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TECHNICAL NOTE / NOTA TÉCNICA

QUALITY CONTROL IN FTIR WINE ANALISYS
ACCEPTANCE OF ANALYTICAL RESULTS

CONTROLO DE QUALIDADE EM ANÁLISES DE VINHOS POR FTIR
Aceitação de resultados analíticos

M. L. Ferreira, a. M. Costa, N. Ribeiro, T. Simões, P. Barros

INSTITUTO DOS VINHOS DO DOURO E DO PORTO, IP. Rua de Ferreira Borges, 27. 4050-253 PORTO. Portugal
Corresponding author / Autor para correspondência: Manuel Lima Ferreira, e-mail: mlferreira@ivdp.pt

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SUMMARY

The Fourier Transform Infrared (FTIR) spectrophotometry is, at present, in the front line of routine wine analysis. With this emerging methodology it is possible to obtain a great throughput of information in a very short time. However, it is being commonly accepted that, in order to proceed successfully, a rigorous system of quality control of analytical results must be implemented and meticulously followed, being this, nowadays, a major concern for the international oenological scientific community (OIV 2009).
In the present paper, the authors are proposing a new strategic approach for the quality control of results obtained through analysis with FTIR, which is being followed in routine at Port and Douro Wines Institute (IVDP). Examples are given for wine density (g/mL), alcoholic strength (% v/v), pH, volatile acidity (g/L) and total acidity (g/L).

RESUMO

A espectrofotometria por infravermelho com transformada de Fourier (FTIR) está, presentemente, na linha da frente da análise em rotina de vinho. Com esta metodologia emergente é possível obter-se uma elevada produção de informação num curto período de tempo. Entretanto, é geralmente aceite que, para se progredir com sucesso, deve ser executado e meticulosamente seguido um sistema rigoroso de controlo da qualidade de resultados analíticos, sendo hoje em dia uma preocupação para a comunidade científica internacional enológica (OIV 2009).
Neste trabalho, os autores vêm propor uma nova aproximação estratégica para o controlo da qualidade dos resultados obtidos pela análise do vinho por FTIR que tem vindo a ser seguida em rotina no Instituto dos Vinhos do Douro e do Porto (IVDP). São apresentados exemplos para a massa volúmica (g/mL), titulo alcoométrico volúmico (% v/v), valor de pH , acidez volátil (g/L) e acidez total (g/L).

Key words: wine analysis, FTIR spectrophotometry, quality control, limits, analytical calibration.
Palavras-chave: análise de vinho, espectrofotometria de infra-vermelho com transformada de Fourier, controlo da qualidade, limites, calibração analítica.

INTRODUCTION

The application of FTIR spectrophotometry to wine automatic analysers is an emergent and very promising methodology (Moreira *et al*, 2002a). This technique is based on the absorption of the infrared radiation from the material that is being analysed. Based on the detection of special vibrations associated with chemical bonds, it is possible to quantify some wine compounds if an adequate analytical calibration has been previously established.

With this emerging methodology it is possible to obtain a great throughput of information in a very short time. However, it is being commonly accepted that, in order to proceed successfully, a rigorous system of quality control of analytical results must be implemented and meticulously followed, being this, nowadays, a major concern for the international oenological scientific community (OIV 2009).

The aim of this work is to propose a new strategic approach for the quality control of results obtained by FTIR which is being followed in routine at IVDP. A quality control applied to the determination of wine density (g/mL), alcoholic strength (% v/v), pH , volatile acidity (g/L) and total acidity (g/L) in routine daily work is proposed, based on the establishment of a working range for each analytical parameter, defined tolerances related to the standard deviation (sd) associated with replicates, the checking of the equipment with internal control standards and associated reports for each analytical parameter (pilot samples), as well as a continuous crosschecking of the results obtained from the various methods.

MATERIAL AND METHODS

All analytical data have been obtained with a FOSS Winescan FT-120 FTIR spectrophotometer equipped with an autosampler (5027 Sampler FOSS Tecator). This spectrophotometer is equipped with an operative basic multitasking software used to set running conditions, to fix analytical coordinates and to obtain analytical data.

An in-house specific database for still wines, has been created based on approximately 800 observations of samples obtained mainly from the Douro Demarcated Region and collected between 2006 and 2008. For fortified wines, also an in-house specific database has been created based on approximately 4000 observations of samples collected between 2003 and 2008. The aim was establishing a preliminary analytical calibration and, in a simple run, to determine simultaneously all the analytical parameters for which this analytical calibration is optimised. In fact, each analytical parameter has a "specific calibration" that is concatenated in a "global analytical calibration". It was created a "global analytical calibration" for still wines and a different one for fortified wines.

