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Analytical Methods

Determination of ppq-levels of alkylmethoxypyrazines in wine by stirbar sorptive extraction combined with multidimensional gas chromatography-mass spectrometry

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ABSTRACT

Alkylmethoxypyrazines are powerful odorants in many food products. A new method for analysing 3-isopropyl-2-methoxypyrazine, 3-s-butyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine has been developed and applied to wine. The analytes were extracted from 5 mL of wine using stirbar sorptive extraction followed by thermal desorption and multidimensional gas chromatography-mass spectrometry analysis in a single oven. The extraction conditions were optimized in order to obtain a high recovery of the 3-alkyl-2-methoxypyrazines (MP). The detection limits of the method in all cases were under 0.08 ng/L, well below the olfactory thresholds of these compounds in wine. The reproducibility of the method was adequate (below 10%), the linearity satisfactory and the recoveries in all cases close to 100%. The method has been applied to the analysis of 111 Spanish and French wine samples. The levels found suggest that MP have a low direct impact on the aroma properties of wines from the regions around the Pyrenean massif.

1. Introduction

3-Alkyl-2-methoxypyrazines (MP) are a family of compounds whose presence has been amply reported in a variety of food products (Maga, 1992). These compounds are also well known for their contribution to wine aroma. The importance of MP in the aroma of wine has been widely studied since the first report of 3-isobutyl-2-methoxypyrazine (IBMP) in Cabernet Sauvignon grapes in 1975 (Bayonove, Cordonnier, & Dubois, 1975). The presence of IBMP, 3-s-butyl-2-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) has been related with the green and vegetative aromas characteristic of some wines made with Cabernet Sauvignon, Sauvignon blanc, Merlot or Cabernet Franc grapes (Allen, Lacey, Harris, & Brown, 1991; Chapman, Thorngate, Matthews, Guinard, & Ebeler, 2004; Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Preston et al., 2008; Roujou de Boubée, van Leeuwen, & Dubourdieu, 2000; Sala, Busto, Guasch, & Zamora, 2005). Although there are some wine styles with notable MP levels, it has been demonstrated that these compounds exert a negative influence on the perception of wine fruitiness (Campo, Ferreira, Escudero, & Cacho, 2005; Hein, Ebeler, & Heymann, 2009). They have also been found to take part of negative vectors of quality in premium Spanish red wines (Ferreira, San Juan, Escudero, & Culleré, 2009) and IBMP has even been considered as a marker for grape unripeness (Roujou de Boubée et al., 2000). The origin of these compounds is mostly endogenous as they form part of the chemicals produced in the first stages of grape development, their levels being strongly correlated with vine vigor and shade conditions (Ryona, Pan, Intrigliolo, Lakso, & Sacks, 2008). Nevertheless, the isopropyl isomer may have its origin in the infestation of the vine by the multicolored Asian lady beetle, Harmonia axyridis (Botezatu, Kotseridis, Inglis, & Pickering, 2013).

MP have extremely low sensory detection thresholds. In the case of IBMP, detection thresholds of 10 ng/L in red wine have been reported (Kotseridis, Beloqui, Bertrand, & Doazan, 1998), although there is evidence of IBMP modifying the aroma of wine in concentrations as low as 1 ng/L (Allen et al., 1991). IPMP may be still more powerful since its thresholds can be as low as 0.3 ng/L in white wine or 2.3 ng/L in red (Pickering, Karthik, Inglis, Sears, & Ker, 2007). In addition, these compounds can act additively (Campo et al., 2005), or can even interact with some oxidation compounds to intensify unpleasant aroma attributes (Coetzee et al., 2015). Thus, MP have a significant impact on wine aroma at very low concentrations levels, and this has led to a continuous improvement in their analytical determination. Early determination strategies involved laborious methods of sample preparation based on distillation and selective isolation in cation-exchange resins

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(Lacey, Allen, Harris, & Brown, 1991). Methods developed in the last decade are simpler and mainly based on headspace solid-phase microextraction (HS-SPME) (Callejon, Ubeda, Ríos-Reina, Morales, & Troncoso, 2016) or solid-phase extraction (SPE) (Culleré, Escudero, Campo, Cacho, & Ferreira, 2009; Lopez, Gracia-Moreno, Cacho, Ferrreira, & Ferreira, 2011).

