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Accurate determination of 3-alkyl-2-methoxypyrazines in wines by gas chromatography quadrupole time-of-flight tandem mass spectrometry following solid-phase extraction and dispersive liquid–liquid microextraction

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ABSTRACT

A new reliable method for the determination 3-alkyl-2-methoxypyrazines (MPs) in wine samples based on the sequential combination of solid-phase extraction (SPE), dispersive liquid–liquid microextraction (DLLME) and gas chromatography (GC) quadrupole time-of-flight accurate tandem mass spectrometry (QTOF-MS/MS) is presented. Primary extraction of target analytes was carried out by using a reversed-phase Oasis HLB (200 mg) SPE cartridge combined with acetonitrile as elution solvent. Afterwards, the SPE extract was submitted to DLLME concentration using 0.06 mL carbon tetrachloride (CCl_4) as extractant. Under final working conditions, sample concentration factors above 379 times and limits of quantification (LOQs) between 0.3 and 2.1 ng L^{-1} were achieved. Moreover, the overall extraction efficiency of the method was unaffected by the particular characteristics of each wine; thus, accurate results (relative recoveries from 84 to 108% for samples spiked at concentrations from 5 to 25 ng L^{-1}) were obtained using matrix-matched standards, without using standard additions over every sample. Highly selective chromatographic records were achieved considering a mass window of 5 mDa, centered in the quantification product ion corresponding to each compound. Twelve commercial wines, elaborated with grapes from different varieties and geographical origins, were processed with the optimized method. The 2-isobutyl-3-methoxypyrazine (IBMP) was determined at levels above the LOQs of the method in half of the samples.

1. Introduction

Alkyl methoxypyrazines (MPs) are natural heterocyclic compounds contributing to the vegetative or earthy odor of various fresh vegetables such as peas, asparagus, lettuce, potatoes, and others [1–4]. MPs have also been detected in several grape cultivars and their wines, where they can have a positive impact on the aroma profile of certain varieties. The most prevalent MP found in wine, 2-isobutyl-3-methoxypyrazine (IBMP), imparts a characteristic green bell pepper aroma to specific wine grape varieties (Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc and Merlot) having a sensory threshold in the range of 8–15 ng L^{-1}. The other compounds of this family that are commonly studied include 2-isopropyl-3-methoxypyrazine (IPMP), 2-sec-butyl-3-methoxypyrazine (SBMP), 2-ethyl-3-methoxypyazine (EMP). Winemaking industry often monitors the levels of these compounds due to (1) the relevant organoleptic impact on wine quality and (2) the need to meet consumer's demands related to wines with lower levels of MPs.

Determination of MPs in wines presents two major challenges, the first related to sensitivity due to the low expected concentration of target compounds (low ng L^{-1} range). The second is associated with complexity of wine matrix. These both difficulties need to be managed by a combination of high efficiency and selective sample preparation approaches. Concurrently, accurate mass spectrometry techniques (MS) can dramatically reduce isobaric interferences when quantification ions are extracted using a mass window of a few milidaltons (mDa) [5].

Diverse sample preparation approaches have been proposed for MPs isolation and concentration, being headspace solid-phase

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microextraction (HS-SPME), SPE and wine distillation with several liquid–liquid extraction steps the most used [1,6–13]. Among the above techniques, SPE offers a good compromise between robustness, rapidity, clean-up efficiency, scope for automation and solvent consumption. The relatively low breakthrough volumes of SPE sorbents for wine (compared to those attained for less complex matrices such as environmental water samples) can be compensated with a further dispersive liquid–liquid microextraction (DLLME), aiming not only to increase the obtained enrichment factors, but also to remove some undesired species from the primary SPE extract [5,14].

