVIN AA1 (batch number A 03171222 1) whose acidity value declared by the manufacturer is 4.01±0.25 g/L and TITRIVIN AA4 (batch number A 03171222 4) with a total acidity of 8.59±0.12 g/L. All the acidity values are reported as g/L of tartaric acid. The choice of the two standards was made in such a way as to test the repeatability of the method at both low and high values of total acidity. For each standard 5 consecutive analyses were performed, repeating the test for 5 consecutive days.

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Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	4.0	4.2	4.0	4.0	4.0
	3.9	4.3	4.0	3.9	4.0
	3.9	4.2	3.9	3.9	3.9
	4.1	3.9	3.8	3.9	4.2
	4.2	3.8	3.9	3.9	3.9
Average	4.0	4.1	3.9	3.9	4.0
Standard deviation	0.1	0.2	0.1	0.1	0.1

Table 3.2: Total acidity results obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	3.8	4.3	4.0	0.1

Table 3.3: Reproducibility of the total acidity measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVIN AA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	8.3	8.6	8.2	8.3	8.6
	8.3	8.3	8.2	8.3	8.5
	8.5	8.4	8.4	8.4	8.5
	8.5	8.3	8.4	8.3	8.4
	8.4	8.3	8.4	8.4	8.6
Average	8.4	8.4	8.3	8.3	8.5
Standard deviation	0.1	0.1	0.1	0.1	0.1

Table 3.4: Total acidity results obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	8.2	8.6	8.4	0.1

Table 3.5: Reproducibility of the total acidity measurement obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

The average acidity value measured in TITRIVIN AA1 is found to be 4.0 g/L±0.2 g/L and 8.4 g/L±0.2 g/L in TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed with a 95% confidence range (coverage factor k=2). A good repeatability and reproducibility of the method and the acidity values obtained with CDR WineLab® are observed and that agree with the values of the standards.

4 DETERMINATION OF L-MALIC ACID

4.1 Malic acid in wine

L-malic acid originates in the grape and its synthesis is linked to the weather conditions, the characteristics of the soil and those of the vine. Its concentration in the grape decreases rapidly and regularly from the moment of veraison and more slowly thereafter. In wine, L-malic acid maintains the concentration it had in grapes if the product does not undergo malolactic fermentation, while it decreases reaching concentrations of below 0.2 g/L if this fermentation occurs.
Malolactic fermentation is the process that naturally allows maintaining of the biological stability of a wine. Despite being called "fermentation", the degradation of L-malic acid is an enzymatic process by which this acid, aggressive and pungent, is converted into the more delicate L-lactic acid. This generally allows the obtaining of a softer and more balanced wine, with greater aromatic complexity and greater persistence. Essential to guarantee the biological stability of red wine, this process is usually avoided in white wine in order to keep the freshness and acidity of the product, even if in some white wines, characterised by ageing processes in barrique, it is still used to confer to the wine a remarkable complexity and a rich and buttery taste.
Malolactic fermentation occurs after alcoholic fermentation thanks to the action of certain lactic bacteria such as *Oenococcus Oeni* and *Lactobacillus*, which are naturally present in the must and which are reactivated in the presence of ideal conditions of pH (optimal between 4.2 and 4.5), temperature (18-20°), quantity of ethyl alcohol (not exceeding 15%) and sulphur dioxide (<5mg/L). However, the decrease in L-malic acid can occur in a variable percentage from 10 to 30% even during alcoholic fermentation with the mechanism of malo-alcoholic fermentation.
The determination of L-malic acid is therefore important for assessing the initial concentration present in wine, obtaining information on the previous fermentation process and during malolactic fermentation to follow its development.
The main chemical methods for the quantification of this acid are spectrophotometric enzymatic analysis and analysis for HPLC (High Performance Liquid Chromatography).

4.2 System precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*) obtained by performing the analyses with CDR WineLab® and with HPLC according to the reference method OIV-MA-AS313-16.

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	Malic Acid (g/L)	
	CDR WineLab ®	Reference
Sample 1	2.03	1.65
Sample 2	1.72	1.35
Sample 3	1.44	1.19
Sample 4	2.69	2.26
Sample 5	0.36	0.42
Sample 6	2.12	1.65
Sample 7	0.50	0.58
Sample 8	2.55	2.25
Sample 9	2.03	1.77
Sample 10	2.73	2.37
Sample 11	0.79	0.63
Sample 12	< 0.05	0.13
Sample 13	< 0.05	< 0.1
Sample 14	< 0.05	0.20
Sample 15	< 0.05	< 0.1
Sample 16	< 0.05	< 0.1
Sample 17	0.38	0.43
Sample 18	< 0.05	< 0.1
Sample 19	< 0.05	< 0.1
Sample 20	< 0.05	< 0.1
Sample 21	< 0.05	< 0.1
Sample 22	2.93	2.31

Table 4.1: L-malic acid results obtained with CDR WineLab® and with the reference method.

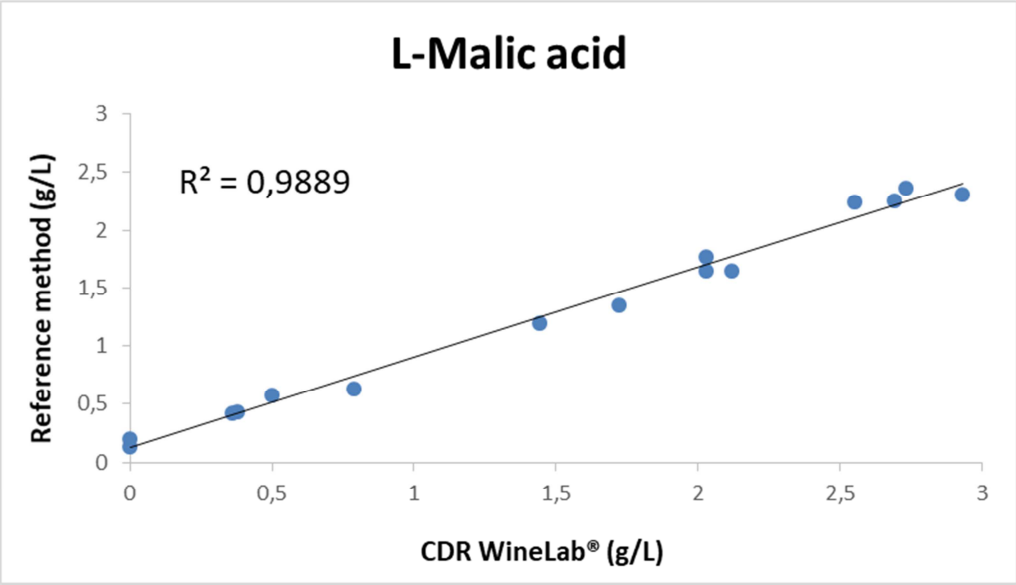

Figure 4.1: Correlation between CDR WineLab® and reference method

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The analyses were performed on all 22 samples but the samples that showed a concentration of L-malic acid lower than the Detection Limit of the reference method (LOQ= 0.1 g/L) and of the CDR WineLab® (LOQ= 0.05 g/L) instrument were not shown in the graph.
The two methods gave highly correlated results (R^2= 0.9889).