However, it has been acutely pointed out (Schmarr et al., 2010) that both HS-SPME and SPE have limited extraction selectivity in a complex matrix such as wine. Considering the low detection limits required for MP monitoring, together with the matrix complexity, one-dimensional gas chromatographic analysis with mass spectrometric detection (GC–MS) involves a high risk of coelution in critical cases (Schmarr et al., 2010). For this reason, various authors have developed different two-dimensional chromatographic techniques for MP analysis in wine. In 2005, Ryan et al. described the application of GCxGC coupled with time of flight MS (TOF-MS) or NPD for the determination of IBMP (Ryan, Watkins, Smith, Allen, & Marriott, 2005). Similarly, GCxGC coupled with TOF-MS was used for the determination of IPMP and IBMP in wine grapes (Ryona, Pan, & Sacks, 2009). Also in wine grapes, Legrum et al. recently applied *Enantio*-GCxGC–MS for the enantiodifferentiation of SBMP in different species (Legrum, Slabizki, & Schmarr, 2015). The same research group had previously applied GCxGC–MS to the analysis of MP in wine (Schmarr et al., 2010). In spite of its very high separation efficiency, GCxGC is probably not the simplest approach for a limited number of target analytes as is the case with MP. Due to its simpler experimental setup and easiness for data processing, heart-cut multidimensional chromatography (MDGC) has also been applied to the analysis of MP in wine. Culleré et al. used MDGC combined with SPE to achieve very low detection limits for MP in wine (Culleré et al., 2009). While MP extraction with HS-SPME-MDGC was faster, it provided slightly higher detection limits (Botezatu, Pickering, & Kotseridis, 2014; Koegel, Botezatu, Hoffmann, & Pickering, 2015). Even more selectivity could be obtained combining on-line liquid chromatography with the MDGC–MS technique (Schmarr et al., 2010), or through tandem mass spectrometry MDGC–MS/MS (Legrum et al., 2014). Ochiai et al. proposed a MDGC–MS combined with olfactometry and with preparative fraction collection for the determination of IBMP among other off-flavors (Ochiai & Sasamoto, 2011).

Among the variety of sample preparation techniques applied to wine aroma analysis, stirbar sorptive extraction (SBSE) (Baltussen, Sandra, David, & Cramers, 1999) has several advantages that make it a good choice for the analysis of MP in wine. Compared with SPE, SBSE is more easily automated and requires no solvent; on the other hand, SBSE has a much greater amount of sorbent than SPME, which results in a higher sample extraction capacity and consequently better sensitivity and fewer matrix effects (David & Sandra, 2007). To the best of our knowledge, Franc et al. reported the first application of SBSE to IBMP analysis in wine (Franc, David, & de Revel, 2009). Comparing it with other aroma extraction methods, Gamero et al. found that SBSE was the most sensitive extraction method for IBMP (Gamero, Wesselink, & de Jong, 2013). Very recently, SBSE has also been applied to the determination of MP in Chinese Syrah wines (Zhao, Gao, Qian, & Li, 2017). Due to the increase in the amount of volatiles extracted by SBSE compared with other techniques, the risk of interference and column overloading is also increased. Hjelmeland et al. addressed this challenge coupling SBSE with GC–MS/MS for a more selective method of MP determination (Hjelmeland, Wylie, & Ebeler, 2016). In the present study, we also propose a method for the determination of MP in wine by means of SBSE, but the required additional selectivity is provided by a simpler and more affordable experimental setup based on MDGC–MS using only one chromatographic oven. The method optimization and validation for the quantitative determination of MP at pg/L levels is presented, together with its application to a large number of wines.

