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GENERAL PAPER

Proficiency-testing scheme for haloanisoles and halophenols in oak wood

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Abstract
Haloanisoles are responsible for musty or mouldy off-flavours in wine. These molecules are extremely odorous and they alter wines in an irreversible way. The origin of haloanisoles can be attributed to the biodegradation of halophenols, which can be found in winery environments. Various materials including oak products (wood tanks, barrels, chips, staves) may be contaminated by haloanisoles and halophenols. Once polluted, these materials may release these molecules into wine. Requests for analyses of haloanisoles and halophenols in oak wood have gradually increased in recent years, above all from the coopers, who want to prove the quality of their products. However, the lack of an official testing method is an obstacle for the performance monitoring of laboratories. In response to these challenges, BIPEA organises, since October 2013, regular proficiency-testing schemes (PTS) for the detection and quantification of these molecules in oak wood. For each test, the statistical treatment of laboratories' results is performed according to ISO 13528. These PTS enable the participating laboratories to compare with each other, draw up a general inventory of their analytical skills and improve their performances for the detection and quantification of haloanisoles and halophenols in oak wood.

Keywords Proficiency-testing schemes · Haloanisoles and halophenols · Oak wood · Wine quality control · Laboratory performance

Introduction

Haloanisole contamination is a serious problem for wine quality [1]: even trace amounts of 2,4,6-tricloroanisole (3CA), 2,3,4,6-tetrachloroanisole (4CA), pentachloroanisole (5CA) and 2,4,6-tribromoanisole (3BA) can cause musty or mouldy off-flavours in wine [2]. Each of these haloanisoles has a similar odour but possesses different sensory thresholds. These compounds are not naturally occurring wine constituents. The origin of haloanisoles can be attributed to the biodegradation of 2,4,6-tricholorophenol (3CP), 2,3,4,6-tetrachlorophenol (4CP), pentachlorphenol (5CP) and 2,4,6-tribromophenol (3BP), respectively, which can be found in winery environments [3]. Several materials, including barrel oak wood, may be contaminated and release these molecules into wine. The coopers' need to prove the quality of manufactured barrels increases the requests for analyses of haloanisoles and halophenols (HAHP) in oak wood. However, coopers and laboratories face difficulties in results interpretation due to the lack of an official testing method. Different analytical methods, more or less comparable, have been implemented by laboratories. The main goal of setting up this PTS is to develop an evaluation process for laboratory performances, considering that laboratories can perform extraction (composition) or migration analyses, the results of which are not equivalent. Analyses on corks, which have similar aims to those on oak wood, are mostly migration ones, because the product impact makes the extraction analysis less relevant for producers' needs. Both methods have advantages and disadvantages; migration analysis is more similar to the real migration conditions in barrels because it reflects the thermodynamic equilibrium of haloanisoles and halophenols between wood chips and the model wine solution. However, migration times are unknown and long. From the cooper's perspective, this delay is problematic since corrective actions are similarly delayed. Extraction analyses take less time than migration ones, and results are generally more accurate. On the other hand, the interpretation of results is difficult, because the migration

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and equilibrium conditions of the organohalogen compounds in wood and in wine or alcoholic solution are still unknown.

This work describes the design and the implementation of a PTS for the analyses of haloanisoles and halophenols in oak wood samples, with a focus on the results of the proficiency test of March 2018.

Experimental

Sample production and shipment

Production of homogenous and stable samples is a crucial point for the implementation of a proficiency test. Homogeneity and stability of the samples must be demonstrated to avoid misjudging laboratory performance owing to lack of sample homogeneity. The preparation of oak-wood samples spiked with haloanisoles and halophenols was set up to reach a stable equilibrium between free and absorbed molecules. Woodchips are soaked in a diluted ethanol solution of haloanisoles and halophenols in a closed container during a period of 15 days, to allow the migration of halophenols and haloanisoles into the wood. A slow evaporation of the solvent with a consequent stabilization of the spiked product follows this first step. Finally, the woodchips are shaken to ensure proper homogeneity, protected by aluminium foil and packaged in a bag under vacuum.

