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Rapid headspace solid-phase microextraction/gas chromatographic/mass spectrometric assay for the quantitative determination of some of the main odorants causing off-flavours in wine

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Abstract

In this study we present a rapid and simultaneous assay method using headspace (HS) solid-phase microextraction (SPME)/gas chromatography (GC)/electron impact (EI) mass spectrometry (MS) (selected ion monitoring) for contaminants causing the principal organoleptic defects of wine (2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole, pentachloroanisole, 2,4,6-tribromoanisole, 1-octen-3-ol, geosmin, 2-methylisoborneol, 3-isopropyl-2-methoxypyrazine, fenchol, fenchone, 2-methoxy-3,5-dimethylpyrazine, 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol, 4-vinylguaiacol, 3-isobutyl-2-methoxypyrazine, guaiacol and ethyl acetate). The method was validated according to protocols NF ISO 5725-1, 2 and NF V03-110. Its characteristics (limit of detection (LOD), limit of quantification (LOQ), uncertainties) were determined after having optimised the SPME parameters. The target contaminants were quantified in the wines below their threshold of perception with a satisfactory relative standard deviation for all the analytes except ethyl acetate (RSD = 36%); for that, the assay method permits clear differentiation of the wines that are at risk of presenting an acescent character, i.e. containing more than 120 mg L^{-1} ethyl acetate. The target volatile and odorous substances were determined at concentrations significantly below their threshold of perception in a hydroalcoholic context and their threshold of recovery in wines.

Keywords: Wine; Off-flavours; SPME; Validation of method; GC–MS

1. Introduction

The control of the organoleptic quality of wines is indispensable if the consumer is to be presented with a high-quality product. To date, sensory analysis is a subjective technique subject to a number of problems. In addition, qualification of an organoleptic defect is very difficult in the absence of objective data regarding the identification and precise quantification of the contaminants responsible for the disorder that has been detected. Finally, when using these data, it is always necessary to link them to the thresholds of perception of the substances in question in order to be able to draw conclusions actually on the probable responsibility and origin of the adulteration. From this perspective it is interesting to employ a rapid analytical method that enables the simultaneous quantification of the principal molecules identified as responsible for the main olfactory disorders of wines.

The main organoleptic defects of wines involve molecules that have thresholds of perception that range from some 100 mg L^{-1} for ethyl acetate to nanograms per litre for 2,4,6-trichloroanisole. In order to determine the origin of these contaminants, it is necessary to quantify the molecules responsible on this side of their thresholds of perception in the wines by means of a method that is sufficiently simple and sensitive.

Solid-phase microextraction (SPME), developed by Arthur and Pawliszyn in 1990 [1], used in headspace mode (HS-SPME) and coupled with high-performance gas chromatography (GC), allows the quantification of a large number of molecules with sufficiently low limits of detection and good linearity over a considerable dynamic range. Mass spectrometry used in specific fragmentometry after ionization by constant-energy electron impact and in the selected ion monitoring mode (EI-MS–SIM) allows detection that is simultaneously versatile, sensitive and very specific. In addition, this method, easily automated and reliably repeatable, uses no solvent and little or no preparation of

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Table 1
Indicative threshold of olfactory perception of the molecules responsible for the principal organoleptic defects of wine

	Odour	Olfactory threshold in the wine	Origin	Refs.
2,4,6-Trichloroanisole (TCA)	Mould	3 ng L^{-1}	Bio-methylation of corresponding phenol, source of pollution variable	[2–5]
2,3,4,6-Tetrachloroanisole (TeCA)	Mould-dust	15 ng L^{-1}	Bio-methylation of corresponding phenol, source of pollution variable	[3–5]
2,4,6-Tribromoanisole (TBA)	Mould	3 ng L^{-1}	Bio-methylation of corresponding phenol, source of pollution variable	[6]
Pentachloroanisole (PCA)	Dust	10 000 ng L^{-1}	Bio-methylation of corresponding phenol, source of pollution variable	[4]
1-Octen-3-ol	Fungus	40 μg L^{-1}	Fungal flora on the grape	[7,8]
1-Octen-3-one	Fungus	70 ng L^{-1}	Fungal flora on the grape	[7,8]
2-Methylisoborneol (2MIB)	Muddy	55 ng L^{-1}	Fungal flora on the grape	[9–12]
Geosmin	Muddy	50 ng L^{-1}	Fungal flora on the grape	[9–12]
2-Isopropyl-3-methoxypyrazine (IPMP)	Muddy	15 ng L^{-1}	Endogenous presence in the grape	[13]
(+)-Fenchone	Muddy	500 μg L^{-1} (water)	Fungal flora on the grape	[12,14]
(+)-Fenchol	Muddy	50 μg L^{-1} (water)	Fungal flora on the grape	[12,14]
2-Methoxy-3,5-dimethylpyrazine (2M35DP)	Corky	2.1 ng L^{-1}	Bacterial or fungal contamination of the cork	[15]
Ethyl-4-phenol (E4P)	Phenolic	430 μg L^{-1}	Formed by *Brettanomyces Dekkera* yeasts	[16–18]
Ethyl-4-guaiacol (E4G)	Phenolic-spicy	33 μg L^{-1}	Formed by *Brettanomyces Dekkera* yeasts	[16–18]
Ethyl-4-catechol (E4C)	Phenolic	50 μg L^{-1}	Formed by *Brettanomyces Dekkera* yeasts	[19]
Vinyl-4-guaiacol (V4G)	Pharmaceutic-spicy	380 μg L^{-1}	Formed by the yeast *Saccharomyces cerevisiae*	[20]
Vinyl-4-phenol (V4P)	Pharmaceutic-gouache	1500 μg L^{-1}	Formed by the yeast *Saccharomyces cerevisiae*	[20]
2-Isobutyl-3-methoxypyrazine (IBMP)	Vegetal-green pepper	15 ng L^{-1}	Endogenous presence in the green grape	[21,22]
Ethyl acetate	Acescent/sour	120 mg L^{-1}	Formation by acetic bacteria	[23]
Guaiacol	Smoky	50 μg L^{-1}	Bacterial (or thermic) transformation of the vanillin in guaiacol	[24]