2. Materials and methods

2.1. Wine samples

A commercial 2013 vintage Crianza red wine from La Rioja was used to optimize the extraction conditions. After optimization of the experimental parameters, a synthetic wine and four commercial Spanish wines of different grape varieties (Tempranillo red wine, Cabernet Sauvignon red wine, Cabernet Sauvignon rosé wine and Sauvignon Blanc white wine) were used for the validation of the proposed method.

A total of 111 different wine samples were analyzed with the proposed method. These samples were elaborated using non-commercial, recently identified grape cultivars from the regions around the Pyrenean massif. The 56 French wine samples comprised 8 white, 24 rosé and 24 red wines, while the 55 Spanish wine samples were made up of 11 white, 9 rosé and 35 red wines. All the wines were produced in the same conditions and with the same yeast starter cultures for each category (white, rosé and red).

2.2. Reagents and standards

3-Isopropyl-2-methoxypyrazine (IPMP), 3-s-butyl-2-methoxypyrazine (SBMP), 3-isobutyl-2-methoxypyrazine (IBMP), citric acid and trisodium citrate dihydrate were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol was supplied by Merck (Darmstadt, Germany) and tartaric acid was provided by Panreac (Barcelona, Spain). Water was purified in a Milli-Q system supplied by Millipore (Bedford, Germany). The citrate buffer was prepared with 8% (v/v) of 0.5 M citric acid and 92% (v/v) of 0.5 M trisodium citrate dihydrate.

Deuterated standards of MP (3-alkyl-2-[$^2H_3$]methoxypyrazines) were chosen as internal standards. The deuterated MP were synthesized in-house as described previously (Schmarr, Sang, Ganß, Koschinski, & Meusinger, 2011). The internal standards solution was prepared with the deuterated MP in ethanol.

Stirbars coated with 126 µL polydimethylsiloxane (PDMS, 20 mm length × 1.0 mm thickness) were obtained from Gerstel (Müllheim an der Ruhr, Germany). Before the first use, each stirbar was conditioned at 300 °C under constant helium flow for 2 h.

2.3. Sample preparation

2.3.1. Optimization

A Spanish Crianza red wine was used to optimize the extraction parameters: dilution factor, pH and extraction time. Such sample was first spiked with deuterated and non-deuterated MP at 40 ng/L. Then, three 5 mL-volumes of the spiked sample were taken, the first one was directly extracted, the second diluted with 1 mL of milli-Q water (1.2 dilution factor), and the third with 5 mL (2.0 dilution factor). Then, with the optimum dilution factor, the effect of pH was studied by adding 1 mL of milli-Q water or citric acid-sodium citrate buffer to adjust the pH to 5.4. Finally, extraction times of 15, 30 and 60 min were evaluated under the optimized dilution and pH conditions. Each condition was analyzed in triplicate.

2.3.2. Proposed method

Five mL of sample were transferred into a clean 25 mL Erlenmeyer flask, and 1 mL of 0.5 M citric acid-sodium citrate buffer was added to the same flask to adjust the pH to 5.4. Then 50 µL of the internal standards solution were added. A conditioned stirbar was inserted into the flask using tweezers. The closed flask was placed onto a 20-position magnetic stirrer (Gerstel, Mülheim an der Ruhr, Germany), then stirred at room temperature and 750 rpm for 30 min. After extraction, the stirbar was removed from the flask, rinsed briefly with Milli-Q water and dried with a lint-free tissue. Each stirbar was then transferred into a thermal desorption tube which was placed in the autosampler tray for

analysis.

2.4. Thermal desorption

The stirbar was desorbed using a thermal desorption unit (TDU) and a cryo-cooled injection system (CIS 4) with a programmable temperature vaporization (PTV) inlet (Gerstel, Müllheim an der Ruhr, Germany) equipped with a MPS auto-sampler from Gerstel (Müllheim an der Ruhr, Germany). The stirbar was thermally desorbed in the TDU in splitless mode. The TDU temperature was programmed from 25 °C (held for 1 min) at 60 °C/min to 270 °C (held for 7 min). The transfer line of the TDU was kept at 250 °C. The initial temperature of the CIS was set at -80 °C using liquid nitrogen. The CIS was then heated to 250 °C at a rate of 12 °C/s and held for 30 min to inject the trapped compound into the capillary columns in solvent vent mode. Complete desorption of the MP under these conditions was checked by running a blank analysis of a recently used stirbar.