Woodchips sent to laboratories are 2 mm in size, with spiking concentrations between 0.5 ng/g and 5.0 ng/g for haloanisoles and between 2.0 ng/g and 20 ng/g for halophenols.

The homogeneity of the samples is verified by experimental studies on 10 samples in duplicate, taken randomly across the batch of samples, according to the requirements of ANNEX B of the ISO 13528 standard [4]. The results are analysed through several statistical tests:

- Fisher test (variance analysis): observed F value < critical F value;
- Test of significant non-homogeneity: between-sample variance < critical c value;
- Study of the ratio of the between-sample standard deviation (s_s) and the standard deviation for proficiency assessment (σ_{pt}): $s_s \leq 0.3\sigma_{pt}$.

The stability of the samples during the test period is checked on the packaged samples stored at 5 °C ± 3 °C. According to the ISO 13528 standard [4], the samples can be considered stable if the absolute difference between the means at t_0 and t_1 (t_0 + 3 weeks, the test period accorded to participants) is inferior or equal to 0.3 × the standard deviation for proficiency assessment ($|y_0 - y_1| \leq 0.3\sigma_{pt}$).

The processed batches were proven to be homogeneous and stable at 5 °C ± 3 °C.

Results collection and data statistical treatment

Once the homogeneity and the stability have been demonstrated, the samples are shipped at 5 °C ± 3 °C to all participants, who are invited to analyse the samples as soon as possible after reception.

The results of laboratories are collected via a reply form available online over a period of 3 weeks. Migration and extraction analyses are divided and evaluated separately: to harmonise migration analyses between all the laboratories, participants are asked to perform the analysis according to the following conditions (inspired by the ISO 20752 standard [5] for cork stoppers adapted to the needs of coopers):

- Solvent: model wine solution (ethanol 20 % by volume, adjusted to pH 3.4 with tartaric acid)
- Temperature: room temperature (20 °C ± 2 °C), without stirring
- Soaking time: 24 h
- Woodchips ratio in the model wine solution: 50 g/L.

The soaking time was chosen after some previous studies had demonstrated that equilibrium between the concentration of these molecules in a model wine solution and woodchips is reached in 24 h.

No instructions are given for extraction analyses, but laboratories are invited to provide information about the method performed in the reply form.

The statistical treatments of the returned results are conducted according to ISO 13528 [4]. The assigned values (x_{pt}) are estimated using the robust means of all results from the application of robust algorithm A. Laboratories with a lack of traceability are not taken into account in the statistical estimations.

Data interpretation

Performances of each laboratory are evaluated using robust standard deviations (s^*) set as the standard deviation for performance assessment (σ_{pt}). This value is used to identify an interval around the assigned value. Results in this range are considered as satisfactory. Moreover, laboratory results (x) are also evaluated through z-scores (z). The z-score for a result x_i is calculated as:

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

where σ_{pt} is the standard deviation for proficiency assessment. Laboratories with a "z score $\leq |2|$" or "z

score > | 3 | " are considered having reported "Satisfactory" or "Unsatisfactory" results, while the remaining laboratories reported "Questionable" results. Results are published in a specific interlaboratory comparison report distributed to all participants who can then classify their results and implement some corrective and/or preventive actions if necessary.