Note: 4-ethylcatechol and 1-octen-3-one, which require special procedures [8,19] of analysis in GC/MS (stage of derivation and/or specific extraction in SPE) were not determined by the rapid method described in this study.

the sample. We have used a HS-SPME/GC/EI-MS–SIM combination to quantify by internal standardization 18 molecules that are of interest because they have been identified as being responsible for a large number of faults encountered in today's wine industry (Table 1). The method was validated in intra-laboratory trials according to the International validation criteria NF ISO 5725-1, 2 and NF V03-110 [25–27] with the aim of defining the characteristics of the method [linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, margin of error] for each analyte under consideration assayed simultaneously.

2. Materials and methods

2.1. Chemical standards

2,4,6-Trichloroanisole [87-40-1] 99%, 2,3,4,5-tetrachloroanisole [938-86-3] 98% (in equivalence of 2,3,4,6-TeCA), 2,3,4,5,6-pentachloroanisole [1825-21-4] 98%, 4-ethylphenol [123-07-9] 99%, 4-ethylguaiacol [2785-89-9] >98%, 4-vinylphenol [2688-17-3] 10% in polypropylene glycol, 4-vinylguaiacol [7786-61-0] 98%, guaïacol [90-05-1] 98%, 1-octen-3-ol [3391-86-4] 98% and L-(+)-tartaric acid 99% from Aldrich, geosmin [19700-21-1] 100 μg mL^{-1} in methanol, 2-methylisoborneol [2371-42-8] 100 μg mL^{-1} in methanol, 3-isobutyl-2-methoxypyrazine [24683-00-9] 100 μg mL^{-1} in methanol and 3-isopropyl-2-methoxypyrazine [25773-40-4] 100 μg mL^{-1} in methanol from Supelco, fenchol [2217-02-9] >99%, fenchone [4695-62-9] >99.5%, methyl-4-pentan-2-ol (4M2P) [108-11-2] 97% and sodium chloride >99.5% from Fluka, sodium hydroxide pellets 99% and absolute ethanol 99.8% from Riedel-de Haën were supplied by Sigma–Aldrich (Saint Quentin Fallavier, France); [$^2H_5$]2,4,6-tribromoanisole [607-99-8] and [$^2H_{10}$]4-ethylphenol [352431-18-6] from CDN isotopes (Pointe-Claire, Canada); ethyl acetate [141-78-6] 99.8% from SDS (Peypin, France); the water used was of Milli-Q quality (18.2 MΩ cm) supplied by a Simplicity 185 system (Millipore, Bedford, MA, USA); the 2-hydroxy-3,5-dimethylpyrazine and [$^2H_3$]2-hydroxy-3,5-dimethylpyrazine were synthesized according to the method described by Simpson et al. [15]. The deuterated chloroanisole analogues ([$^2H_3$]TCA, [$^2H_3$]TeCA, [$^2H_3$]PCA) were synthesized according to the method described by Chatonnet et al. [5].

SPME fibres coated with divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm (reference 57329-U) and polydimethylsiloxane 100 μm (reference 57301) were purchased from Supelco (Saint Quentin Fallavier; France).

2.2. Reference solutions

A solution is obtained containing the different internal standards in absolute ethanol at a concentration of 50 μg L^{-1} for [$^2H_3$]TCA (standard for TCA), [$^2H_3$]TeCA (standard for TeCA),

[$^2H_3$]PCA (standard for PCA), [$^2H_5$]TBA (standard for TBA), at 1 g L^{-1} for [$^2H_{10}$]E4P (standard for E4P, E4G, V4P, V4G), at 250 mg L^{-1} for 4M2P (standard for ethyl acetate, guaiacol, fenchol, fenchone, 2MIB, geosmin, 1-octen-3-ol) and at 100 μg L^{-1} for [$^2H_3$]2M35DP (standard for 2M35DP, IBMP, IPMP).

The concentrations of the analytes in the standard reference solution are as follows: 1 μg L^{-1} for TCA, TeCA, PCA and TBA; 10 μg L^{-1} for IBMP and IPMP; 20 μg L^{-1} for geosmin and 2MIB; 5 μg L^{-1} for 2M35DP, 25 mg L^{-1} for guaiacol; 1 mg L^{-1} for octen-3-ol, fenchol and fenchone; 10 mg L^{-1} for E4P, V4P and V4G; 50 mg L^{-1} for E4P and 10 g L^{-1} for ethyl acetate.