2.5. Multidimensional gas chromatography

The analysis was performed using an Agilent 7890A gas chromatograph equipped with a Deans switch device (Agilent Technologies, USA) allowing the selective transfer of heart cuts from the first column to the second. The oven temperature was first held at 45 °C for 4.5 min and then increased by 6 °C/min to 220 °C.

The first column was a DB-5MS column (15 m length, 250 μm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA) combined with a flame ionization detector (FID) and the Deans switch. An uncoated, deactivated column (6.7 m length, 180 μm i.d.) from Agilent was used as a restrictor between the FID detector and the Deans switch. The carrier gas helium was delivered at a constant pressure of 36 psi. The FID was kept at 280 °C and operated with 40 mL/min hydrogen and 450 mL/min air. Under the described conditions, the MP and their corresponding deuterated standards were eluted from column 1 between 13.7 and 17.0 min, and consequently the Deans switch system was programmed for two cuts. The first cut between 13.7 min and 14.2 min was for IPMP and IPMP-d3, and the second cut was from 16 min to 17 min for SBMP, IBMP and the deuterated standards for both compounds.

The second column was a SAPIENS-WAX MS (Teknokroma, Barcelona, Spain) (30 m length, 250 μm i.d., 1 μm film thickness) directly connected to an Agilent 5975C mass spectrometer. The pressure was kept constantly at 31 psi. A quadrupole mass detector was operated in selected ion monitoring mode (SIM) with electron ionization. The temperature of the ion source was set at 230 °C and the transfer line was kept at 240 °C. Quantifier ions were *m/z* 137, 138 and 124 for IPMP, SBMP and IBMP, respectively, and 140, 141, and 127 for their related deuterated standards. Qualifier ions were 152 (36%) and 124 (23%) *m/z* for IPMP (155 (38%) and 127 (25%) for IPMP-d3), 151 (46%) and 124 (58%) for SBMP (127 (55%) for SBMP-d3) and 151 (18%) for IBMP (154 (16%) for IBMP-d3). Values in brackets are relative proportions of abundance (%) to base peak.

2.6. Method validation

A set of six concentrations ranging from 0.1 to 15 ng/L of the studied MP (Table 1) and 5 ng/l of the deuterated MP were spiked into both the model wine (13% vol. Ethanol, pH 3.4 and 5 g/L tartaric acid) and a Tempranillo red wine, and then analyzed by the optimized SBSE-TD-MDGC-MS method in duplicate. The matrix effect was evaluated by comparing the slopes in both matrices with a *t*-test. Limits of detection (LOD) were defined as the amount of MP in a spiked red wine free of MP that produces, with the proposed method, a peak with a height equivalent to three times the average standard deviation of the baseline in the surrounding area to the ion peak. The lowest concentration of the calibration curves (Table 1) was considered as the limit of quantification. The method reproducibility was calculated by spiking a commercial red wine free of the analytes with 1 ng/l of each MP, and analyzing this wine 6 times during a period of three weeks.

Table 1
Linearity, limits of detection and reproducibility of the method.

Compound	Concentration Range (ng/L)	Slope[a]	r^{2a}	LOD[b] (ng/L)	Reproducibility[b] (RSD%)
IPMP	0.22–14.9	0.2507	0.9999	0.07	10
SBMP	0.13–15.6	0.6044	0.9999	0.02	2
IBMP	0.11–14.7	3.161	0.9996	0.02	11

[a] Measurement was made in model wine.
[b] Data was measured in a commercial red wine spiked at 1 ng/L level.