Results and discussion

Results of the proficiency test of March 2018 are examined in detail. Eight laboratories reported their results for migration analyses and 10 for extraction ones. Table 1 summarises the statistical data of this test for each compound analysed. Assigned values (x_{pt}) were estimated for all compounds except for the migration analysis of pentachlorophenol due to the wide dispersion of the results. Uncertainties, $u(x_{pt})$, that allow quantification of the confidence that can be given to the assigned value, were calculated as indicated in paragraph 7.7 of the ISO 13528 standard [4]. Due to the low number of results and their dispersion, the ratio between uncertainties and standard deviations are ≥ 0.44 for all studied compounds. It has to be noted that laboratory results obtained for migration and extraction analyses are significantly different and reflect the different approach of these two methods. Concerning laboratory performances, only two results were unsatisfactory for migration analyses of 2,4,6-trichloroanisole and 2,3,4,6-tetrachlorophenol and only one for extraction analyses of 2,4,6-trichlorophenol and pentachlorophenol.

Figure 1 describes graphically the degree of dispersion and skewness in data for the extraction and migration analyses of all compounds, except for pentachloroanisole and pentachlorophenol. In these boxplots, median, minimal and maximal values are also indicated. The range of dispersion varies according to the organohalogen compound analysed and the concentration levels. This dispersion can be mainly linked to the nature of the product (woodchips) and then analysis procedures of the laboratories.

For extraction analyses, laboratories returned information about the solvent, the temperature and time of extraction. This collection of information highlights that the extraction conditions of the laboratories are quite varied, soaking time ranges from 15 min to 24 h and 5 different solvents are used (diethyl ether, dichloromethane, methanol, ethanol and 40 % ethanol). All laboratories work at room

Table 1 Main statistical parameters of the proficiency test of March 2018

Compound	Analysis	$p(x_{pt})$a	x_{pt}[b] (ng/g)	$u(x_{pt})$[c] (ng/g)	σ_{pt}[d] (ng/g)	σ_{pt} (%)	$u(x_{pt})/\sigma_{pt}$	p_S[e]	p_Q[f]	p_U[g]	Ratio of x_{pt}[h] (%)
2,4,6-Tribromoanisole	Extraction	8	1.97	0.28	0.63	32	0.44	9	0	0	12
	Migration	6	0.24	0.05	0.09	38	0.56	8	0	0	
2,4,6-Trichloroanisole	Extraction	7	3.67	0.91	1.92	52	0.47	9	0	0	31
	Migration	6	1.15	0.09	0.18	16	0.50	5	1	2	
2,3,4,6-Tetrachloroanisole	Extraction	8	2.21	0.29	0.66	30	0.44	10	0	0	14
	Migration	5	0.30	0.09	0.15	50	0.60	8	0	0	
Pentachloroanisole	Extraction	8	18.11	3.88	8.78	48	0.44	10	0	0	6
	Migration	5	1.07	0.29	0.51	48	0.57	8	0	0	
2,4,6-Tribromophenol	Extraction	8	4.22	0.79	1.79	42	0.44	10	0	0	16
	Migration	5	0.68	0.18	0.32	47	0.56	8	0	0	
2,4,6-Trichlorophenol	Extraction	7	5.19	0.67	1.42	27	0.47	9	0	1	24
	Migration	6	1.27	0.38	0.74	58	0.51	8	0	0	
2,3,4,6-Tetrachlorophenol	Extraction	8	8.11	1.31	2.97	37	0.44	10	0	0	20
	Migration	4	1.63	0.17	0.28	17	0.61	6	0	2	
Pentachlorophenol	Extraction	7	70.51	9.08	19.22	27	0.47	9	0	1	–
	Migration	Due to the wide dispersion of the results, no assigned value was estimated									

[a] $p(x_{pt})$: Number of results taken into account for the estimation of the assigned value

[b] x_{pt}: Assigned value

[c] $u(x_{pt})$: Standard uncertainty of the assigned value: $u(x_{pt}) = 1.25 * s^* / \sqrt{p(x_{pt})}$

[d] σ_{pt}: Standard deviation for proficiency assessment: $\sigma_{pt} = s^*$

[e] p_S: Number of satisfactory results

[f] p_Q: Number of questionable results

[g] p_U: Number of unsatisfactory results

[h] Ratio of x_p: Ratio of assigned values (x_{pt} migration)/(x_p extraction).