Calibration was achieved by adding 10, 20, 50, 100 and 250 μl of the standard solution to 10 ml of a hydroalcoholic solution at 6% (v/v) of ethanol containing 5 g L^{-1} of tartaric acid adjusted to pH 7 with 0.5 M NaOH.

2.3. Preparation of the sample

Fifty milliliters of wine was adjusted to pH 7 (with a 36 M sodium lye as far as pH 6 then adjusted precisely to pH 7 with 0.5 M NaOH on a pH meter). Five milliliters of the sample was placed in a 20 mL SPME vial with 5 mL Milli-Q water at pH 7 and 3.0 g (+/− 0.1 g) of NaCl. Ten microliters of internal standard solution was added, and the vial was mounted with a capsule fitted with a silicone/PTFE joint. The vial was analysed by HS-SPME/GC/MS with a DVB/CAR/PDMS fibre previously treated according to the recommendations of the supplier (1 h at 270 °C). The vial had been equilibrated for 3 min at 45 °C (250 rpm) then extracted for 60 min at 45 °C (250 rpm). Desorption of the fibre in the injector proceeded at 270 °C for 5 min.

2.4. Materials

Gas chromatography coupled with mass spectrometry was performed on a 6890 chromatograph coupled with a 5973Inert Agilent mass detector equipped with a MPS2 Gerstel carrier and injector. The carrier was used in the SPME configuration.

2.5. Chromatographic analysis and detection conditions

GC/MS analysis was performed on a Varian FactorFourVF-5 ms column 30 m × 0.25 mm I.D., with 0.25 μm film thickness and a EZ-Guard 10 m protective column of deactivated silica. The carrier gas was Helium (Helium N55, Air Product, Toulouse, France) programmed to flow at a constant linear speed of 45 cm s^{-1} during all the run (flow 1.5 mL min^{-1}). The injector was a standard split/splitless operated in splitless mode at 270 °C (gas shield at 5.90 min flow 15 mL min^{-1}) with a Supelco SPME borosilicate glass insert of 0.75 mm internal diameter. The oven program started at an initial temperature of 50 °C for 2.0 min. Temperature was then increased at a rate of 3.0 °C min^{-1} to 190 °C, then at 50 °C min^{-1} to 320 °C, the oven was held to 320 °C for 1 min. Detection was achieved with a HP-5973Inert quadrupolar mass detector working with EI ionization (EI, source temperature 230 °C, temperature of quaduropole 150 °C, energy of constant ionisation 70 eV, multiplier of electrons 1600 V) and was operated in the SIM mode (SIM) on the selected ions characteristic of each molecule (the quantification ions are detailed below). The analysis takes 52 min, and a sample was injected every 63 min. With the aim of improving the sensitivity, 10 groups of ions were formed in order to work with the longest possible Dwell time on each ion of quantification. In addition, the electron multiplier operating at 1600 V was boosted by +200 V to optimise the detection of the 2M35DP.

Group 1 [0.0 8.0 min]: ethyl acetate [43 (dwell 10 ms), 61(100), 70(10)]; 4M2P [45(100), 69(100), 87(10)].
Group 2 [8.0 14.0 min]: 1-octen-3-ol [57(10), 72(100), 85(10), 99(10)].
Group 3 [14.0 16.6 min]: [$^2H_3$]2M35DP [141(100), 112(10), 123(10), 140(10)]; 2M35DP [138(100), 137(10), 120(10), 109(10)].
Group 4 [16.6 19.2 min]: fenchone [152(50), 81(10), 69(10), 109(10)]; fenchol [80(50), 81(10), 121(10), 111(10)]; guaiacol [124(50), 109(10), 81(10)]; IPMP [137(100), 152(10), 124(10)].
Group 5 [19.2 24.6 min]: [$^2H_{10}$]E4P [113(50), 131(10)]; E4P [107(50), 122(10)]; IBMP [124(10), 151(100), 94(10)]; 2MIB [135(100), 95(10), 108(10), 168(10)]; V4P [120(50), 91(10), 119(10)].
Group 6 [25.0 27.5 min]: E4G [137(100), 152(10)]; V4G [150(100), 135(10), 107(10)].
Group 7 [27.5 35.5 min]: [$^2H_3$]TCA [213(100)]; TCA [210(100), 212(10)]; geosmin [182(100), 112(10), 149(10), 125(10)].
Group 8 [35.5 37.5 min]: [$^2H_3$]TeCA [249(100)]; TeCA [244(10); 246(100)].
Group 9 [37.5 42.0 min]: [$^2H_5$]TBA [349(100)]; TBA [346(100), 344(10)].
Group 10 [42.0 45.0 min]: [$^2H_3$]PCA [283(100)]; PCA [280(100), 282(10)].

2.6. Validation of the assay method

The proposed analytical method was evaluated by comparison, on the one hand, with the validation protocol of an ordinary method and the methods of calculation described in the OIV frame of reference (2001), and on the other hand, with the French criteria for intra-laboratory validation NF ISO 5725-1 and NF V 03-110 (AFNOR, 1998). Table 2 shows for each experimental design the number of samples, of replications and of measurements.