To assess the method accuracy, three commercial Spanish wines with different matrices were spiked with 0.5 ng/L of IPMP, SBMP and 4 ng/L of IBMP, after which the spiked and unspiked samples were analyzed in triplicate using the proposed method. Recovery was defined as the ratio (in %) between the amount of target analytes determined in the spiked sample minus that determined in the corresponding unspiked original sample to the exact concentration added in the wine sample.

3. Results and discussion

3.1. Extraction optimization

The goal of the present analytical method was to determine MP in wine at sub-ng/L levels. With this objective, the following SBSE parameters were optimized during the development of the method: sample dilution factor, sample pH and extraction time.

Sorptive extraction with PDMS coating is based on the partition coefficient of the MP between the hydroalcoholic wine matrix and the PDMS phase. Since this silicone material is essentially an apolar phase, it is expected that the extraction efficiency of the MP will increase when decreasing the ethanol content of the wine matrix, which can be achieved by diluting the sample. To examine the effects of the matrix dilution on the signal, 3 different dilution conditions were tested: dilution factor of 1 (no dilution), 1.2 and 2. The results are shown in Fig. 1A. It was found that dilution had a significant effect. A dilution factor of 1.2 increased the recovery of all MP. However, a larger dilution of 2 not only did not improve the signal strength but produced a significant decrease. In spite of the lower solubility of the MP in the most diluted sample, the larger phase ratio between the PDMS on the stirbar and the sample led to a worse recovery of the analytes (Baltussen et al., 1999). Therefore, dilution of the 5 mL of wine with 1 mL of water was chosen as the optimum.

MP have acid-base properties that can influence their extraction efficiency (Franc et al., 2009; Hjelmeland et al., 2016; Lopez et al., 2011). For that reason, the pH of the sample was adjusted to 5.4 by adding 1 mL of a citrate buffer and compared to the same sample with its original pH of 3.6 but diluted with 1 mL of water. When the extraction was performed (Fig. 1B), the results showed that the extraction efficiency of the MP increased significantly at higher pH. Because MP are very weak alkalis with a pKa of around 0.5 (Boutou & Chatonnet, 2007), it is likely that most of the improvement observed in the extraction was due to an increase of the ionic strength rather than a change in the state of ionization of the MP. In any case, it was decided to choose a pH of 5.4 not only because of the better extraction but also to standardize the sample pH.

Finally, with the optimal conditions of dilution and pH, three different extraction times between 15 and 60 min were tested. The extraction time curves (Fig. 2) illustrated an increasing trend of the signal responses for all the studied MP over time. The results showed an increase of 35% ~45% for all the analytes comparing results from 15 min