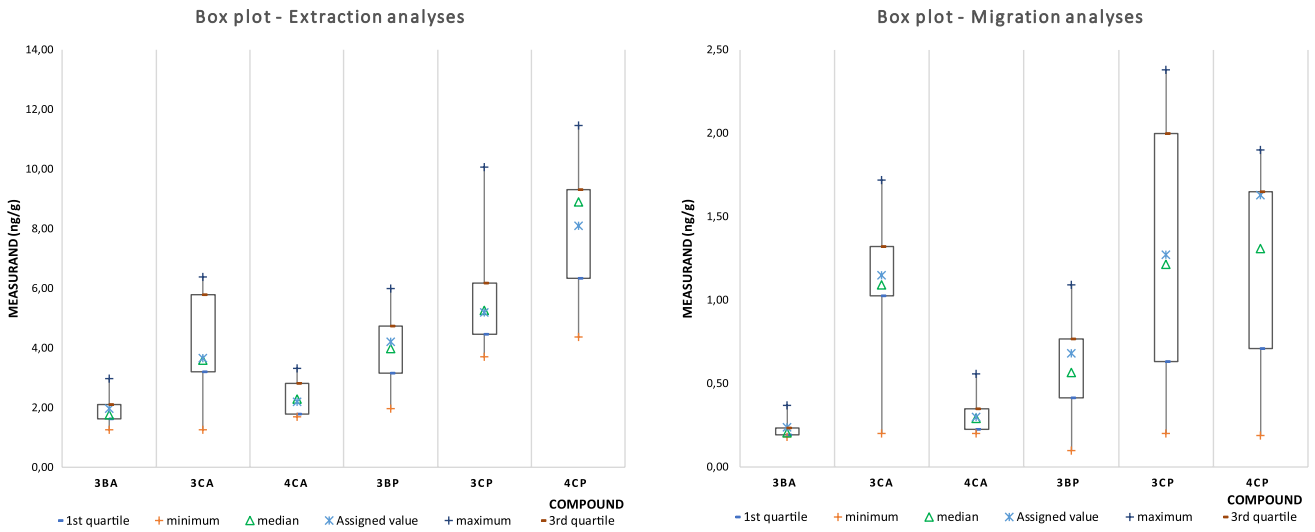
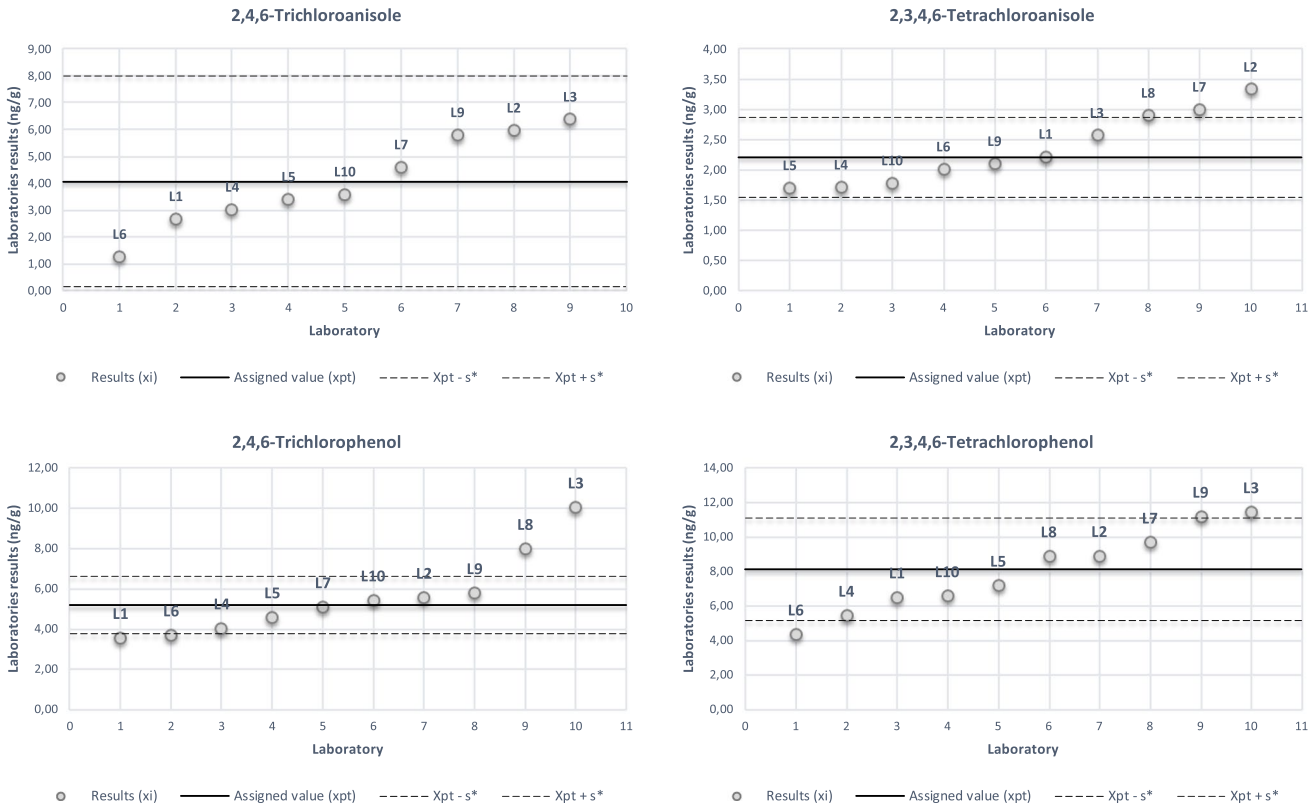