3. Results

3.1. Optimisation of the method

The parameters of the method were optimised by focusing on 2M35DP, the molecule on which it was the most difficult to obtain a detection limit in keeping with its threshold of perception in wines (2.1 ng L^{-1}). It was verified that all the

Table 2
Number of measurements performed for the different experimental designs for intra-laboratory validation of the assay methods

Experimental design	Caractéristic measured	Samples	Replications	Measures
A	Linearity, limits of determination	5 or 6	5	25–30
B	Specificity	10	1	10
C	Reliability and precision	15	2	30
D	Internal reproductibility	10	2	20
	Total			90

analytes considered presented rates of recovery and levels of detection compatible with their thresholds of perception and the concentrations expected in wine. For the optimisation, we worked with vials supplemented with 100 μl of the solution of the various standards in a hydroalcoholic solution (concentration of the analytes in the vial: ethyl acetate 85 mg L^{-1}; 1-octen-3-ol 10 μg L^{-1}; fenchone 11 μg L^{-1}, fenchol 12 μg L^{-1}, guaiacol 243 μg L^{-1}; 2MIB 202 μg L^{-1}; geosmin 206 μg L^{-1}; 2M35DP 53 ng L^{-1}; IPMP 50 ng L^{-1}; IBMP 49 μg L^{-1}; TCA 10 ng L^{-1}; TeCA 10.5 ng L^{-1}; TBA 13 ng L^{-1}; PCA 10 ng L^{-1}; E4P 435 μg L^{-1}; E4G 103 μg L^{-1}; V4P 108 μg L^{-1} and V4G 100 μg L^{-1}).

3.1.1. Choice of fibre

Taking account of the physico-chemical characteristics of the contaminants under consideration, we tested two types of fibre (PDMS and DVB/CAR/PDMS) among those used most routinely for assaying each family of molecules (Fig. 1). We chose the DVB/CAR/PDMS fibre, which was found to be the most effective for all the target molecules.

3.1.2. Dilution of the sample

The goal of dilution is to lead the ethanol of the sample around 6% (v/v) in order to improve the headspace of the analytes, thus assisting the extraction by fiber SPME and having for consequence an increase of the signal to noise ratio. This result is not inevitably true for all the molecules (it is what we checked in our paper) and then, according to our experiment, a more important dilution never involves an improvement of the signal to noise ratio for compounds at the traces level (nanogram per liter). More trials with more dilution ratio have been tried with no improvement (data not published). That is why we have chosen the dilution to ½ which improves the extraction of all the molecules (Fig. 2) except those of the haloanisoles which remain stable.

3.1.3. Optimisation of pH

pH is an important parameter when the aim is to quantify the molecules belonging to many families of molecules in solution. Taking their pK_a into account, the pyrazines (p$K_a \approx 0.50$) are generally very easily analysed in the gaseous phase at a neutral or basic pH, whereas the phenols (p$K_a \approx 10$) or the esters (p$K_a \approx 25$) are best analysed at an acid pH. The optimum pH (Fig. 3), corresponding to the maximum extraction of 2M35DP without significant loss for the volatile phenols (salifiable) or ethyl acetate (saponifiable), was fixed at pH 7 (Fig. 3).

3.1.4. Optimisation of the temperature of extraction

The temperature of extraction in SPME controls the phenomena of vaporisation of molecules (passage of the liquid phase to the gaseous phase) as well as the phenomena of adsorption onto the fibre (Fig. 4). The value that we selected (45 °C) corresponds to the maximum of extraction of the pyrazines, notably of 2M35DP, without negatively affecting the other compounds.

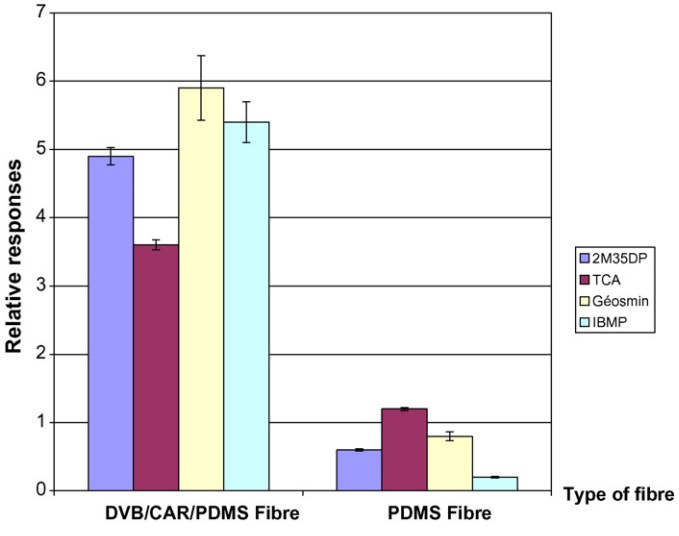

Fig. 1. Choice of SPME fibre between 100 μm PDMS and 50/30 μm DVB/CAR/PDMS (three replicates). Dilution of sample to ½, pH 7, temperature of extraction 45 °C, duration of extraction 60 min.