It is possible to observe a summary of statistical data related with the study of crosschecking results between FTIR and IVDP Accredited Methods (AM), respectively in still wines (TABLE I) and fortified wines (TABLE II). Through the observation of the results obtained for each parameter, confidence specific ranges had been fixed (TABLE III).

TABLE I

Statistical data summary of crosschecking results study in still wines

Resumo de dados estatísticos do estudo de cruzamento de resultados em vinhos tranquilos

		Density (g/mL)	Alcoholic Strength (% v/v)	pH	Volatile Acidity (g/L)	Total Acidity (g/L)
Analytical working range (*)		From 0.9878 to 0.9950	From 10.83 to 15.13	From 2.99 to 3.85	From 0.16 to 0.98	From 3.93 to 6.52
Number of samples		139	150	148	138	130
|FTIR-AM|	Max.	0.0004	0.15	0.10	0.10	0.20
	Min.	0.0000	0.00	0.00	0.00	0.00
	Average	0.0001	0.04	0.02	0.03	0.07
	Sd	0.0001	0.03	0.02	0.03	0.05

(*) Maximum and Minimum values obtained by AM

TABLE II

Statistical data summary of crosschecking results study in fortified wines

Resumo de dados estatísticos do estudo de cruzamento de resultados em vinhos licorosos

		Density (g/mL)	Alcoholic Strength (% v/v)	pH	Volatile Acidity (g/L)	Total Acidity (g/L)
Analytical working range (*)		From 0.9913 to 1.0463	From 17.84 to 22.86	From 3.10 to 3.88	From 0.14 to 1.24	From 2.46 to 7.03
Number of samples		188	195	129	188	187
|FTIR-AM|	Max.	0.0004	0.15	0.10	0.10	0.20
	Min.	0.0000	0.00	0.00	0.00	0.00
	Average	0.0001	0.05	0.03	0.04	0.06
	Sd	0.0001	0.04	0.02	0.03	0.04

(*) Maximum and Minimum values obtained by AM

TABLE III

Example of analytical working ranges for FTIR wine analysis

Exemplos de gamas de trabalho analíticas para análises por FTIR

	Still wine		Fortified wine	
Analytical Parameter	*Lower Limit*	*Upper Limit*	*Lower Limit*	*Upper Limit*
Density (g/mL)	0.9880	0.9950	0.9915	1.0450
Alcoholic Strength (% v/v)	10.80	15.00	18.50	21.95
pH	3.00	3.80	3.20	3.80
Volatile Acidity (g/L)	0.15	1.00	0.16	0.98
Total Acidity (g/L)	4.00	6.50	3.14	6.00

The standard deviation (sd) associated with the results of the two replicates of each sample has been defined based on a repeatability study made on different white, red and rosé still wines, and different samples of Portuguese fortified wines.

In the repeatability study, for each analytical parameter, has been made series of 12 determinations in the same sample, in the same day, and using the same method and equipment. This methodology was followed in all levels (different types of wines) and different analytical parameters of the study. Each sd obtained was multiplied by 2,8 to obtain the repeatability results. The most unfavourable values obtained were chosen as the repeatability for each analytical parameter.

In order to check the daily work of the FTIR spectrophotometer, different pilot samples corresponding to Internal Control Standards (ICS) are used in routine to confirm the overall stability of the analytical calibrations. To achieve a large spectrum of matrices, different types of white and red fortified wines (old, young, dry and sweet) and different types of still wines (white and red) are normally used as ICS.

RESULTS AND DISCUSSION

For specific calibrations, the equipment selects the wave lengths with the higher statistical variation for the reference values of this analytical parameter (Moreira *et al*. 2002b) and gives a result that must validated in depth.

Analytical working range

First of all, as an obligatory precondition is the adjustment of the analytical working range. In order to establish the analytical working range for each parameter, analytical results have been crosschecked between AM and FTIR analysis. This comparison has been performed both over still wines (white, red and rosé) and different samples of Portuguese fortified wines. Concerning still wines, 139 samples were analysed for density (g/mL), 150 samples for alcoholic strength (% v/v), 148 samples for pH value, 138 samples for volatile acidity (g/L) and 130 samples for total acidity (g/L).