Fig. 1 Boxplots with whiskers from minimum to maximum describing the results obtained for extraction and migration analyses, test of March 2018 (ng/g)



Key

L1	Not specified	**L6**	Et_2O, 50 min
L2	EtOH, 24h	**L7**	MeOH, 15 min
L3	EtOH, 24h	**L8**	MeOH, 15 min
L4	Et_2O, 30 min	**L9**	CH_2Cl_2, 2h
L5	Et_2O, 30 min	**L10**	EtOH 40%, 24h

Fig. 2 Graphs describing results obtained for extraction analyses of 2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole, 2,4,6-trichlorophenol, 2,3,4,6-tetrachloropenol—test of March 2018

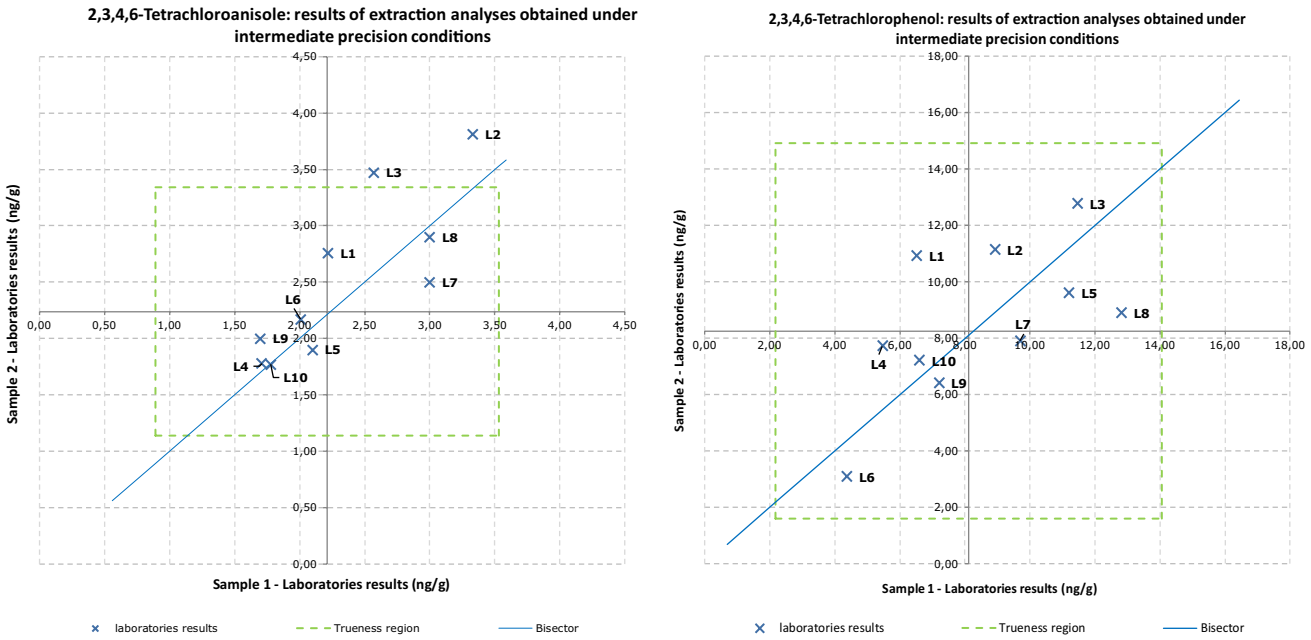


Fig. 3 Extraction analyses: Youden graphs comparing laboratory results obtained under intermediate precision conditions for 2,3,4,6-tetrachloroanisole and 2,3,4,6-tetrachlorophenol, test of March 2018

temperature (between 20 and 25 °C) and use a mass spectrometer for the final quantification. Results of extraction analysis of 2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole, 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol are shown graphically in Fig. 2. The extraction solvent and soaking time used by each participant are indicated in the key below these graphs. Generally, laboratories using diethyl ether as solvent tend to underestimate the concentration of haloanisoles and halophenols. On the contrary, higher results are obtained using ethanol and methanol. However,