4.3 Evaluation of the repeatability and reproducibility of the system

The repeatability and reproducibility of the CDR WineLab® method are evaluated by analysing two different certified reference solutions: TITRIVIN AA1 (batch number A 03171222 1) which contains a quantity of L-malic acid equal to 0.24±0.06 g/L and TITRIVIN AA4 (batch number A 03171222 4) which has a concentration of 2.51±0.14 g/L.
The choice of the two standards was made in such a way as to test the repeatability of the method both at low and high concentrations of L-malic acid. For each standard, 5 consecutive analyses were performed, repeating the test for 5 days.
Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	0.18	0.17	0.17	0.17	0.20
	0.18	0.20	0.18	0.18	0.22
	0.20	0.20	0.20	0.20	0.17
	0.17	0.20	0.22	0.16	0.21
	0.18	0.18	0.18	0.18	0.18
Average	0.18	0.19	0.19	0.18	0.20
Standard deviation	0.01	0.01	0.02	0.01	0.02

Table 4.2: L-malic acid results obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.16	0.22	0.19	0.02

Table 4.3: Reproducibility of the L-malic acid measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVIN AA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	2.61	2.60	2.61	2.60	2.60
	2.62	2.56	2.53	2.62	2.62
	2.61	2.59	2.60	2.59	2.59
	2.59	2.52	2.51	2.51	2.53
	2.61	2.56	2.58	2.56	2.63
Average	2.61	2.57	2.57	2.58	2.59
Standard deviation	0.01	0.03	0.04	0.04	0.04

Table 4.4: L-malic acid results obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	2.51	2.63	2.58	0.04

Table 4.5: Reproducibility of the L-malic acid measurement obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

The mean value measured in TITRIVIN AA1 is found to be 0.19 g/L±0.04 g/L and 2.58 g/L±0.08 g/L in TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed with a 95% confidence range (coverage factor k=2). The low standard deviation indicates good repeatability and reproducibility of the method.
Furthermore CDR WineLab® provides concentrations of L-malic acid in accordance with those present in the standards.

5 DETERMINATION OF THE LACTIC ACID CONTENT

5.1 Lactic acid in wine

Within wine it is possible to find both isomers of lactic acid but it is important to differentiate them as the D(-)- lactic acid is produced by yeast, while the L(+)-lactic acid is obtained from the metabolism of lactic bacteria.
The small amounts found prior to malolactic fermentation of the L(+) isomer are produced during the initial stage of sugar fermentation, but later the yeast mainly produces the D(-) isomer. It is during the following malolactic fermentation that the lactic acid bacteria in wine transform the L(+)-malic acid exclusively into L(+)-lactic acid, which is more stable and has a more delicate taste; during this phase the concentration of the L(+) isomer increases even reaching concentrations of 5 g/L.
Quantifying lactic acid is essential during malolactic fermentation to monitor the process, but it is also important to measure the concentration present in the must and wine to assess the need of whether or not to add this acid to the product. Lactic acid is in fact added to correct the acidity of musts and wines as an alternative to tartaric acid. In particular it can be added up to a maximum of 2.25 g/l on musts and 3.75 g/l on wines with the aim of rebalancing natural acidity, improving

preservation, taste and promoting correct biological evolution.
The standard methods for the quantification of this acid are spectrophotometric enzymatic analysis and analysis for HPLC (High Performance Liquid Chromatography).
However, the HPLC analysis, in addition to determining the L-lactic acid, also detects its D(-) isomer, formed by the yeasts during alcoholic fermentation. Therefore, unlike the enzymatic method which allows direct quantification of the concentration of L(+)-lactic acid, the HPLC analysis does not allow knowing the actual concentration of L(+)-lactic acid, which is the only indicative parameter of the start of **malolactic fermentation.**

5.2 Method precision evaluation

The precision of the system developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 5.1*) obtained by performing the analyses with CDR WineLab® and with HPLC according to the reference method OIV-MA-AS313-16.

	Lactic Acid (g/L)	
	CDR WineLab ®	Reference
Sample 1	0.49	0.69
Sample 2	<0.05	0.12
Sample 3	0.38	0.46
Sample 4	0.07	0.15
Sample 5	0.98	1.03
Sample 6	0.57	0.71
Sample 7	1.12	0.75
Sample 8	0.27	0.37
Sample 9	0.06	0.20
Sample 10	0.11	<0.1
Sample 11	1.12	1.15
Sample 12	0.68	0.93
Sample 13	0.99	1.14
Sample 14	0.98	0.91
Sample 15	1.09	1.52
Sample 16	1.12	1.22
Sample 17	0.81	1.03
Sample 18	1.05	1.52
Sample 19	1.1	1.29
Sample 20	1.03	1.07
Sample 21	1.57	1.55
Sample 22	0.14	0.25

Table 5.1: Lactic acid results obtained with CDR WineLab® and with the reference method.

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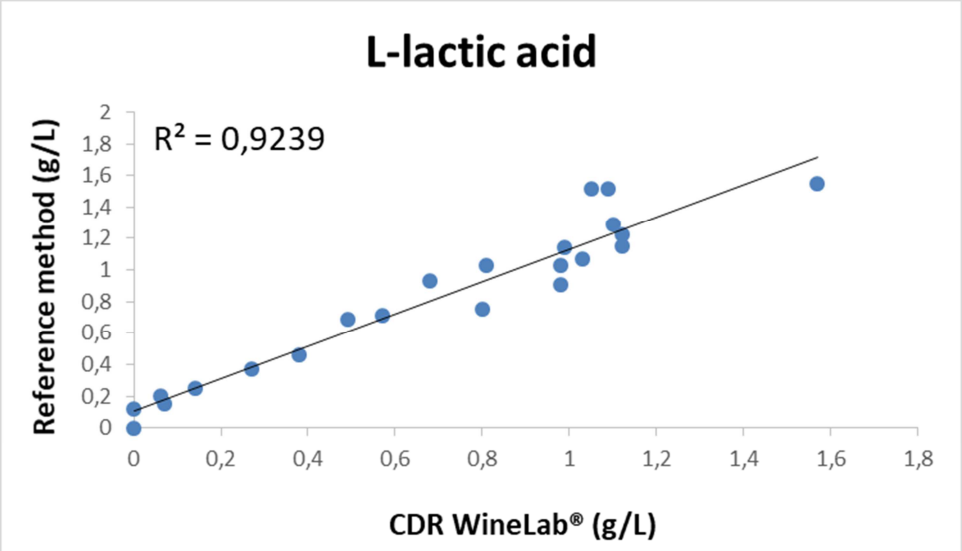

Figure 5.1: Correlation between CDR WineLab® and reference method

The correlation coefficient is not optimal (R^2 = 0.9239).
However, we must consider that the two methods are not perfectly comparable due to the fact that, as previously mentioned, the HPLC analysis detects both isomers of lactic acid unlike the enzymatic reaction exploited by CDR WineLab® with which only the L(+) isomer is quantified, which we remember as being the only indicative parameter of the start of **malolactic fermentation.**

To estimate to what extent this difference affects the result obtained, CDR has provided a further method for the determination of both isomers of lactic acid using CDR WineLab®. The test was performed on the two samples that showed the worst correlation with the results obtained by HPLC (*Table 5.2*). The values obtained show the presence in solution of a non-negligible quantity of the D isomer.