Relative to the determination step, most procedures are based on gas chromatography (GC) coupled with different detection systems, with a high percentage of reports using MS detection [4]. Due to MPs analysis suffer the presence of interferences that difficult accurate quantification, recent developments using two-dimensional GC for chromatographically resolving target analytes and increasing selectivity have been proposed [1,10,15]. Another alternative to discriminating between co-eluting peaks (not isomers) is to determine the exact m/z ratios of their characteristics ions either in MS or MS/MS modes. Hybrid mass spectrometers, such as quadrupole time-of flight (QTOF) systems, are emerging as an appealing alternative to triple quadrupole (QqQ) instruments providing accurate mass measurements and the possibility to record full scan MS/MS (product ion scan) spectra at the expense of slightly higher quantification limits than QqQ instruments, operated in the MRM mode. Some sensitive applications of GC-QTOF-MS instruments have been recently developed for the determination of pesticides in some food commodities [16–18] and trace organic pollutants in complex environmental samples [19].

The aim of the present work was to develop a sensitive and selective method for the accurate identification and quantification of 3-alkyl-2-methoxypyrazines in wines with detection limits at, or below than, their sensorial thresholds. The proposed approach involves sequential combination of SPE, as selective extraction technique, with the concentration capabilities of DLLME, and final accurate determination of target compounds by GC combined with a hybrid QTOF MS instrument. Conditions affecting the efficiency of extraction and concentration steps were systematically studied and their effects in the overall performance of the method discussed. GC-QTOF-MS parameters were adjusted to allow the sensitive and unequivocal determination of selected MPs. Finally, the applicability of the developed method was demonstrated with analysis of commercial wines from different varieties.

2. Experimental

2.1. Standards, solvents and sorbents

The standards of IBMP (98% purity) and EMP (99% purity) were purchased from Alfa Aesar (Karlsruhe, Germany). The IPMP standard (99.5% purity) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). The SBMP (97% purity) and the internal standard (IS) 2-ethoxy-3-isopropylpyrazine (EPP, 95% purity) were purchased from TCI (Tokyo, Japan). Individual solutions of each compound were prepared in ethanol and maintained at −20 °C. Diluted mixtures were prepared in ethanol (for wine spiking) or carbon tetrachloride (calibration standards), and stored at −20 °C.

Methanol, acetonitrile and acetic acid (HPLC grade) were obtained from Merck (Darmstadt, Germany). Pesticide grade acetone, toluene, dichloromethane, chloroform (CCl_3H) and carbon tetrachloride (CCl_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (NaCl) and dipotassium hydrogen phosphate were acquired from Merck. Sodium acetate and ammonium hydroxide were obtained from Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA).

OASIS HLB (divinylbenzene-*co*-*N*-vinylpirrolidone polymer) 200 mg, OASIS MAX (dimethylbutylamine functionalized divinylbenzene-*co*-*N*-vinylpirrolidone polymer) 150 mg and OASIS MCX (sulfonic-acid functionalized divinylbenzene-*co*-*N*-vinylpirrolidone polymer) 150 mg SPE cartridges were acquired from Waters (Milford, MA, USA).

Red and white wine samples, from different geographic origins and grape varieties, were obtained from local markets. Synthetic wine was prepared by addition of tartaric acid (3.5 g L^{-1}) to 13% ethanolic solutions in ultrapure water, followed by pH adjustment to 3.5 with NaOH (0.1 M).

2.2. Sample preparation

The sample preparation method developed in this study involves two consecutive steps. First, wine samples were passed through SPE cartridges to concentrate and to separate target analytes from other matrix components, particularly pigments in the case of red wines, disturbing phase separation during DLLME. Afterwards, the analytes contained in the SPE extract were concentrated by DLLME.

Under optimized conditions, 40 mL of wine were diluted with the same volume of ultrapure water and passed through HLB SPE cartridges, previously conditioned with acetonitrile and ultrapure water (10 mL each). Then, cartridges were rinsed with 5 mL of synthetic wine, dried for 20 min using a gentle stream of nitrogen and eluted with 1 mL of acetonitrile.

Operating DLLME conditions involved dilution of the acetonitrile SPE extract with 8 mL of ultrapure water and addition of 1.5 g of NaCl. Thereafter, 1 mL of the extraction solution, consisted in 0.94 mL of MeOH and 0.06 mL of CCl_4, was added. The DLLME tubes were capped, shaken for 1 min and centrifuged for 5 min at 3000 rpm. The CCl_4 settled extract was recovered and transferred to an insert for direct injection in the GC-QTOF-MS system.