In fortified wines, the results of 188 samples for density (g/mL), 195 samples for alcoholic strength (% v/v), 129 samples for pH value, 188 samples for volatile acidity (g/L) and 187 samples for total acidity (g/L) were analysed.

FTIR methodology, as all other spectrophotometric methods, can only produce secure results if a specific working analytical range is respected. Only results that fall between the lowest and the highest analytical defined range should be considered. When dealing with FTIR methodology, the panoply of sample types and profusion of results usually discourages this complex job, but the daily experience says it is unavoidable.

Analytical working ranges for some parameters are given as an example (TABLE III), both in the case of still wine samples and fortified wine samples. Those limits are based on a meticulous study in which the results of several AM had been crosschecked with results obtained in FTIR (TABLE I and TABLE II)**.** As we can confirm in TABLE III, the analytical ranges chosen for each analytical parameter are within the maximum value and the minimum value that was observed in the study of crosschecking results for still wines (TABLE I) and fortified wines (TABLE II) referred above.

As a consequence, when performing routine analysis all analytical results that fall outside the defined and validated working ranges should be repeated using AM.

Standard Deviation (sd) associated with replicates

During the process of validation of the method, a maximum sd has been considered, based on the calculation of the repeatability for each analytical parameter. As criteria, the fixed limits must never exceed the repeatability value considered as it can be confirmed by comparing data on TABLE IV.

The maximum admissible standard deviations of the two replicates for density (g/mL), alcoholic strength (% v/v), pH value, volatile acidity (g/L) and total acidity (g/L) are shown in TABLE IV.

In routine analysis, it must be considered that, if the result of an analytical parameter exceeds the maximum established standard deviation, the reading for that parameter must be repeated.

As an example, a report of an analytical result (Run 1) showing the sd tolerance exceeded (0,036 % v/v) for alcoholic strength, and a repetition of this analytical result (Run 2) showing an acceptable sd (0,011% v/v) are presented in TABLE V. In Run 2 the sd was satisfactory, so, therefore, the mean result of the two replicates has been considered as correct.

Internal control standards (Pilot Samples)

Pilot samples corresponding to Internal Control Standards (ICS) are normally used to check if calibrations remain stable overtime. Alert and action limits must be stated for all analytical parameters being considered in the set of analysis. To establish reference values (Ref) of the control charts, AM must be used to perform sample analysis.

Action limits were applied to reference values, based on the maximum differences in absolute value obtained for each analytical parameter in the study of crosschecking results, for still wines (Table I) and fortified wines (Table II) referred above in Material and Methods.

Alert limits were defined, based on daily work experience in order to provide an effective control of the equipment and the calibrations and also to give indication of systematic deviations that might be

TABLE IV

Repeatability Study for still wine and fortified wine in FTIR and maximum sd established

Estudo de repetibilidade para Vinho Tranquilo e Vinho Fortificado em FTIR e definição de sd máximo

	Density (g/mL)	Alcoholic Strength (% v/v)	pH	Volatile Acidity (g/L)	Total Acidity (g/L)
Repetibility - still wine	0.0001	0.03	0.02	0.02	0.04
Maximum sd established for still wine	0.0001	0.03	0.02	0.02	0.04
Repetibility - fortified wine	0.0002	0.06	0.02	0.02	0.04
Maximum sd established for fortified wine	0.0001	0.03	0.02	0.02	0.04

TABLE V

Report of an analytical result (Run 1) showing the sd tolerance exceeded for alcoholic strength (%v/v), and repetition of this result (Run 2) showing an acceptable sd

Relatório de um resultado analítico (Run 1) que evidencia que a tolerância do sd foi excedida para titulo alcoométrico (%v/v) e em que a repetição do resultado (Run 2) apresenta um sd aceitável