	Lactic acid (g/L)		
	Reference	CDR WineLab®	
	Isomer D+L	Isomer L	Isomer D+L
Sample 15	1.52	1.09	1.69
Sample 18	1.52	1.05	1.68

Table 5.2: Lactic acid results obtained with CDR WineLab® and with the reference method

5.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the CDR WineLab® method are evaluated by analysing two certified reference solutions: TITRIVIN AA1 (batch number A 03171222 1) which contains a quantity of L-lactic acid equal to 0.90±0.14 g/L and TITRIVIN AA4 (batch number A 03171222 4) which has a concentration of 2.91±0.22 g/L.
The choice of the two standards was made in such a way as to test the method at different concentrations of L-lactic acid. For each standard, 5 consecutive analyses were performed, repeating the test for 5 different days.

Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	0.95	0.94	0.94	0.94	0.93
	0.95	0.93	0.94	0.94	0.94
	0.96	0.93	0.95	0.95	0.95
	0.94	0.96	0.97	0.94	0.94
	0.95	0.94	0.93	0.94	0.95
Average	0.95	0.94	0.95	0.94	0.94
Standard deviation	0.01	0.01	0.02	0.01	0.01

Table 5.3: Lactic acid results obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.93	0.97	0.94	0.01

Table 5.4: Reproducibility of the L-lactic acid measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVIN AA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	3.07	3.10	3.10	3.10	3.08
	3.10	3.07	3.10	3.11	3.08
	3.11	3.06	3.06	3.11	3.06
	3.08	3.07	3.10	3.07	3.05
	3.07	3.06	3.07	3.07	3.10
Average	3.09	3.07	3.09	3.09	3.07
Standard deviation	0.02	0.02	0.02	0.02	0.02

Table 5.5: L-lactic acid results obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.70	0.76	0.73	0.02

Table 5.6: Reproducibility of the acetic acid measurement obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

The average value of L-lactic acid measured in TITRIVIN AA1 is found to be 0.94 g/L±0.02 g/L and 3.08 g/L±0.04 g/L in TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed as uncertainty extended to a 95% confidence range with coverage factor k=2. The CDR WineLab® system provides a concentration in accordance with that of the standard and demonstrates good repeatability and reproducibility in the determination of L-lactic acid.

6 DETERMINATION OF THE ALCOHOL CONTENT

6.1 Alcohol in wine

Ethanol (or ethyl alcohol) is, after water, the quantitatively most important compound of those present in wine. Its content is expressed by means of the alcoholic degree which represents the percentage by volume of alcohol in the wine.
The **ethyl alcohol** in wine is produced by the **alcoholic fermentation** of the sugars contained in the musts: the sweeter the grapes are at the time of harvest, the more sugars there will be in the musts and the more alcoholic the wine will be.
The action of ethanol, combined with that of acidity, allows the wine to be kept for a long time without appreciable alteration, but alcohol also contributes to the characterisation of the wine in other ways.
During the vinification, its solvent power allows dissolution of the phenolic compounds of the solid parts of the grapes. Furthermore, alcohol, reacting with acids, forms esters, which contribute to enhancing the bouquet of the wine with tertiary aromas.
On the palate, alcohol gives an immediate sensation of warmth that enhances the softness of the wine; if the wine is well balanced, we will perceive a pleasant and enveloping warmth spread in the mouth.
Wines with an alcohol content of **up to 10%** are generally defined as "**light**", while wines with an alcohol content increasing to an alcohol content of 16% are often defined as fairly "**warm**", considered the maximum limit of yeast resistance to alcohol.
The immediate perception of alcohol on the palate does not always depend on the high value of the actual alcoholic strength, but on how much the alcoholic component is in balance or not with the other components of the wine. There are wines that despite being at 14% we do not perceive as alcoholic, because they are supported by structure, body, tannins and acidity, capable of creating a perfect balance. At other times, however, we immediately perceive a strong heat that connotes the tasting in an almost pungent and unpleasant way. These are wines that are not necessarily very alcoholic but which are undoubtedly unbalanced in which the other components are too weak and slight to counteract the alcoholic effect.
By law, the sale for consumption of musts and wines with an overall alcohol content of less than 9 degrees is prohibited and, for DOC or DOCG wines, a minimum alcohol content is envisaged, i.e. a specific alcohol content below which it is not possible to sell that particular wine.
According to legislation, it is possible to increase the alcohol content by a maximum of 2 units by cutting. The addition of sugars to the must to compensate for the lack of natural sugar is **prohibited** by most of the specifications of the denominations in Italy, but correction in the form of **adding rectified concentrated musts** of wine origin **is permitted**.
The knowledge of the alcohol meter, therefore, is of great interest both from a legal and a commercial point of view and must necessarily appear on the labels of table wines intended for sale.
The official methods for measuring the actual alcoholic strength by volume include a double distillation of the alkalised wine (to avoid the interference of acetic acid, sulphur dioxide, aldehydes and other volatile substances) and the subsequent measurement of the density of the hydroalcoholic solution obtained, by pycnometry, by electronic densimetry or by means of a hydrostatic balance.

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6.2 Method precision evaluation

The precision of the system developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*) obtained by performing the analyses with CDR WineLab® and with the distillation method provided for by the reference method OIV MA-AS312-01A R2016 4.B.

	Alcohol content (% vol.)	
	CDR WineLab ®	Reference
Sample 1	12.4	12.27
Sample 2	12.1	11.99
Sample 3	13.2	13.43
Sample 4	12.1	12.07
Sample 5	10.4	10.47
Sample 6	10.8	10.52
Sample 7	12.2	12.44
Sample 8	11.6	11.38
Sample 9	13.4	13.16
Sample 10	12.5	12.50
Sample 11	11.4	11.54
Sample 12	13.1	13.75
Sample 13	12.6	12.79
Sample 14	12.5	12.58
Sample 15	14.8	14.62
Sample 16	11.8	11.24
Sample 17	11.5	11.38
Sample 18	13.0	12.97
Sample 19	13.4	13.59
Sample 20	12.8	13.00
Sample 21	10.3	10.15
Sample 22	10.4	10.42

Table 6.1: Alcohol content results obtained with CDR WineLab® and with the reference method.