2.3. Determination conditions

The GC-QTOF-MS instrument comprised of a 7890A gas chromatograph and a 7200 QTOF mass spectrometer, both acquired from Agilent (Wilmington, DE, USA), equipped with an electron ionization (EI) source. The TOF mass analyzer was operated in the 2 GHz mode (mass resolution varied from 4900 at m/z 131 to 7500 at m/z 414). Automated recalibration of the mass axis was carried out every 5 injections, by infusion of PFTBA in the EI source. Two different columns were considered for compounds separation: a BP-5MS type capillary column (30 m × 0.25 mm i.d. 0.25 μm film thickness) and a DB-WAXETR (30 m × 0.25 mm i.d. 0.5 μm film thickness), both acquired from Agilent. Under final conditions, compounds were separated using the former column. Helium was used as carrier gas at a constant flow of 1.2 mL min^{-1} and the column temperature was programed as follows: 40 °C (1 min), rated at 5 °C min^{-1} to 110 °C (5 min), and finally at 15 °C min^{-1} to 270 °C with a hold time of 3 min. The transfer line and the EI source were set at 280 and 230 °C, respectively. Injections (1 μL) were made in the pulsed splitless mode (25 Psi, 1 min) with the injection chamber set at 260 °C. The splitless time and the split flow were 1 min and 60 mL min^{-1}, respectively. The solvent delay was fixed at 6 min.

The MS and MS/MS modes were considered for compounds detection. In both cases, spectra were recorded every 0.3 s as the average of 3900 transients per spectrum. The MS mode was employed during preliminary steps of method development. Validation of the overall sample preparation procedure and analysis of wine samples was carried out in the product ion scan mode. During MS/MS experiments, precursor ions were isolated within a window

Table 1
GC–QTOF–MS/MS accurate-mass database including retention times (RT) for the BP-5 column, precursor and fragmentation of the studied MPs.

Analyte	RT (min)	[a]Molecular ion (*m*/*z*)	[a]Precursor ion (*m*/*z*)	[b]CE (eV)	[a]Quantification product ion (*m*/*z*)	[a]Additional product ions (*m*/*z*)
EMP	11.2	138.0829	138.0829	12	123.0535	119.0597
IPMP	12.0	152.0970	137.0689	14	109.0753	105.0438
SBMP	14.6	165.1027	138.0829	12	123.0546	119.0602
IBMP	14.9	166.1090	124.0643	12	94.0538	81.0460
IS	14.3	166.0618	166.0618	12	123.0563	151.0878

[a] Experimental *m*/*z* values obtained for a 100 ng mL^{-1} standard prepared in CCl_4.
[b] CE, collision energy, eV.

of 1.3 Da and re-fragmented by collision with nitrogen gas. Selective chromatographic records were obtained considering a mass extraction window of 5 mDa around the precursor ion (usually the base peak in the MS spectrum) or the selected product ion of each compound, Table 1.

2.4. Evaluation of method performance and quantification of wine samples

Evaluation of the two main steps (SPE and DLLME) involved in the sample preparation was carried out with different spiked wine (red and white) samples. The recoveries of the SPE step were calculated as the ratio between responses obtained for each MP in samples spiked before (wine aliquots) and after the SPE step (cartridge extracts), submitted to identical DLLME conditions. The extraction efficiencies (EEs, %) of the DLLME step were evaluated following an indirect method using liquid–liquid extraction (LLE) of aqueous solutions (spiked SPE extracts plus 8 mL of water and 1.5 g NaCl) with 2 mL CCl_4 for samples submitted and non-submitted to DLLME. Thus, EEs were calculated as: $EE(\%) = (A_{ns} - A_{es})/A_{ns})^*100$, being A_{es} and A_{ns} the responses for each compound in the LLE extracts from aqueous solutions previously submitted (A_{es}) and not submitted (A_{ns}) to DLLME concentration, respectively. The enrichment factors (EFs) provided by the overall sample preparation step were defined as: $EF = (40/0.045) \times EE$, with 40 and 0.045 mL representing the volumes of wine sample and the settled volume of the CCl_4 phase.