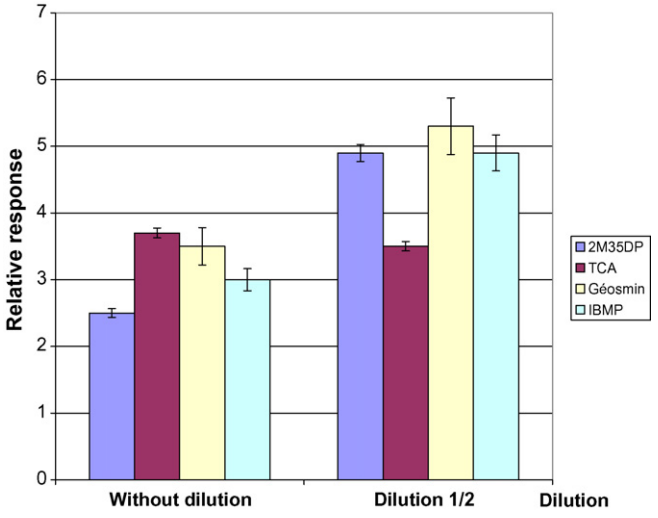

Fig. 2. Dilution test (three replicates). DVB/CAR/PDMS fibre, pH 7, temperature of extraction 45 °C, duration of extraction 90 min.

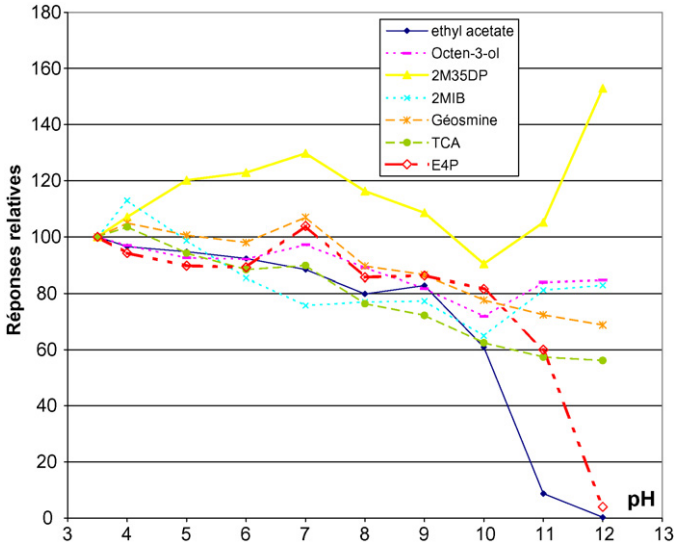

Fig. 3. Optimisation of pH of the sample (three replicates). DVB/CAR/PDMS fibre, dilution to ½, temperature of extraction 45 °C, duration of extraction 90 min.

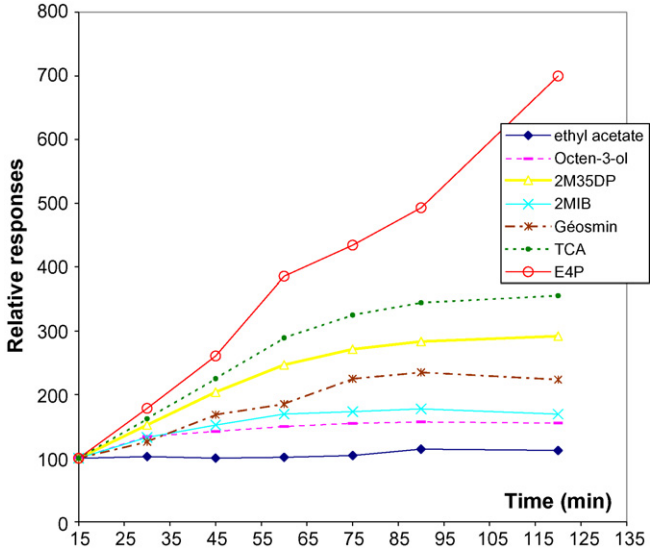

Fig. 5. Optimisation of extraction time for the SPME fibre. DVB/CAR/PDMS fibre, dilution to ½, pH 7, temperature of extraction 45 °C.

3.1.5. Optimisation of the extraction time

The duration of exposure of the fibre to the headspace is a parameter that strongly influences the quantitative adsorption on the stationary phase of the SPME fibre. We observed a phenomenon of saturation for ethyl acetate after 15 min of extraction (Fig. 5). Apart from this molecule, always abundant in wines (>20 mg L^{-1}), the equilibrium of adsorption is reached between 75 and 90 min. However, this equilibrium is still not reached after 2 h extraction for the volatile phenols. In order to increase productivity, we selected a time of 60 min.

3.2. Results of the validation

The validation was performed with a red wine selected for its low content of the target contaminants. The initial pH of the wine was 3.40 with 12.1% (v/v) ethanol and an index of total polyphenols, estimated by its absorbance at 280 nm, of 64.

The characteristics of the method are shown in Table 3. Each target molecule passed the tests of evaluation of the method. It is important to note that the uncertainty associated with ethyl acetate (+/−55 mg L^{-1} for a concentration of 80 mg L^{-1}, relative standard deviation, RSD = 36%) is not acceptable for precisely quantifying this substance over the whole range studied (4–250 mg L^{-1}), but it nevertheless allows successful evaluation of the risk that the taste of the wine will be adjudged to be acescent (tending towards sourness) or will not be (a quantity above or below 120 mg L^{-1}).