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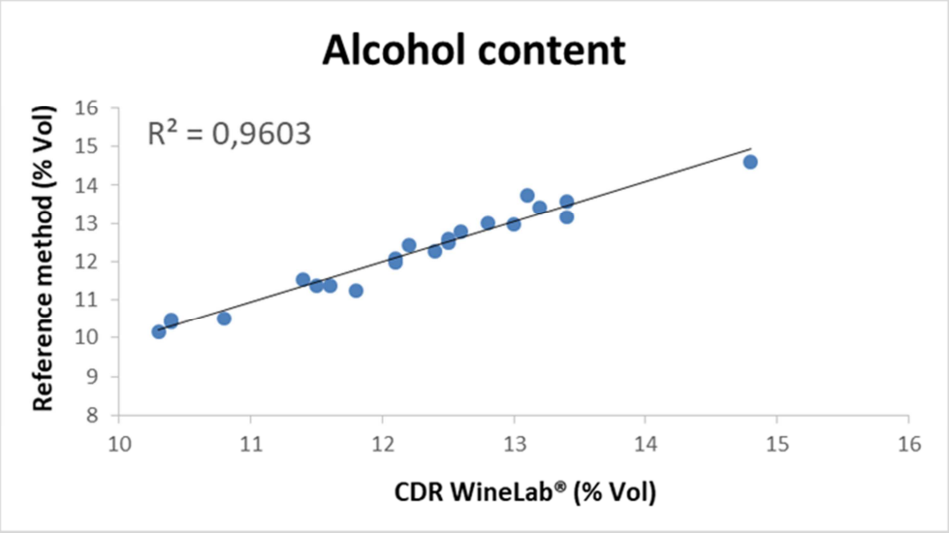

Figure 6.1: Correlation between CDR WineLab® and the reference method in the analysis of alcohol content.

The two methods demonstrate a good correlation coefficient (R^2= 0.9603).
In the sample 15 ethyl alcohol was added to increase the alcohol content of the sample. The alcohol contents of the samples were all between 10.3% and 13.4% and a low dispersion of the samples would have negatively affected the correlation estimate.

6.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the CDR WineLab® method are evaluated by analysing two different certified reference solutions: TITRIVIN AA1 (batch no. A 03171222 1) for which an alcohol content of 14±0.05% vol is declared and TITRIVIN AA4 (batch number A 03171222 4) with an alcohol content of 9.46±0.06% vol.
The choice of the two standards was made in such a way as to test the repeatability of the method at both low and high alcoholic strength values. For each standard, 5 consecutive analyses were performed, repeating the test for 5 days.
Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	9.40	9.50	9.40	9.50	9.30
	9.40	9.30	9.40	9.40	9.30
	9.60	9.50	9.50	9.60	9.60
	9.40	9.40	9.40	9.40	9.40
	9.60	9.50	9.70	9.70	9.50
Average	9.50	9.40	9.50	9.50	9.40
Standard deviation	0.11	0.09	0.13	0.13	0.13

Table 6.2: Alcoholic degree values obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (% vol.)	Max. value (% vol.)	Average (% vol.)	Standard deviation (% vol.)
25	9.3	9.7	9.5	0.1

Table 6.3: Reproducibility of the alcohol content measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVIN AA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	14.10	14.00	14.10	14.10	13.90
	14.00	14.10	13.90	13.90	14.00
	14.00	13.90	13.90	13.90	14.20
	13.90	13.80	13.90	13.90	14.20
	14.00	13.80	13.80	13.80	14.10
Average	14.00	13.90	13.90	13.90	14.10
Standard deviation	0.07	0.13	0.11	0.11	0.13

Table 6.4: Alcoholic degree values obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (% vol.)	Max. value (% vol.)	Average (% vol.)	Standard deviation (% vol.)
25	13.8	14.2	14.0	0.1

Table 3.5: Reproducibility of the alcohol content measurement obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

The average value measured for TITRIVIN AA1 is 9.5% vol. ±0.2% vol. and 14.0% vol. ±0.2% vol. for TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed with a 95% confidence range (coverage factor k=2). CDR WineLab® has good reproducibility and repeatability in the measurement of alcohol content considering the low standard deviation obtained and the average value measured with CDR WineLab® and is perfectly in agreement with the alcohol content of the two analysed standards.

7 TOTAL SO_2 DETERMINATION

7.1 SO_2 in wine

Sulphur dioxide (or sulphur dioxide), due to its numerous properties, is a fundamental tool in the production of wines.
In the correct quantities this substance prevents the proliferation of bacterial flora. This is especially important during fermentation and storage, when this compound prevents the birth of micro-organisms that could damage the wine in terms of taste and colour. It is also an antioxidant and has antioxidant properties, a fundamental characteristic in every phase of the wine production and preservation process. The SO_2 protects wines from excessive oxidation of phenolic compounds and of

certain aromatic substances, helps to maintain a low redox level that is favourable to the evolution of sensory characteristics during storage and ageing and inhibits the action of oxidase enzymes, protecting the musts from oxidation before the start of fermentation.
In addition to these properties, it combines acetaldehyde and other similar compounds, preserving the flavour and aroma of the wine and at the same time preventing the taste of mould.
To achieve these positive effects, however, sulphur dioxide must be added when the alcoholic fermentation is completely finished. If it is added too soon with respect to the end of fermentation, that is when the temperature of the wine is still too high, it can develop unpleasant aromas and tastes of sulphur dioxide, mercaptan and rotten eggs.
However, its use must be limited both with regard to the negative effects on health and because an excessive quantity of sulphur dioxide modifies the organoleptic characteristics of the wine. The maximum quantities permitted by the European Union are 160 mg/l for red wines and 210 mg/l for white and rosé wines (there are exceptions that allow member States to raise this value for a maximum of 40 mg/l in unfavourable years).
Taking into account the multiplicity of chemical reactions involved, it is not always easy to determine the ideal dose of use in order to benefit as much as possible from the advantages without having to worry about the negative effects. For this reason it is important to evaluate the concentration in the different stages of production.
The Official EEC method for the determination of this compound in wine (EEC Regulation no. 2676/90, Official Journal of the European Communities L 272 of 3/10/90) provides that sulphur dioxide is carried away by a current of nitrogen and is fixed and oxidised, by bubbling, in a dilute and neutral solution of hydrogen peroxide. The sulphuric acid formed is dosed with a titrated solution of sodium hydroxide. The total sulphur dioxide is extracted from the wine by hot entrainment (100°C).
However, the Ripper–Schmitt method is usually employed, which involves the volumetric determination of the SO_2 by iodometric titration, carried out directly on the wine at PH <1. The determination of total sulphur dioxide is performed by alkalising the solution in order to split the aldehyde-sulphur compounds and then acidifying again before performing the titration as reported in the OIV-MA-AS323-04B method.

7.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*).obtained by performing the analyses with CDR WineLab® and with the OIV-MA-AS323-04B method

	total SO2 (mg/L)	
	CDR WineLab ®	Reference
Sample 1	70	74
Sample 2	80	87
Sample 3	90	91
Sample 4	95	110
Sample 5	123	126
Sample 6	102	112
Sample 7	132	154

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Sample 8	123	148
Sample 9	95	105
Sample 10	98	102
Sample 11	106	114
Sample 12	130	147
Sample 13	125	132
Sample 14	98	91
Sample 15	65	70
Sample 16	40	38
Sample 17	110	122
Sample 18	40	45
Sample 19	20	14
Sample 20	40	37
Sample 21	61	52
Sample 22	50	66

Table 7.1: Results of total SO_2 obtained with CDR WineLab® and with the reference method.

