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Subject : Validation of the methods of Winelab CDR

Volatile acidity

The tests for this parameter included linearity, recovery, repeatability and the statistical correspondence with the official method.

Linearity was assessed using a matrix standard (red wine with a high content of polyphenols) with values ranging from a minimum of 0.2 to a maximum of 1.2 g/l. Within this range, the method showed a good linearity.

Recovery (assessed using a matrix standard of red wine with a high content of polyphenols) was calculated using 3 concentration levels and through 10 repetitions, and always resulted statistically equivalent to 100%.

Repeatability tests were carried out using 3 concentration levels and through 10 repetitions. 2 values failed to pass Dixon's test due to the presence of aberrant values, but were still regarded as valid because they passed the Student's and standard deviation tests. The repeatability limit (max. offset between two repeated tests with a probability of 95%) was higher than that of the official method (0.08 vs. 0.04), but was regarded acceptable for the purpose for which it is used.

The results obtained with the reference method (OIV MAFAS31302ACIVOL) were compared on 46 samples of different kinds (wine, red and passito wines). Samples were selected so as to have a wide range of values (from 0.10 to 1.12 g/L). The two methods were statistically equivalent with a probability of 95%. 6 values with an offset above 0.1 g/L were found.

L-Malic acid

The tests for this parameter included linearity, recovery, repeatability and the statistical correspondence with the HPLC method.

Linearity was assessed using a matrix standard (red wine with a high content of polyphenols) with values ranging from a minimum of 0.17 to a maximum of 1.7 g/l (values calculated in L-Malic acid). Within this range, the method showed a good linearity. The additional test carried out on white wines (non reported indicative test) suggests an excellent linearity up to 2.5 g/L.

Recovery (assessed using a matrix standard of red wine with a high content of polyphenols) was calculated using 3 concentration levels and through 10 repetitions. Although recovery appeared to fall as concentration increased, it was above 85 up to 3 g/L. Indicative tests, that have not been reported, show a higher recovery value for white wines.

Repeatability tests were carried out using 3 concentration levels and through 10 repetitions. Even in this case 2 values failed to pass Dixon's test, but were still regarded as valid because they passed the Student's and standard deviation tests. The repeatability limit (max. offset between two repeated tests with a probability of 95%) was 0.10 g/L, which is far more than sufficient for the purpose for which it is used.

The results obtained with the reference method (internal ISVEA HPLC method) were compared on 58 samples of different kinds (wine, red and passito wines). Samples were selected so as to have a wide range of values (from 0 to 1.85 g/L). The two methods were statistically equivalent with a probability of 95%. Only 2 values with an offset above 0.3 g/L were found.

Glucose

The tests for this parameter included linearity, recovery, repeatability and the statistical correspondence with the HPLC method.

Linearity was assessed using a matrix standard (red wine with a high content of polyphenols) with values ranging from a minimum of 1 to a maximum of 3 g/l. Within this range, the method was absolutely linear.

Recovery (assessed using a matrix standard of red wine with a high content of polyphenols) was calculated using 3 concentration levels and through 10 repetitions, and was statistically above 100% up to 3 g/L.

Repeatability tests were carried out using 3 concentration levels and through 10 repetitions. 1 value failed the Dixon's and Student's tests with a probability of 95%. The value was however considered valid, because it was close to the acceptability limit, because of the rather limited number of tests performed and the fact that it passed the standard deviation test. The repeatability limit (max. offset between two repeated tests with a probability of 95%) was 0.10 g/L, which is far more than sufficient for wines at the end of the fermentation phase. For musts and passito wines, for which samples need to be significantly diluted (more than 20 times), it is advisable to perform a duplicate test and carefully perform the manual operations during the testing phase.

The results obtained with the reference method (internal ISVEA HPLC method) were compared on 46 samples selected taking into account that the method is valid up to values of 3 g/L of glucose. The red and white wine samples selected had a content of reducing sugars ranging from 1 to 6 g/L. Unfortunately, almost none of the samples contained glucose, as confirmed with both the Winelab and reference methods. Therefore, in order to have a significant volume of comparable data, we also diluted some passito wine samples with a high concentrations of sugars. The two methods are statistically equivalent with a probability of 95%.

Fructose

The tests for this parameter included linearity, recovery, repeatability and the statistical correspondence with the HPLC method.