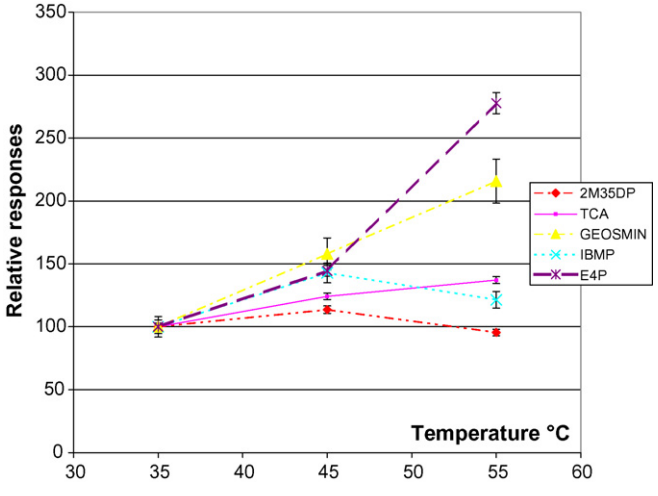

Fig. 4. Optimisation of the temperature of extraction of the fibre (three replicates). DVB/CAR/PDMS fibre, dilution to ½, pH 7, duration of extraction 90 min.

3.2.1. Design A—linearity and characteristics of standardization

In all cases, the regression test is satisfactory (Fisher's test is acceptable, i.e. the F_I values are higher than $F_{critique}$ with a 1% α risk for 1 and $p(n-1)$ degrees of freedom, where p is the number of levels tested and n is the number replicates). Nor is there any error of the model in the selected domain

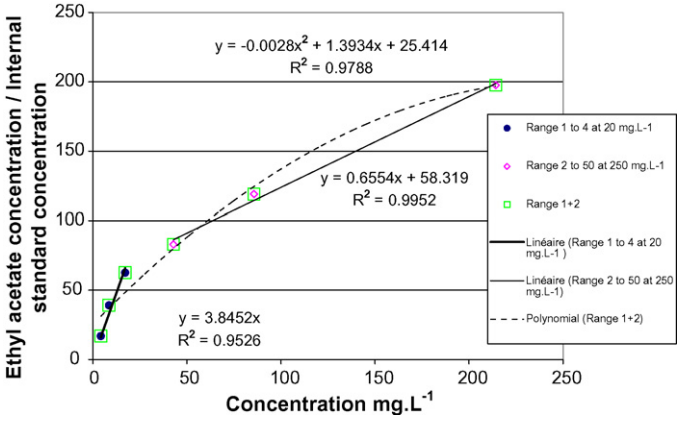

Fig. 6. Effect of saturation of the fibre on the linearity of the ethyl acetate range.

Table 3
Table summarizing the characteristics of the method with the results of validation according to criterion NF V 03–110

	Ethyl acetate ($mg\,L^{-1}$)	1-Octen-3-ol ($\mu g\,L^{-1}$)	Fenchone ($\mu g\,L^{-1}$)	Fenchol ($\mu g\,L^{-1}$)	Guaiacol ($\mu g\,L^{-1}$)	2MIB ($ng\,L^{-1}$)	Geosmin ($ng\,L^{-1}$)	2M35DP ($ng\,L^{-1}$)	IPMP ($ng\,L^{-1}$)	IBMP ($ng\,L^{-1}$)	TCA ($ng\,L^{-1}$)	TECA ($ng\,L^{-1}$)	PCA ($ng\,L^{-1}$)	TBA ($ng\,L^{-1}$)	E4P ($\mu g\,L^{-1}$)	E4G ($\mu g\,L^{-1}$)	V4P ($\mu g\,L^{-1}$)	V4G ($\mu g\,L^{-1}$)
Retention time (minute)	2.81	12.11	17.24	18.68	17.08	21.36	32.09	15.29	17.14	21.36	28.2	36.25	43.56	39.95	20.89	25.87	23.33	27.44
Dynamic range	47.6–215	1–26	1.8–27	1.6–31	14.9–600	5.1–500	1.5–500	0.7–130	3.1–250	3.1–250	0.3–25	0.1–25	0.5–25	0.2–32	11.5–1100	3.8–260	2.3–270	1.0–250
Slope (range)	0.1351	0.0723	0.1532	0.3197	0.0580	2.2E − 05	0.0002	0.0030	0.0106	0.0215	0.0192	0.0216	0.0168	0.0184	0.0090	0.0018	0.0002	3.04E − 5
Bias (range)	7.00	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
r^2	0.9905	0.9984	0.9907	0.9892	0.9856	0.9910	0.9959	0.9986	0.9976	0.9913	0.9997	0.9945	0.9990	0.9992	0.9989	0.9953	0.9992	0.9987
Design A sensitivity	0.81	1.01	0.94	1.01	1.08	1.14	1.06	0.95	0.90	0.86	1.02	1.07	1.03	1.02	1.00	1.14	1.06	1.02
Design A blank	29.00	0.48	0.92	0.63	6.59	−2.82	−6.07	0.21	0.76	−1.80	0.14	−0.16	0.40	0.04	5.66	1.69	−3.83	−1.95
Design A test regression	847.0	4610.5	2249.8	2312.8	2271.6	1495.0	2262.9	43397.9	4362.0	1123.0	90157.3	30094.6	59217.4	59183.2	88470.4	5091.2	4155.3	41062.6
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Design A test model	3.80	1.96	2.93	2.17	3.10	0.16	2.38	4.25	0.91	3.25	1.72	4.05	0.98	2.26	3.80	1.46	1.70	4.18
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Design A L_D	47.6	1.0	1.8	1.6	14.9	5.1	1.5	0.7	3.1	3.1	0.3	0.1	0.5	0.2	11.5	3.8	2.3	1.0
Design A L_Q	74.9	2.3	3.8	3.8	35.4	22.7	18.3	1.8	8.5	9.4	0.5	0.7	0.9	0.7	25.1	9.1	16.0	4.7
Design B significance test of the line	2.09	0.96	2.44	1.70	0.38	0.69	1.04	0.09	2.57	2.43	2.71	1.70	1.93	3.21	3.21	2.46	1.02	3.04
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Design B significance test of origin	0.31	1.29	1.67	1.39	0.83	1.17	0.46	1.21	0.84	1.55	2.19	0.28	2.59	0.70	2.94	2.17	0.74	0.54
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Design C precision test equality	0.66	1.26	0.22	0.85	0.94	0.86	0.19	1.03	0.26	0.40	1.20	1.02	2.12	1.12	2.47	1.74	0.70	1.14
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Design D standard deviation																		
Internal reproducibility (mean of the level)	27.3 (76)	0.67 (7.0)	0.65 (7.0)	0.70 (7.7)	20.0 (208)	10.2 (147)	11.3 (139)	0.8 (31)	3.8 (49)	2.5 (45)	0.12 (6)	0.4 (6.4)	0.11 (7.9)	0.12 (6.7)	8.8 (289)	2.8 (71)	5.3 (68.6)	3.8 (48)
RSD (%)	36	9.6	9.3	9.1	9.6	7.0	8.1	2.6	7.8	5.5	2	6.5	1.3	1.8	3.0	3.9	7.8	7.9