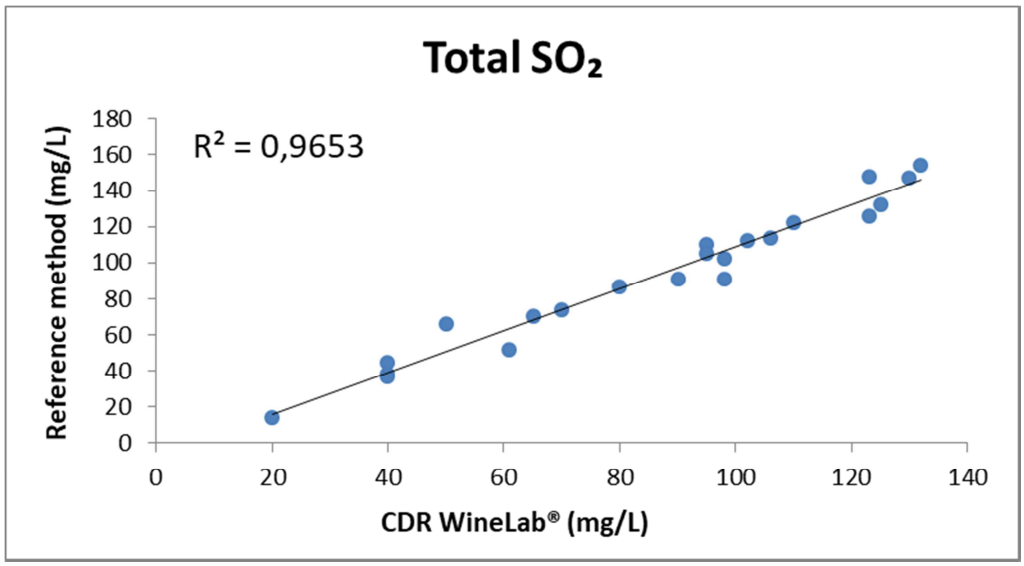

Figura7.1: Correlation between CDR WineLab® and reference method

The two methods showed a good correlation (R^2= 0.9653) considering the non-optimal repeatability of both measurement methods.

7.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the method are evaluated by carrying out 5 consecutive analyses for 5 days of the total sulphur dioxide concentration in the dry white wine sample 21-RT-003 sent by the Ring Test-Lab circuit (analysis circuit coordinated by Unione Italiana Vini) in February 2021 to CDR s.r.l., which supplied the sample to the University of Florence to perform the test.
For this parameter there are no commercial standard solutions and therefore it was chosen to test

the repeatability/reproducibility of the measurement using a sample sent for an interlaboratory comparison test (Ring Test) to CDR s.r.l.

	Day 1	Day 2	Day 3	Day 4	Day 5
	119	120	118	118	118
	118	117	119	117	120
	117	122	119	118	119
	119	117	122	119	121
	119	118	120	118	123
Average	118	118	120	118	119
Standard deviation	1	1	2	1	2

Table 7.2 Measurements of the SO_2 total concentration obtained from the analysis of sample 21-RT-003 with CDR WineLab®

Total number of analyses	Min. value (mg/L)	Max. value (mg/L)	Average (mg/L)	Standard deviation (mg/L)
25	117	123	119	2

Table 7.3: Reproducibility of the total SO_2 measurement with CDR WineLab®

The mean value measured for sample 21-RT-003 is 119 mg/L±4 mg/L (measurement uncertainty is expressed as uncertainty extended to a 95% confidence range with coverage factor k=2) . The value obtained with CDR WineLab® shows a standard deviation and therefore a repeatability that is not optimal but better than the repeatability obtained with the reference method OIV-MA-AS323-04B.
The total sulphur content obtained with CDR WineLab® is perfectly in agreement with the value obtained in the Ring Test (123.4 mg/L±12.5 mg/L) confirming the correlation with the standard method.

8 DETERMINATION OF FREE SULFUR DIOXIDE

8.1 Free SO_2 in wine

Correct management of the SO_2 in wine it is essential, in fact this compound is difficult to replace in the vinification and preservation of wine due to its innumerable properties (*Chapter 7.1*).
The sulphur dioxide contained in wine is present in different forms, not all of which are equally significant from an oenological point of view.
The term "free sulphur dioxide" indicates the forms that can be released by acidification, namely:

- H_2SO_3 or molecular sulphur
- HSO_3^- or bisulphite ion
- SO_3^{2-} or sulphite ion

Instead, when we talk about combined sulphur dioxide we mean that part of sulphur dioxide that is generally bound with certain wine compounds such as acetaldehyde, sugars, ketonic acids, uronic

acids and anthocyanins. Depending on the stability of the bond, a further distinction is made between:

- SO_2 combined, permanently bound with acetaldehyde;
- SO_2 deposit bound to compounds with medium or low affinity and which, dissociating by heating, can generate free SO_2 .

There is an equilibrium between free and combined sulphur dioxide which is mainly influenced by the temperature and pH of the wine. This latter parameter has a significant influence on the presence of the three forms because the quantity of undissociated sulphuric acid decreases as the pH increases.
It is the free part that performs the important antioxidant and antiseptic effects: for this reason it is essential that sulphur dioxide is combined as little as possible. Sulphur dioxide combined with compounds with medium and low affinity is, however useful, as in the case in which the free fraction is dispersed, for example during the pouring operations, a part of the combined one is freed, replacing it. A wine must always have a certain amount of free sulphur dioxide to ensure correct preservation.
To ensure a suitable addition of sulphur dioxide to the product it is important not only to evaluate the total concentration of SO_2 , but also to evaluate its free form, essential for obtaining the desired antiseptic and antioxidant effects.
For its determination, the official EEC method provides that free sulphur dioxide is carried away by a current of air or nitrogen and then fixed and oxidised, by bubbling, in a dilute and neutral solution of hydrogen peroxide. The determination is performed by titrating with a sodium hydroxide solution, similarly to that required for the determination of total sulphur dioxide. The free sulphur dioxide is however extracted from the wine by cold entrainment (10°C).
Also in the case of free sulphur, the Ripper – Schmitt method reported in the OIV-MA-AS323-04B method is commonly used but without performing the alkalinisation (*Chapter 7.1*).

8.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*).obtained by performing the analyses with CDR WineLab® and with the OIV-MA-AS323-04B method

	free SO2 (mg/L)	
	CDR WineLab ®	Reference
Sample 1	13	11
Sample 2	27	25
Sample 3	24	27
Sample 4	30	27
Sample 5	12	16
Sample 6	21	17
Sample 7	20	24
Sample 8	58	58
Sample 9	24	29
Sample 10	12	17
Sample 11	25	27

Sample 12	23	21
Sample 13	16	16
Sample 14	15	9
Sample 15	3	7
Sample 16	6	1
Sample 17	10	15
Sample 18	5	2
Sample 19	2	1
Sample 20	7	2
Sample 21	2	2
Sample 22	6	4

Table 8.1: Results of free SO_2 obtained with CDR WineLab® and with the reference method.