	Date	Sample ID	Rep.	Density (g/mL)	Alcoholic Strength (% v/v)	pH	Volatile Acidity (g/L)	Total Acidity (g/L)
Run 1	14-05-2009	Pilot Dry White	1	1.0014	19.81	3.43	0.34	4.05
	14-05-2009	Pilot Dry White	2	1.0014	19.86	3.43	0.33	4.07
	14-05-2009	Pilot Dry White	Mean	1.0014	19.83	3.43	0.33	4.06
	14-05-2009	Pilot Dry White	Sd	0.00003	0.036	0.001	0.004	0.014
Run 2	**Date**	**Sample ID**	**Rep.**	**Density (g/mL)**	**Alcoholic Strength (% v/v)**	**pH**	**Volatile Acidity (g/L)**	**Total Acidity (g/L)**
	14-05-2009	Pilot Dry White	1	1.0014	19.86	3.43	0.34	4.08
	14-05-2009	Pilot Dry White	2	1.0014	19.88	3.43	0.33	4.10
	14-05-2009	Pilot Dry White	Mean	1.0014	19.87	3.43	0.33	4.09
	14-05-2009	Pilot Dry White	Sd	0.00004	0.011	0.001	0.004	0.015

occurring. Because calibrations are replaced and adjustments are made, these limits must be periodically reviewed.

As a rule, four pilot samples of fortified wine and two pilot samples of still wine must be introduced in a working sequence. In order to obtain reports for each analytical parameter, four fortified wines samples (one dry and one sweet fortified wine, and also a young and an old fortified wine) and two still wine samples (one white and one red wine) are analysed in routine.

Examples of alert and action limits for several analytical parameters are presented (TABLE VI). When those limits are exceeded, specific measures must be taken.

Those specific measures vary according to the different situations that may occur. So, if the results of a pilot sample do not exceed the alert limit, the validation of all the previous working sequence can be assumed.

If the results are above alert limit(s) but do not exceed the action limit(s) the validation of all the previous working sequence can be assumed, but the following concerns should be evaluated: (i) Possibility of an evolution or deterioration of the pilot sample (e.g. sample drawn from the bottom of a bottle, wrong setting for the sample, etc.); (ii) Need to adjust the analytical calibration (systematic deviations), by checking the historic of the method comparisons and, if possible, comparing it with the results of the working day.

On the other hand, if results of a pilot sample are above the action limit all the previous working sequence must be invalidated. However, the following concerns should be evaluated and the final decision must be taken according to the gathered elements: (i) Possibility of an evolution or deterioration of the pilot sample: Carrying out the sample determination

TABLE VI

Examples of alert and action limits for several analytical parameters

Exemplos de limites de alerta e de acção para diversos parâmetros analíticos

Analytical Parameters	Alert Limits	Action Limits
Density (g/mL)	Ref ± 0.0003	Ref ± 0.0004
Alcoholic Strength (% v/v)	Ref ± 0.12	Ref ± 0.15
pH	Ref ± 0.08	Ref ± 0.10
Volatile Acidity (g/L)	Ref ± 0.08	Ref ± 0.10
Total Acidity (g/L)	Ref ± 0.15	Ref ± 0.20

through the reference method for the parameter in question can clarify; (ii) checking if the readings comply with the established criteria by analyzing all available pilot samples in the FTIR spectrophotometer; (iii) carrying out an "equalizer standardization" of the equipment to verify the spectrophotometric condition; (iv) reviewing the history of the method comparisons and checking if it would be necessary to adjust the calibration (systematic deviations) for the parameter(s) in question. In this case, the calibration in use must be adjusted.

As an example, Fig. 1 represents a pilot control report in which it is possible to check that, during a given large period of time, all values of a pilot sample obtained for alcoholic strength (% v/v), fall between the alert limits.

Another example (Fig. 2), represents two values (05 Feb and 06 Feb) overtaking alert limits, but below the action limit adopted (TABLE VI).

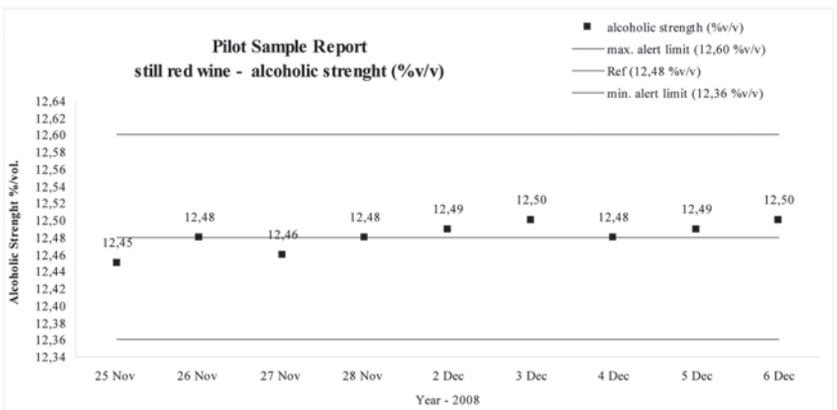

Fig. 1 - Internal control standards (pilot sample) with results between the alert limits for alcoholic strength (% v/v)