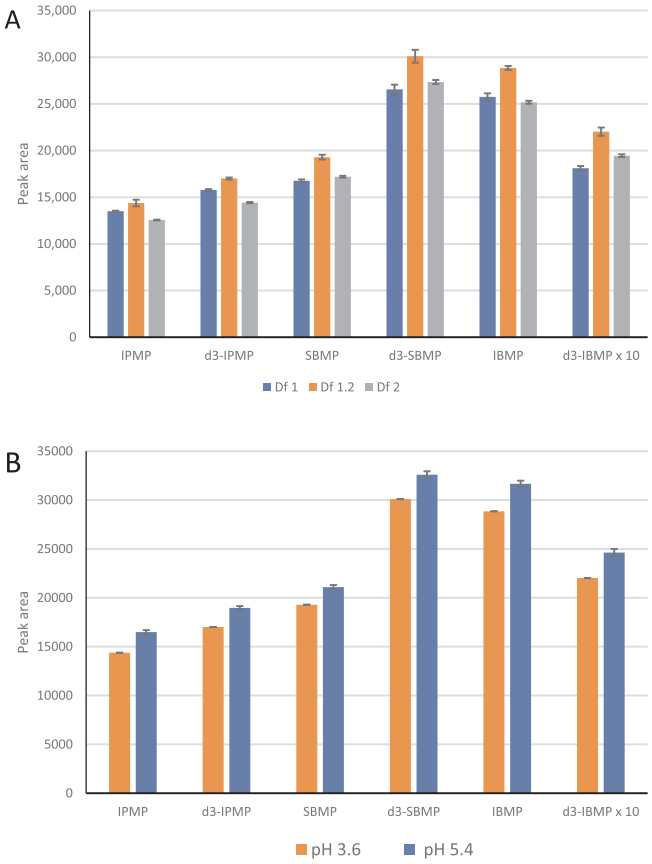

Fig. 1. (A) Effect of dilution factor on MPs recovery. Df 1: no dilution, Df 1.2: 5 mL of wine plus 1 mL of water, Df 2: 5 mL of wine plus 5 mL of water. (B) Effect of pH on MPs recovery. Error bars represent the standard error of the mean.

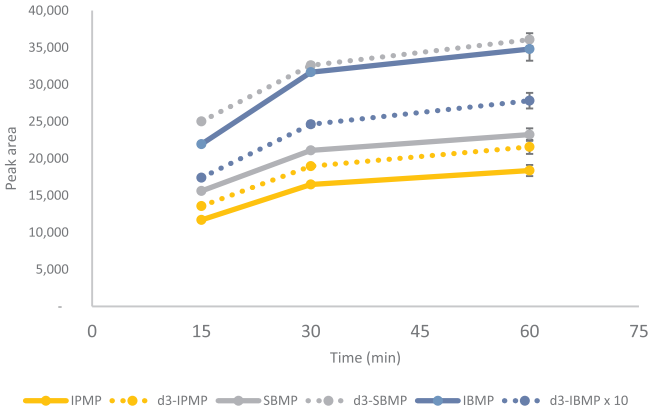

Fig. 2. Effect of extraction time on MPs recovery. Error bars represent the standard error of the mean.

and 30 min extraction. Although not statistically significant, the 60 min extraction showed an increasing trend in the recovery of the MP. As a compromise between acceptable extraction efficiency and sample preparation time, a 30 min extraction time was selected for the proposed method.

3.2. Method validation

The corresponding deuterated isotopologues were used as internal standards for each MP. As shown in the method optimization, these compounds showed a similar behavior to that of the targeted MP in all the procedural steps.