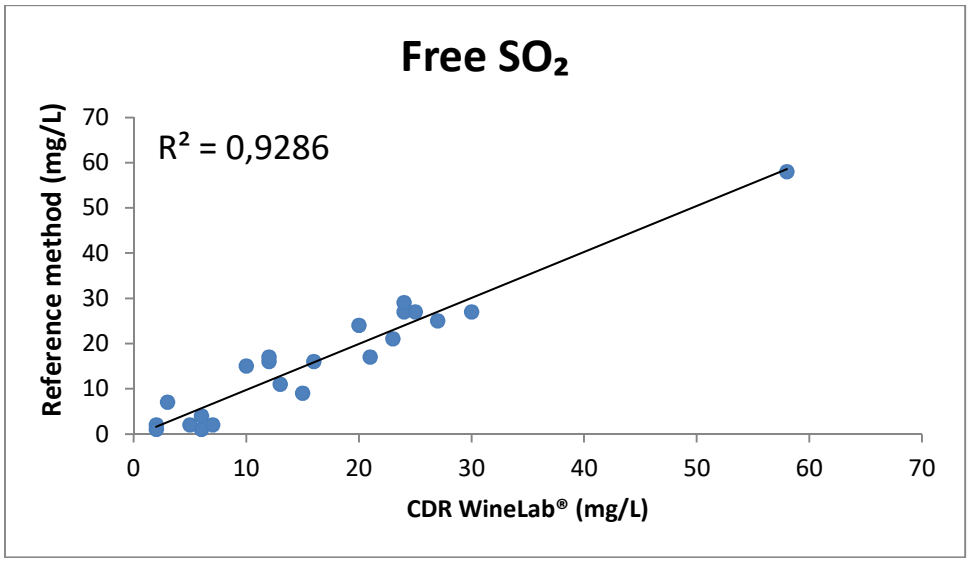

Figure 8.1: Correlation between CDR WineLab® and reference method

The two methods showed a good correlation (R^2= 0.9286) considering the non-optimal repeatability of both measurement methods (*Chapter 8.3*).

8.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the method are evaluated by performing 5 consecutive analyses for 5 days of the total sulphur dioxide concentration in the dry white wine sample 21-RT-003 sent by the RT-LAB circuit in February 2021 to CDR s.r.l., which provided the sample to the University of Florence to perform the test.
For this parameter there are no commercial standard solutions and therefore it was chosen to test the repeatability/reproducibility of the measurement with a sample of a Ring Test.

	Day 1	Day 2	Day 3	Day 4	Day 5
	22	20	22	25	22
	23	23	25	21	20
	24	23	24	23	23
	22	22	24	22	23
	22	22	22	24	23
Average	23	22	23	23	22
Standard deviation	1	1	1	2	1

Table 7.2: Measurements of free SO_2 concentration obtained from the analysis of sample 21-RT-003 with CDR WineLab®

Total number of analyses	Min. value (mg/L)	Max. value (mg/L)	Average (mg/L)	Standard deviation (mg/L)
25	20	25	23	1

Table 7.3: Reproducibility of the free sulphur dioxide measurement with CDR WineLab®

The mean value measured for sample 21-RT-003 is 23 mg/L±2 mg/L (measurement uncertainty is expressed as uncertainty extended to a 95% confidence range with coverage factor k=2) . The result obtained with CDR WineLab® has a good reproducibility in the measurement of the concentration of free sulphur dioxide if compared with that of the method used as a reference (OIV-MA-AS323-04B). The free sulphur content obtained with CDR WineLab® is perfectly in agreement with the value obtained in the Ring Test (20.6 mg/L±6 mg/L) confirming the correlation with the standard method.

9 DETERMINATION OF SUGARS

9.1 Fermentable sugars in wine

Knowledge of the sugar content is a parameter that is used to monitor the state of ripeness of the grapes in their various stages and to identify the exact time for the harvest.
Determining the quantity of sugars present in musts is one of the most important analyses as the alcohol content of the future wine will depend on the greater or lesser sugar content.
The potential alcoholic degree of a wine is 0.66 alcoholic degrees by volume for each gram of fermentable sugars present in the must, therefore the monitoring of sugars during alcoholic fermentation enables evaluation of the course.
Furthermore, the determination of sugars is essential in the preparation of special wines (liqueur, sparkling, flavoured, etc.) or sweet wines to meet the relevant legal and technological requirements.
For example, in the case of **sparkling wine**, the addition of sugar is essential for good refermentation in the bottle. The quantity of sugar added will determine the pressure due to CO_2 in the bottle. After the addition, the winemaker can determine the total fermentable sugars in the sample to be sure of the **correct sugar level in the wine.**
If the fermentation is not completed **by fermenting all the sugars present in the must**, the resulting **sugar residue** determines the greater or lesser sweetness of the wine in question. The presence or

absence of sugar in wine determines its style and organoleptic orientation. A quantity of sugar greater than 50 g/L classifies the wine as sweet, the absence (or negligible quantity) classifies it as dry (<10 g/L) and intermediate quantities determine particular sensory profiles.
In grapes there are different types of sugars, however the main ones, which represent the greatest quantity, are glucose and fructose (fermentable sugars). Not all sugars present in grape pulp affect the alcoholic fermentation process. Some of these, called infermentable, are not converted into alcohol and carbon dioxide by the yeasts and remain in the wine to contribute to its sweetness. This sweetness, produced by the so-called residual sugars, is not always perceptible when tasted, both because they are balanced by other substances, and because they are present in negligible quantities and such as not to exceed the level of the perceptibility threshold.
In Italy sugaring is prohibited, but, in countries where it is permitted to add sucrose to increase the potential alcohol, the winemaker can analyse the fermentable sugar content **to verify if the added quantity is correct.**
The most commonly used methods for the determination of fermentable sugars are the enzymatic method (OIV-MA-AS311-02) and the OIV-MA-AS311-03 method through HPLC that are used to determine glucose and fructose, excluding the detection of **pentoses**.

9.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*).obtained by performing the analyses with CDR WineLab® and with HPLC according to the reference method OIV-MA- AS311-03

	Sugar content (g/L)	
	CDR WineLab ®	Reference
Sample 1	1.3	1
Sample 2	2	2.2
Sample 3	1.8	2.2
Sample 4	0.8	1
Sample 5	< 1	16
Sample 6	7.7	17
Sample 7	1.4	1.4
Sample 8	2.8	3.1
Sample 9	2.5	2.8
Sample 10	3.9	4.1
Sample 11	7.4	8.1
Sample 12	2.1	1.9
Sample 13	1.5	1.4
Sample 14	0.4	< 1
Sample 15	0.9	< 1
Sample 16	15	18
Sample 17	6.5	6.6
Sample 18	1.1	< 1
Sample 19	0.1	< 1

Sample 20	< 0.1	< 1
Sample 21	11.1	13
Sample 22	0.3	< 1

Table 9.1: Sugar concentration results obtained with CDR WineLab® and with the reference method