Padrão de controlo interno (amostra piloto) com resultados dentro dos limites de alerta para titulo alcoométrico (% v/v)

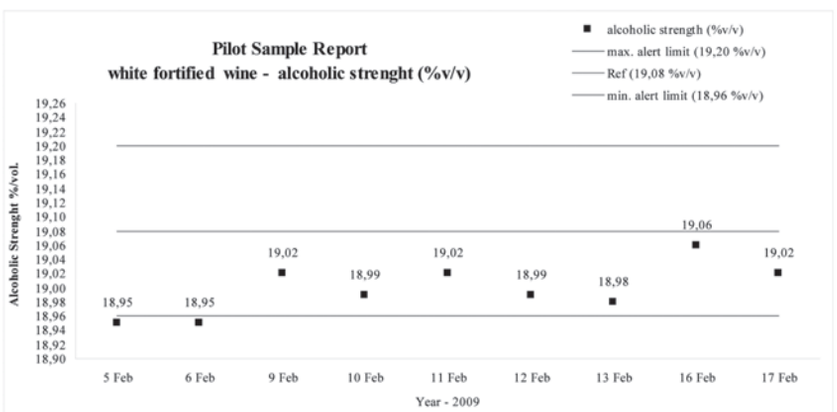

Fig. 2 - Internal control standards (pilot sample) with first two results above the alert limit but below action limit for alcoholic strength (% v/v)

Padrão de controlo interno (amostra piloto) com dois primeiros resultados ultrapassando o limite de alerta mas abaixo do limite de acção para título alcoométrico (% v/v)

After comparing results of alcoholic strength by volume obtained by FTIR and accredited method (TABLE VII) in samples of fortified wines and in the same days that the alert limits were exceeded, we have observed that the absolute differences (|a-b|) were far below the alert limits and also by the analysis of differences (a-b), we have concluded that there was not any systematic deviation that demanded an adjustment, and so, therefore, we have concluded that a calibration adjustment was unnecessary. This quite easy confirmation procedure has avoided a laborious recalibration work procedure.

TABLE VII

Comparison of alcoholic strength (% v/v) results between methods

Comparação de resultados entre métodos para titulo alcoométrico (% v/v)

		Alcoholic Strength (%v/v)			
Date	**Wine**	**FTIR**	**AM**	**Differences**	
		a	b	a-b	|a-b|
5 Feb	Fortified Wine 1	19.19	19.26	-0.07	0.07
5 Feb	Fortified Wine 2	20.22	20.15	0.07	0.07
6 Feb	Fortified Wine 3	19.87	19.86	0.01	0.01
6 Feb	Fortified Wine 4	19.67	19.60	0.07	0.07

Continuous crosschecking of results

The results obtained with FTIR spectrophotometer are regularly compared with those obtained from AM. By routine, an average of one sample a day, must be compared for all the analytical parameters, using both methods.

As values of the analyzed samples became more consistent with the defined working ranges (TABLE III), the differences in results are, in absolute terms, increasingly lower than the action limits (TABLE V).

However, if results are higher than the established action limits, the following concerns should be evaluated and the final decision, must be taken according to the gathered elements: (i) checking if there is a valid reason for the differences, namely whether the samples correspond to matrices different from those included in the calibration (e.g. non-stabilized, not clear, with gas), etc.; (ii) checking, if possible, the differences between results obtained by different methods for the parameter in question in two additional samples of the working day; (iii) reviewing the history of latest crosschecking of results and considering if a calibration adjustment is in fact necessary (analysis of systematic deviations).

CONCLUSIONS

The use of FTIR technology is extremely useful in wine laboratories because it provides precise and accurate results, provided that a strategy for the quality control of the analytical results obtained is implemented.

This strategy requires the implementation of different kinds of limits and criteria, namely: working range limits, standard deviation (sd) limits associated with the results of the two replicates of each sample, alert and action limits for control charts, etc. We have implemented specific measures for the cases where these limits are exceeded.

It is also very important to continuously crosscheck the results with accredited methods. FTIR technologies require that the alternative methods are still operating on a permanent basis. With the results of the continuous crosschecking, it is possible to detect and adjust systematic errors that might be occurring. In routine daily work, these strategies allow us to have significant control of the equipment, as well as great confidence in the results produced.

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