Method linearity, repeatability, detection and quantitation limits

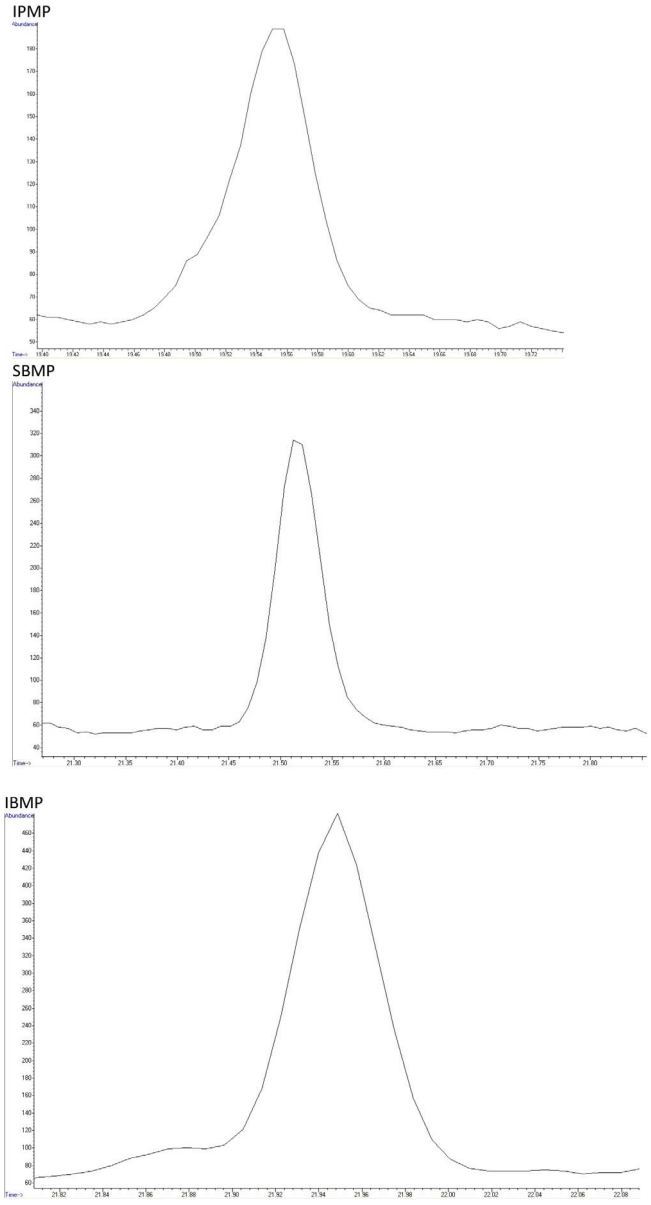

Fig. 3. SPE–GC–MS chromatograms obtained in the analysis following the proposed procedure, of a red wine containing 0.7 ng/L IPMP (m/z 137), 0.3 ng/L SBMP (spiked) (m/z 138) and 1 ng/L IBMP (m/z 124).

were assessed by spiking a model wine and a red wine free of the analytes with levels between 0.1 ng/L and 15 ng/L. In order to evaluate the absence of matrix effects, a statistical comparison of the slopes of the calibration curves between the model and the red wines was carried out. The statistical results showed no significant differences (results not shown). Linearity covered all the range tested with determination coefficients above 0.999 in all cases (Table 1), which can be considered highly satisfactory. Method detection limits were extremely low thanks to the separation power of multidimensional chromatography (Fig. 3), to the selectivity of MS detection and to the high recovery efficiency of SBSE. In fact, the method detection limits were 0.02 ng/L for SBMP and IBMP, and 0.07 for IPMP (Table 1), which, to the best of our knowledge, are the lowest published detection limits for MP in wine. These improved detection limits are possible first, because in the optimized analytical strategy a large fraction of all the MP present in 5 mL of wine are transferred to the GC–MS, thanks to the large extraction capacity of the SBSE twister. Although extraction recoveries were not calculated, theoretical calculations based on logP and extraction times (Baltussen

et al., 1999) suggest that the fraction extracted was in all cases above 80%. I.e., nearly all analytes present in 4.5 mL of wine are introduced into the system, which is 1–2 orders of magnitude above what can be extracted by SPME from wine or what is usually introduced in the normal injection (1–2 μL) of a concentrated SPE extract. Second, the heart-cut MDGC makes it possible to sort out the serious column overload associated with the introduction of all the material extracted by the twister. By transferring selected fractions of the overloaded separation obtained in the first dimension to the second one, perfectly resolved chromatographic peaks and very clean baselines are obtained. This cannot be attained with MS/MS approaches, which can solve the question of the selectivity of the signal, but cannot counteract the distortion of the chromatographic peaks caused by column overload.

Method reproducibility was calculated by repeated analysis of a sample spiked at 1 ng/l on six different days spanning three weeks. The results were good with RSD values around 10% in the three cases (Table 1), which can be considered satisfactory for this low concentration level and the experimental conditions.

Method accuracy was determined by a standard recovery experiment carried out on 3 different commercial wines spiked with 4 ng/L of the analytes. The results of this experiment are shown in Table 2. As shown in the table, average recoveries are in all cases close to 100% which confirms that the method is accurate and free from matrix effects. The RSD obtained in the experiment, between 2% and 7%, provides a good estimate of the overall method reproducibility. These values can be considered satisfactory for the low levels of the analytes.