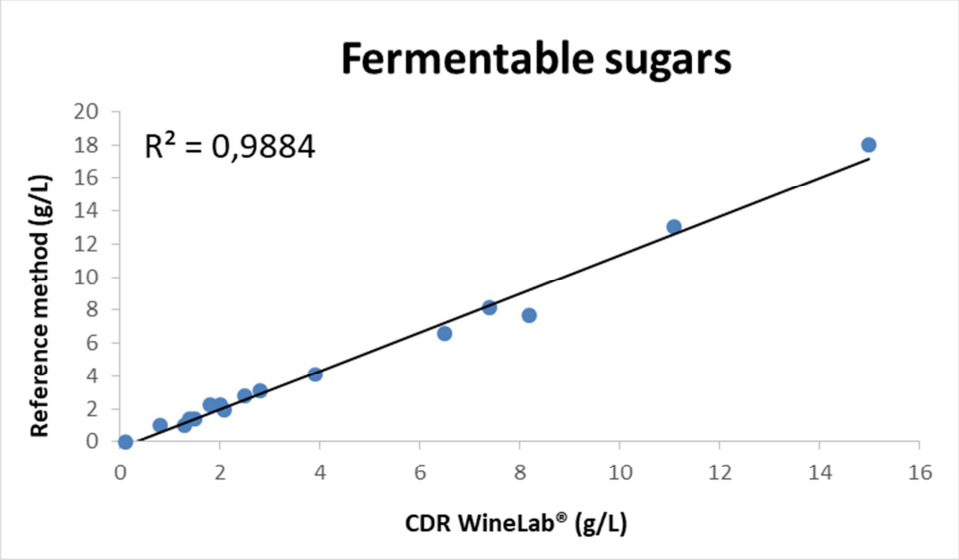

Figure 9.1: Correlation between CDR WineLab® and reference method

The two methods gave highly correlated results (R^2= 0.9884).

9.3 Evaluation of repeatability and reproducibility of the method

The repeatability and reproducibility of the CDR WineLab® method are evaluated by analysing two different certified reference solutions: TITRIVIN AA1 (batch number A 03171222 1) for which a value of sugars equal to 0.87±0.08 mg/L is declared and TITRIVIN AA4 (batch number 03171222 4) which has a concentration of 8.70±0.26 mg/L.
The choice of the two standards was made in such a way as to test the repeatability of the method at both low and high sugar values. For each standard, 5 consecutive analyses were performed, repeating the test for 5 different days.

Here are some of the data obtained:

TITRIVIN AA1:

	Day 1	Day 2	Day 3	Day 4	Day 5
	0.8	0.7	0.9	0.9	1.1
	0.8	0.9	1.0	0.9	1.0
	0.9	0.8	0.9	0.9	1.0
	0.8	0.9	0.9	0.9	0.9
	0.9	0.9	0.9	0.9	1.0
Average	0.9	0.9	1.0	0.9	1.0
Standard deviation	0.1	0.1	0.1	0.1	0.1

Table 9.2: Sugar values obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	0.7	1.0	0.9	0.1

Table 9.3: Reproducibility of the sugar measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

TITRIVINAA4:

	Day 1	Day 2	Day 3	Day 4	Day 5
	8.9	8.7	8.7	8.7	8.8
	8.8	8.8	8.7	8.9	8.9
	8.9	8.6	8.6	8.9	8.7
	8.8	8.8	8.7	8.8	8.8
	8.7	8.8	8.8	8.8	8.8
Average	8.8	8.7	8.7	8.8	8.8
Standard deviation	0.1	0.1	0.1	0.1	0.1

Table 9.4: Sugar values obtained from the analysis of TITRIVIN AA4 with CDR WineLab®

Total number of analyses	Min. value (g/L)	Max. value (g/L)	Average (g/L)	Standard deviation (g/L)
25	8.6	8.9	8.8	0.1

Table 9.5: Reproducibility of the sugar measurement obtained from the analysis of TITRIVIN AA1 with CDR WineLab®

The sugar value measured for TITRIVIN AA1 is found to be 0.9 mg/L±0.2 mg/L and 8.8 mg/L±0.2 mg/L for TITRIVIN AA4. The value obtained with CDR WineLab® is reported with a measurement uncertainty expressed with a 95% confidence range (coverage factor k=2). CDR WineLab® has

excellent reproducibility and repeatability in the measurement of sugars and the measured value is perfectly in agreement with the declared sugar concentration of the two analysed standards.

10 IPT (Total Polyphenol Index)

10.1 Index of total polyphenols in wine

After carbohydrates and acids, polyphenols are the most abundant group of chemical species present in grapes and play a fundamental role in oenology.
Polyphenolic compounds are one of the most important quality parameters of wine, thanks to their contribution to the organoleptic characteristics such as colour, astringency and aroma of the product. Furthermore, benefiting our organism, they possess bactericidal, antioxidant, vitamin and protective properties against cardiovascular diseases.
From the chemical point of view, the study of phenolic compounds in wine is fairly complex and in-depth due to the wide diversity of structures that are part of it and the various sensorial contribution they provide.
These substances in fact belong to different categories, such as hydroxycinnamic and hydroxybenzoic acid derivatives, flavonoids, anthocyanins, flavones and tannins.
Polyphenols are contained in the stalk, in the grape seeds, in the peel and, to a lesser extent, in the pulp. The presence of polyphenols in wine mainly depends on the wine-making technique, in particular on the conditions of some stages of wine-making such as maceration and fermentation that affect the extraction of the various constituents of the grape.
However, the quantity of polyphenols extracted also depends on the initial concentration contained in the bunch which is very variable as the presence of polyphenols is influenced by the ripening conditions of the grapes, as well as by the cultivation techniques, the geographical position and the "terroir".
The content of these substances in wine therefore depends on the type of blend and the vinification system; the content of polyphenols in red wines is on average 1.5 g/l, rosé wines can contain 400-800 mg/l and in white wines they are found from 100 to 400 mg/l.
Polyphenols are substrates of a large number of chemical reactions and undergo various changes in structure during the refinement and ageing of wine, modifying its organoleptic characteristics. Therefore, the estimation of the quantity of grape polyphenols that can be extracted during wine-making, the quantification of these compounds in the final product and the knowledge of the distribution of these compounds between the skins and seeds can help the winemaker to optimally set red vinification and to foresee some of the potential problems that could arise during maturation of the product.
In the oenological sector there are numerous studies and methods of analysis for the qualification and quantification of polyphenols. The official method for determining the Total Polyphenol Index (OIV-MA-AS2-10) involves the use of a particular oxidising reagent, called Folin-Ciocalteu reagent, that can assume a blue colour, whose intensity is linearly proportional to the number of phenolic residues present.

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10.2 Method precision evaluation

The precision of the method developed by CDR is evaluated by determining the correlation between the results of 22 wine samples (*Table 1.1*).obtained by performing the analyses with CDR WineLab® and with the OIV-OENO 419D-2015 method

	Total Polyphenol Index (D.O.)	
	CDR WineLab ®	Reference
Sample 1	9	9
Sample 2	8	7
Sample 3	6	6
Sample 4	6	6
Sample 5	6	6
Sample 6	6	6
Sample 7	9	8
Sample 8	7	6
Sample 9	11	10
Sample 10	14	16
Sample 11	42	9
Sample 12	54	43
Sample 13	49	56
Sample 14	51	51
Sample 15	46	51
Sample 16	38	45
Sample 17	33	48
Sample 18	45	35
Sample 19	57	46
Sample 20	48	58
Sample 21	6	6
Sample 22	6	6

Table 10.1: Results of the total polyphenol content obtained with CDR WineLab® and with the reference method.