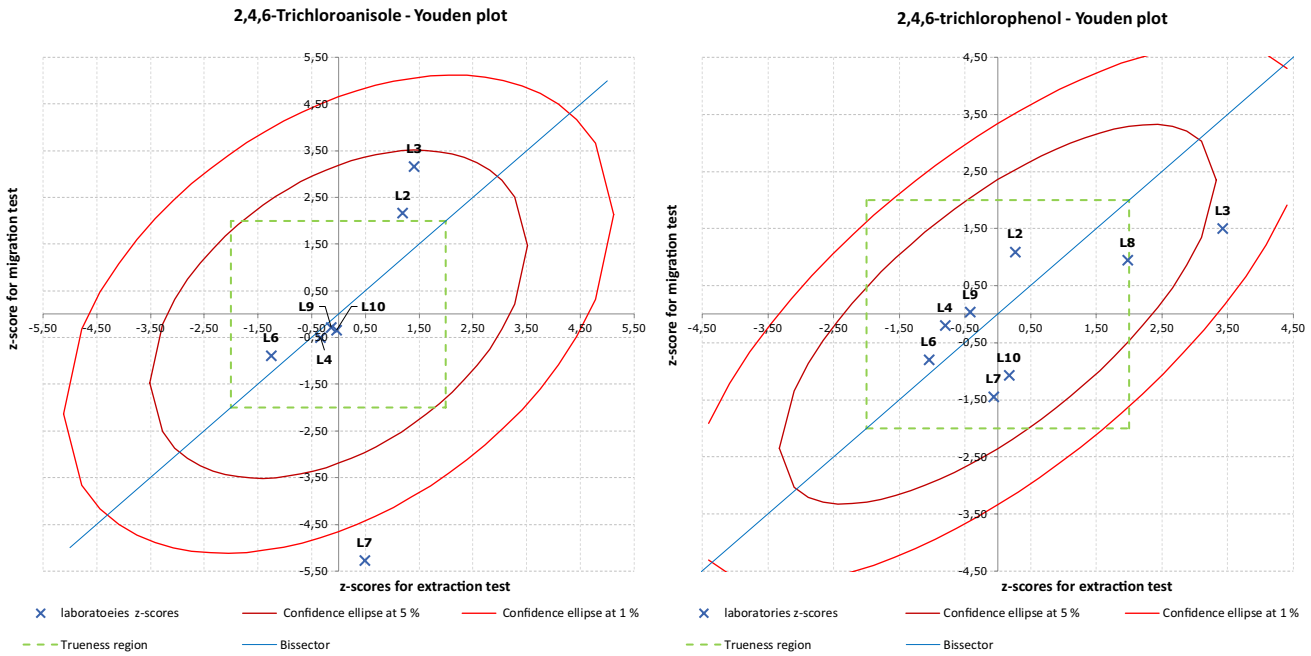


Fig. 4 Youden plot (confidence ellipse based on the Jackson method) comparing laboratories' migration and extraction z-scores for 2,4,6-trichloroanisole and 2,4,6-trichlorophenol, test of March 2018

it remains difficult to highlight a tendency as a function of the procedure performed because many different factors can affect the results.

To have an idea of the within-laboratory variability for these extraction analyses, new samples of the same batch were sent to laboratories 3 weeks after this first trial. Participants were asked to analyse samples under the same extraction conditions preformed on the previous one. Youden graphs were constructed with data collected for these two proficiency tests, which correspond to results obtained under intermediate precision conditions. Figure 3 shows results obtained for 2,3,4,6-tetrachloroanisole and 2,3,4,6-tetrachlorophenol. Most of the laboratories (represented as blue crosses) are close to the bisector, which indicates the same two results.

As the same sample is sent to laboratories for extraction and migration analyses, values of laboratories that returned quantitative results for both methods were also compared through z-scores to check any correlation of the results obtained. Examples of Youden plots comparing laboratories' migration and extraction z-scores for 2,4,6-trichloroanisole and 2,4,6-tricholophenol are shown in Fig. 4: *z*-scores obtained for the migration test are reported on the ordinate axis and those obtained for the extraction on the abscissa. A bisector and two ellipses for the 1 % and 5 % probability levels are also plotted as an aid to interpretation of these plots. Much information can be obtained from these graphs: The first is that results are satisfactory for 2,4,6-trichrophenol, with only