A: result of test of significance was acceptable.

Table 4
Application of the method to a red wine and a white wine

Molecule	Units	White wine $n = 3$ replications					Red wine $n = 3$ replications				
		Concentration by validated method[a,b,c]	Concentration by test method	RSD (%)	Added	Retrieved (%)	Concentration by validated method[a,b,c]	Concentration by test method	RSD (%)	Added	Retrieved (%)
1-Octen-3-ol	$\mu g\,L^{-1}$		1.5	17.6	2.1	100.0		2.8	5.5	2.1	81.1
Fenchone	$\mu g\,L^{-1}$		0.1	0.0	2.2	103.4		nd		2.2	90.1
Fenchol	$\mu g\,L^{-1}$		0.1	35.3	2.5	118.7		nd		2.5	83.5
Guaiacol	$\mu g\,L^{-1}$	11.3[b]	9.1	5.2	48.5	115.6	32.6[b]	30.9	10.4	48.5	93.2
2MIB	$ng\,L^{-1}$		nd		40.4	98.3		nd		40.4	90.2
Geosmin	$ng\,L^{-1}$		nd		41.2	124.9		nd		41.2	104.9
2M35DP	$ng\,L^{-1}$		Traces	6.3	10.7	86.8		Traces	10.4	10.7	88.7
IPMP	$ng\,L^{-1}$		nd		19.9	94.4		nd		19.9	106.5
IBMP	$ng\,L^{-1}$		nd		19.8	114.8		nd		19.8	96.5
TCA	$ng\,L^{-1}$	Traces[a]	0.7	7.3	2.0	90.2	2.2[a]	1.2	13.3	2.0	86.4
TeCA	$ng\,L^{-1}$	2.0[a]	2.2	3.6	2.1	102.2	2.3[a]	2.7	9.8	2.1	88.0
TBA	$ng\,L^{-1}$	nd[b]	nd		2.6	102.1	nd[b]	nd		2.6	104.8
PCA	$ng\,L^{-1}$	5.3[a]	5.0	3.0	2.0	98.2	6.4[a]	6.9	6.3	2.0	84.2
E4P	$\mu g\,L^{-1}$	nd[c]	nd		86.9	101.4	856[c]	742.8	1.0	86.9	95.1
E4G	$\mu g\,L^{-1}$	nd[c]	nd		20.7	101.6	149[c]	137.8	2.4	20.7	111.6
V4P	$\mu g\,L^{-1}$	32.0[c]	31.5	15.2	21.5	76.3	Traces[c]	20.9	10.2	21.5	84.2
V4G	$\mu g\,L^{-1}$	121.0[c]	130.9	2.1	19.9	126.6	Traces[c]	14.8	10.6	19.9	103.1

Comparison with two methods already used in the same laboratory. nd: not detected; traces: $L_d < \text{traces} < L_q$.

[a] Method 1 COFRAC accredited for assay of TCA, TeCA and PCA.

[b] Molecules (guaiacol and TBA) assayed concurrently by method 1.

[c] Method 2 validated for the assay of volatile phenols.