Linearity was assessed using a matrix standard (red wine with a high content of polyphenols) with values ranging from a minimum of 1 to a maximum of 3 g/l. Up to 2.5 g/l the method appeared to be linear though close to the limit, but in correspondence with the value of 3 g/l the loss of linearity was too high. Note: the highest standard contained also 2.5 g/l of glucose and the color of the matrix wine was very intense, which probably contributed to reducing the linearity range of fructose. Therefore, this range of linearity should be considered a limit value obtained in the worst conditions.

Recovery (assessed using a matrix standard consisting of red wine with a high content of polyphenols) was calculated using 3 concentration levels and through 10 repetitions. Up to 2 g/L recovery was statistically equivalent to 100%, but showed a significant reduction in correspondence with 3 g/L though continuing to be acceptable (above 85%). The considerations regarding the linearity range also apply in this case.

Repeatability tests were carried out using 3 concentration levels and through 10 repetitions. All values passed the Dixon's test and 1 value failed the Student's test with a probability of 95%, but passed it with a probability of of 99%. Therefore, all data was considered acceptable. The repeatability limit (max. offset between two repeated tests with a probability of 95%) was 0.08 g/L, which is far more than sufficient for wines at the end of

the fermentation phase. The considerations regarding glucose apply to musts and passito wines.

The results obtained with the reference method (internal ISVEA HPLC method) were compared on 49 samples selected taking into account that the method is valid up to values of 3 g/L. The red and white wine samples selected had a content of reducing sugars ranging from 1 to 6 g/L and additional samples of passito wines with high concentrations of sugars were also diluted. The two methods are statistically equivalent with a probability of 95%.

Total sulphur dioxide

The tests for this parameter included repeatability and the statistical correspondence with the reference distillation method (OIV MAFAS32304DIOSOU par 2.2).

Due to the unavailability of a stable standard for the definition of the content of sulphur dioxide, the validation of this method was based on the comparison with the reference method and the repeatability tests performed at various concentrations.

The comparison was carried out on 65 samples (36 white wine samples and 29 red wine samples): the value of the signal produced by Winelab was correlated with the total values of sulphur dioxide obtained with the reference method. We immediately noticed that white and red wines formed distinct statistical populations and therefore continued the correlation test distinguishing between these two types of wine. We then selected some samples (3 samples of red wine and 6 of white wine separately) that represented the population and repeated the tests on these samples using both the reference and Winelab's methods. The average values were used to calculate the calibration straight line (for red and wine white), which was then employed to calculate the concentrations required for the comparison with the reference method.

Both methods were statistically equivalent (with a probability of 95%) for white whites in all the range observed. 9 samples out of 36 showed however an offset above 10 mg/L as compared to the reference values (outlier).

Both methods were also statistically equivalent (with a probability of 95%) also for red wines. However, in this case the samples with an offset above 10 mg/L as compared to the reference values were 9 out of 29, principally because of their high concentration values, which may suggest a lack of linearity for values above 100 mg/L.

The accuracy of the method was assessed using 3 different concentrations and 10 repetitions, and by selecting a red wine sample for the average concentration and 2 white wine samples for low and high concentration values. As only one value failed to pass the Dixon's test with a probability of 95% (but passed it with a probability of 99%) and all other values passed the Student's test, all values were considered significant. We noticed a substantial difference in the repeatability dependant on the type of wine, with a more than acceptable value for white wines (repeatability limit with a probability of 95% =5 mg/L) for both concentration levels and almost double the value for red wines. This indicates that the test is not very accurate for red wines and suggests that a duplicate test needs to be performed to be able to rely on accurate data.

The limited accuracy found for red wines partly explains the number of outliers resulting from the comparison with the reference method.

FINAL CONSIDERATIONS

The tested methods can be substantially considered valid although they may exhibit some accuracy issues when used in standard operating conditions (specifically for the total amount of sulphur dioxide and the amount of sugars in diluted samples), which could be solved by repeating the tests.

The weak point of the whole system is definitely the precision with which the operator adds the sample to the reagents (which is performed manually using pipettes). Among the methods tested, only the test for the determination of the total amount of sulphur dioxide in red wines exhibited repeatability issues that are related to the method and not to the operator.

The standard supply of material is the only disadvantage: the drilled sample holder only has 10 places for cuvettes   and 1 for reagents, while some methods required 12 places for cuvettes and 2 for reagents.

The cuvette used for reading are almost always scored, though this conditions does not appear to have significantly affect the test repeatability.

Of all the packages tested, only 1 cuvette contained defective reagents (and was immediately identifiable because of the difference in color).

In faith,

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