one laboratory out of the trueness region and the confidence ellipse at 5 %. Concerning 2,4,6-trichloroanisole, three laboratories are out of the trueness region with one out of the confidence ellipse at 1 % (due to an under-estimation for the migration test). These graphs show also that laboratories with high scores for extraction obtained higher results for migration analyses too.

Interesting information provided by the data of these proficiency testing schemes is the amount of haloanisoles and halophenols that can migrate from wood in a standard wine solution at 20 °C, over 24 h.

The migration percentage was estimated for each compound taking into account the ratios of the assigned values of extraction and migration analyses (Table 1). This percentage varies according the molecule, from 31 % for 2,4,6-trichloroanisol to 6 % for pentachloroanisole. It has to be noted that, for each haloanisole and halophenol, this ratio is not linked to contamination levels of the raw material.

Conclusions

PTS on analyses of haloanisoles and halophenols in oak chips have been implemented successfully, from both the homogeneous and stable sample production and the statistical point of view. In the absence of an official method, these tests allow laboratories to compare their results and to obtain recognition of their analytical procedures by coopers and, above all, accreditation bodies according to the ISO 17025 standard [6]. Consistent involvement in PTS helps laboratories to improve their analytical procedures and to have a critical point of view on results obtained by extraction and migration analyses. These two analytical methods are in fact complementary and should be used in parallel according to the results expected and the analytical needs of clients.

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