(F_{nl} is less than Fisher's F with a 1% risk for p–2 and $p(n$–1) degrees of freedom). The sensitivity, the blank and the detection limits and quantification limit (LOD = 3 and LOQ = 10 times the background noise plus the blank/sensitivity) are also determined in this validation procedure. For ethyl acetate, owing to the high concentrations liable to be encountered in wines (milligram per liter) in comparison with the other target contaminants which are present at much lower concentrations (microgram per liter or nanogram per liter), a phenomenon of relatively rapid saturation arises, which leads to a skew (Fig. 6) and poor repeatability (cf. Section 3.2.3). The precise and repeatable assay of ethyl acetate imposes a marked reduction in adsorption time that is incompatible with simultaneous assay of trace contaminants (<milligram per liter). Certainly, the use of isotopically labeled ethyl acetate standard will improve quality (linearity) of proportioning. However, in our method, the rather long time of adsorption of fiber DVB/CAR/PDMS involves surely a phenomenon of saturation of this one. Indeed as one can see it on Fig. 6, the phenomenon of saturation is put in evidence by an important difference of the slope according to whether the weak ones are looked at or the strong concentrations (>50 mg L^{-1}).

3.2.2. Design B—specificity of the method

The specificity of the method was studied for all the analytes with the aim of estimating the matrix effects. Student's t-test can be used to verify whether the slope of the line of regression is equal to 1 and is acceptable for all the molecules (if t_{obs} is less than $t_{critique}$ as read from the tables for a risk of 1–α/2 with $\alpha = 1\%$ with p–2 degrees of freedom). Likewise, the ordinate at the origin of the line of regression does not differ from 0 for each of the analytes. It can be seen that the measured additions of each analyte are thus recovered in a satisfactory manner with regard to the tests that have been performed.

3.2.3. Design C—precision of the method

Design C allows estimation of the possible interval between the mean of the values obtained and a reference value corresponding in our case to the addition carried out. In all cases, the identity test is acceptable (the ratio of the absolute value of the difference between the means to the standard deviation of the differences between means is less than 3.0 with a 1% risk of error). Having neither the material nor the method of reference, it was not possible to evaluate the reliability in terms of criterion NF V03-110.

3.2.4. Design D—internal reproducibility

The internal reproducibility of the method evaluates its reliability when the measurements are made over several (10) days by more than one operator (two operators). With the exception of ethyl acetate with a RSD ≈ 37% owing to the saturation of the fibre, which causes an unstable equilibrium between the molecules present in the headspace and those adsorbed onto the fibre, the relative standard deviation obtained by the method described is less than 10 for all the molecules.

3.3. Comparative analyses and measured additions

Two wines, a red wine, Saint-Emilion Grand Cru 2000 (13.5% ethanol), and a white wine, Crozes-Hermitage 2002 (14.5% ethanol), were analysed by the method described above. The results were compared with results obtained by the two specific methods that we had already used in the laboratory. First of all, the method of assaying the chloroanisoles described by Chatonnet et al. [5] was validated in terms of criterion NF V 03-110 for the analytes TCA, TeCA, PCA, TBA and guaiacol [TCA (LOD = 0.6 ng L^{-1}, LOQ = 1.4 ng L^{-1}, I = +/−0.6 ng L^{-1}); TeCA (0.2 ng L^{-1}, 1.4 ng L^{-1}, +/−0.8 ng L^{-1}); PCA (0.4 ng L^{-1}, 1.5 ng L^{-1}, +/−1.0 ng L^{-1}); TBA (0.7 ng L^{-1}, 1.5 ng L^{-1}, +/−0.5 ng L^{-1}); guaiacol (0.9 μg L^{-1}, 3.1 μg L^{-1}, +/−2.0 μg L^{-1})]. Then the volatile phenols were quantified by a method adapted from the one described by Mejias et al. [18] (use of a polyacrylate fibre in place of a Carbowax/DVB fibre) and also internally validated according to protocol NF V 03-110 [E4P (27 μg L^{-1}, 59 μg L^{-1}, +/−31 μg L^{-1}); E4G (6 μg L^{-1}, 30 μg L^{-1}, +/−18 μg L^{-1}); V4P (1 μg L^{-1}, 27 μg L^{-1}, +/−36 μg L^{-1}); V4G (6 μg L^{-1}, 20 μg L^{-1}, +/−35 μg L^{-1})].

The results obtained (Table 4) with the different methods on the two different wines are similar. The white wine had no defect in terms of taste, and the assay of the different analytes did not detect any molecules above their threshold of perception. For the red wine, the taste had a phenolic characteristic, and the method detected E4P and E4G as being at the origin of the defect since they exceeded their thresholds of perception. The measured additions were recovered in a satisfactory manner in the two types of matrix tested.

4. Conclusion

The proposed assay method is linear, specific, accurate and repeatable with detection limits for all the analytes in accordance with the thresholds of olfactory perception that are generally permitted in wines. The method has too high a variability with ethyl acetate for this molecule to be precisely quantified, but nevertheless it is satisfactory for estimating the risk of perception of an acescent character of the wine. The method here developed permits simultaneous diagnosis and identification of the origin of olfactive defects of widely differing origin in 60 min with a limited test sample (5 mL). This technique can be used to advantage as a control for the results of sensory analysis, and to certify the criteria for acceptance or rejection of bulk buys of wines, to optimise collections of wines or to investigate the origin of organoleptic defects that are not readily identifiable.

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