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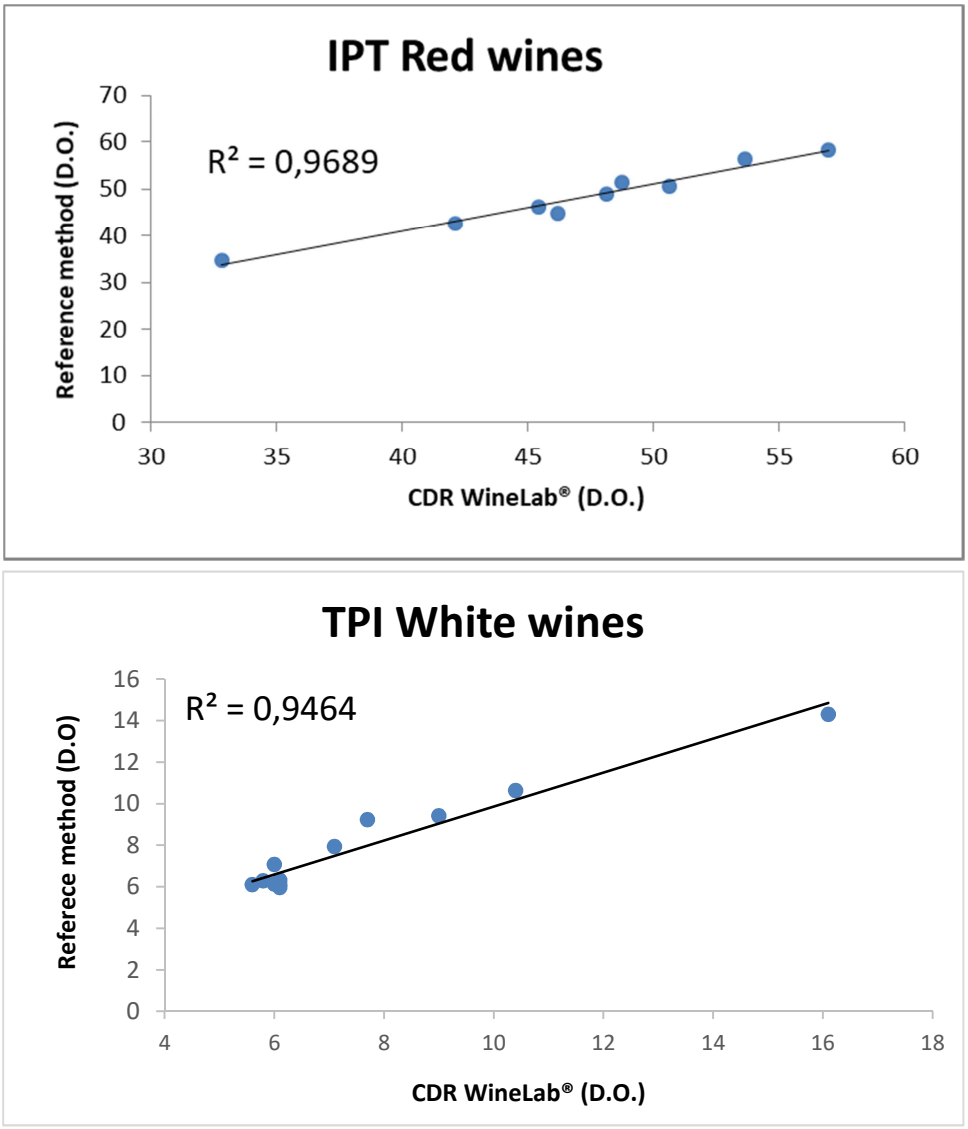

Figure 10.1: Correlation between CDR WineLab® and the reference method.

The concentration of total polyphenols is very variable depending on the wine. The CDR WineLab® tool has two different calibration curves, one for red wines and one for white wines. For this reason, two correlation curves are shown which are very good in both cases (Red wine: $R.^2$= 0.9689; White wine: R^2= 0.9464).

The correlation coefficient R in the case of white wine is lower. However, we must consider that the values obtained from the analysis of white wine do not cover a wide range of values and this negatively affects the estimate of the correlation.

It should be emphasised that the correlation obtained was calculated by eliminating sample 16 from the data set as it was considered an outliers with an anomalous value that caused the coefficient R to vary significantly.

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10.3 Method repeatability evaluation

The repeatability and reproducibility of the method are evaluated by carrying out 5 consecutive analyses for 5 days of the Total Polyphenol Index present in the dry white wine sample 21-RT-003 sent by the RT-LAB circuit in February 2021 to CDR s.r.l., which provided the sample to the University of Florence to perform the test.
Also for this parameter there are no commercial standard solutions and therefore it was chosen to test the repeatability/reproducibility of the measurement with a sample of a Ring Test.
The values reported in *Table 7.2 and 7.3* are expressed as mg/L of gallic acid. This change of measurement unit was necessary to compare the values obtained with the CDR WineLab® method and the results obtained in the Ring Test (193±66 mg/L of gallic acid).
It should be emphasised that the CDR WineLab® system provides the results both in DO and in mg/L of gallic acid and therefore it was not necessary to perform any type of conversion.

	Day 1	Day 2	Day 3	Day 4	Day 5
	141	140	139	141	142
	139	142	142	143	143
	142	142	140	144	142
	140	140	139	140	141
	140	144	140	142	143
Average	140	142	140	142	142
Standard deviation	1	2	1	2	1

Table 10.2: Measurements of the Total Polyphenol Index concentration obtained from the analysis of sample 21-RT-003 with CDR WineLab®

Total number of analyses	Min. value (mg/L)*	Max. value (mg/L)*	Average (mg/L)*	Standard deviation (mg/L)*
25	139	144	141	2

** mg/L of gallic acid*

Table 10.3: Reproducibility of the Total Polyphenol Index measurement with CDR WineLab®

The average value measured with CDR WineLab® of sample 21-RT-003 is 141 mg/L±4 mg/L. The result obtained appears to have good reproducibility and repeatability in the measurement of the Total Polyphenol Index if compared with that of the method taken as a reference (OIV-MA-AS323-04B). The Total Polyphenol Index value obtained with CDR WineLab® was found to be in agreement with the published Total Polyphenol Index values of the Ring Test.

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11 CONCLUSIONS

All analyses tested with the CDR WineLab ® instrumentation provided results that were statistically correlated with those obtained with the official methods.
The detection limits and the reproducibility of the analyses were comparable or better than those obtained with the official methods.
The method of analysis in the case of CDR WineLab® instrumentation is very simple: for each of the analyses carried out, the only sample preparation required is degassing (if necessary). Alternatively, the sample is used as it is except for the analysis of the alcohol content where a dilution is required to be carried out with the dedicated kit supplied. Regarding the actual analysis, the instrument is very simple to use, does not require calibration and is ready to be used to perform the measurement. The operator is assisted by detailed instructions visible on the touch screen of the instrument, present for each method of analysis. This means easy execution of the analysis even by non-expert personnel.
All the material needed to perform the analysis is supplied in specific kits by the manufacturer.
With the CDR WineLab® analysis system there is also a considerably reduced consumption of both sample and reagents compared to some of the corresponding official methods, for example in the analysis of free and total sulphur dioxide.

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