3.3. Wine analysis

The method was applied to the determination of the three compounds in a set of 111 experimental wines produced during 2016 with non-commercial, recently identified grape cultivars from the regions around the Pyrenean massif. The results are shown in Table 3. It should be noted that in spite of the very low detection limit of the method, SBMP was not even detected in the samples and therefore is not mentioned in Table 3. Our results make it possible to state that this compound is not a natural aroma compound of these wines. Regarding IPMP, this compound was found in nearly all the wines below the corresponding odor thresholds (estimated as 0.3 ng/L in white wine and 2.3 ng/L in red wine (Pickering et al., 2007)). In fact, it was found above its sensory threshold in only one Spanish white wine. Considering the values obtained in the set of wine samples, lower concentration standards for the calibration curve and a lower internal standard concentration would be more advisable for IPMP determination. IBMP was the most abundant MP in this set of samples, especially in French wines. In each wine category, the average concentration of IBMP was always higher in French than in Spanish wines. These differences can be associated to the higher humidity and more frequent rainfalls in the French regions which usually produce a higher vine vigor, associated with a larger production of IBMP (Ryona et al., 2008). Despite the higher content of IBMP in the French wines, in only two of them were the levels above the odor threshold of 10 ng/L: the wine elaborated with Gros cabernet grapes with 11.8 ng/L and the wine produced with Bequignol grapes with 41.2 ng/L. These results suggest that, leaving aside these two cases, IBMP is not a key odorant in this set of wines. However, it should not be concluded from these results that this compound does not play any role in the aromatic perception, since even at subthreshold levels it could exert a suppression effect on wine aroma, as suggested by Gas Chromatography-Olfactometry (Ferreira et al., 2009). Specific sensory testing will have to be carried out to assess this.

4. Conclusions

A semi-automated method to analyze MP in wine has been developed. The proposed method utilizes a highly efficient SBSE procedure

Table 2
Recovery and reproducibility of the proposed method.

wine	IPMP				SBMP				IBMP			
	spiked concentration (ng/L)	calculated concentration (ng/L)	spiked recovery (%)	reproducibility (RSD%)	spiked concentration (ng/L)	calculated concentration (ng/L)	spiked recovery (%)	reproducibility (RSD%)	spiked concentration (ng/L)	calculated concentration (ng/L)	spiked recovery (%)	reproducibility (RSD%)
Sauvignon blanc white	0.49	0.53	108	5.9	0.52	0.52	100	6.7	3.60	4.10	114	5.0
Cabernet sauvignon rosé	0.51	0.59	116	7.0	0.54	0.49	110	6.2	3.82	5.59	115	3.9
Cabernet sauvignon red	0.50	0.55	110	7.5	0.52	0.44	85	7.9	3.87	8.80	115	3.4

Table 3
Average, maximum and minimum concentrations (ng/L) of the analytes found in the wine samples from recently identified cultivars. SBMP contents were always below detection limit. Average values were calculated as the arithmetic mean and considering a concentration of 0 ng/L for those samples below the detection limit of the method.

Type of wine	Country of origin	Number of samples	IPMP concentration			IBMP concentration		
			Average	Minimum	Maximum	Average	Minimum	Maximum
White	Spain	11	0.059[a]	0.011[a]	0.41	0.11	< DL	0.29
White	France	8	0.071[a]	< DL	0.24	0.87	0.07[a]	3.16
Rosé	Spain	9	0.052[a]	< DL	0.15[a]	0.17	< DL	0.49
Rosé	France	24	0.034[a]	< DL	0.16[a]	0.76	< DL	4.86
Red	Spain	35	0.047[a]	< DL	0.21[a]	0.41	0.09[a]	1.17
Red	France	24	0.102[a]	< DL	0.46	2.82	< DL	41.2

< DL: below detection limit.
[a] Below quantitation limit.

combined with the selectivity provided by multidimensional chromatography and MS detection. The validated method allows the determination of MP at the ppq-level while using only a small volume of sample and with adequate accuracy. The usefulness of the method has been proved by analyzing 111 French and Spanish wine samples, finding in most cases levels below the threshold, suggesting that MP do not play a relevant role in the aroma of the wines from the regions around the Pyrenean massif.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.02.089.

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