The linearity of the method was evaluated with wine samples spiked at 8 different concentrations, containing a fixed level (25 ng L^{-1}) of the IS. Procedural blanks were prepared with aliquots of synthetic wine (40 mL) submitted to the whole sample preparation process. Identification of target compounds in non-spiked wines was established based on match of the (1) retention time (allowed difference ± 0.05 min) and (2) two product ions (mass window 5 mDa) in sample extracts and authentic standards in CCl_4. The overall recoveries (method accuracy) were calculated as the ratio between concentrations measured for spiked a non-spiked aliquot (n = 3 replicates) of each sample divided by the added concentration and multiplied by 100. The limits of quantification (LOQs) were estimated for a signal to noise of 10, extrapolated from the lowest calibration solution level (1 ng L^{-1} except in case of EMP for which 2.5 ng L^{-1} was used). Concentration levels of target species in wine samples were determined with matrix-matched standards, corresponding to aliquots of red wine (Mencía variety) spiked with increased concentrations of target analytes, from 1 to 100 ng L^{-1} (25 ng L^{-1} for the I.S.), and submitted to the global sample preparation method.

3. Results and discussion

3.1. Optimization of compounds determination

Different assays were carried out in order to optimize compounds separation and detection in presence of the wine matrix surviving to the sample preparation process. As regards GC separation conditions, whatever the employed temperature program, the polyethylenglycol (WAXETR) stationary phase, led to overlapped peaks for the most volatile MPs (EMP and IPMP). On the other hand, the BP-5 type column allowed the baseline separation of the four analytes and the IS.

The instrumental sensitivity of the GC-TOF-MS system, without considering the sample preparation steps, was evaluated with standard mixtures in the range of concentrations between 0.5 and 100 ng mL^{-1}, using MS and product ion scan modes. Although the former one lead to a better sensitivity for standard solutions (higher instrumental calibration curve slopes), it turned unsuitable for the analysis of wine samples due to the extremely complex forest of peak surrounding some MP compounds. Particularly, the signals obtained for different ethyl esters of carboxylic acids existing in wines exactly matching with the retention times of different MPs. As example, the ester of octanoic acid overlapped the peak of IPMP in the polyethylenglycol column, and the ethyl ester of butanoic acid co-eluted with IBMP in the BP-5 column. Obviously, both esters are present in wines at several higher magnitude concentrations orders than those expected for MPs disturbing the chromatographic peaks of the analytes when present at the very low ng L^{-1}. Table 1 summarizes retention times, transitions (both quantifier and qualifier) with optimized collision energies for each analyte and the IS. Product ion scan spectra were recorded for an interval of 1.5 min around the retention time of each MP. GC–MS and GC–MS/MS chromatograms were extracted using a window of 5 mDa around the *m*/*z* values of precursor and product ions compiled in Table 1.

Using MS/MS detection, the system provided linear responses within the range of concentrations comprised between 0.5 and 100 ng mL^{-1} for IBMP, and between 2.5 and 100 ng mL^{-1} for EMP, IPMP and SBMP. The determination coefficients (R^2) of the obtained graphs ranged from 0.993 to 0.998.

3.2. Selection of sample preparation conditions

3.2.1. Solid-phase extraction

Optimization of the SPE step was performed with spiked (2.5 ng mL^{-1}) aliquots of red wine (the most complex matrix) of Mencía variety with a pH of 3.6. Three different types of OASIS SPE cartridges were compared in terms of retention efficiency and extraction selectivity. In case of the MCX sorbent, after sample loading, neutrals were removed with dichloromethane (2 mL) before compounds elution with methanol: NH_3 (98:2), following the strategy proposed by López et al. [11]. The neutral fraction removed, among others, esters present in the wine matrix with similar retention times as pyrazines in the GC column. However, the analytical fraction showed an intense dark appearance, with some particles of precipitate which disturbed phases separation during DLLME.

After sample loading, MAX and HLB cartridges were washed with synthetic wine and then compounds eluted with a polar solvent, compatible with the further DLLME step. The first sorbent strongly retained wine pigments leading to much cleaner extracts than those obtained from the HLB polymer using methanol as elution solvent. On the other hand, it presented breakthrough problems for EMP when processing 20 mL of wine (losses of 18%) and

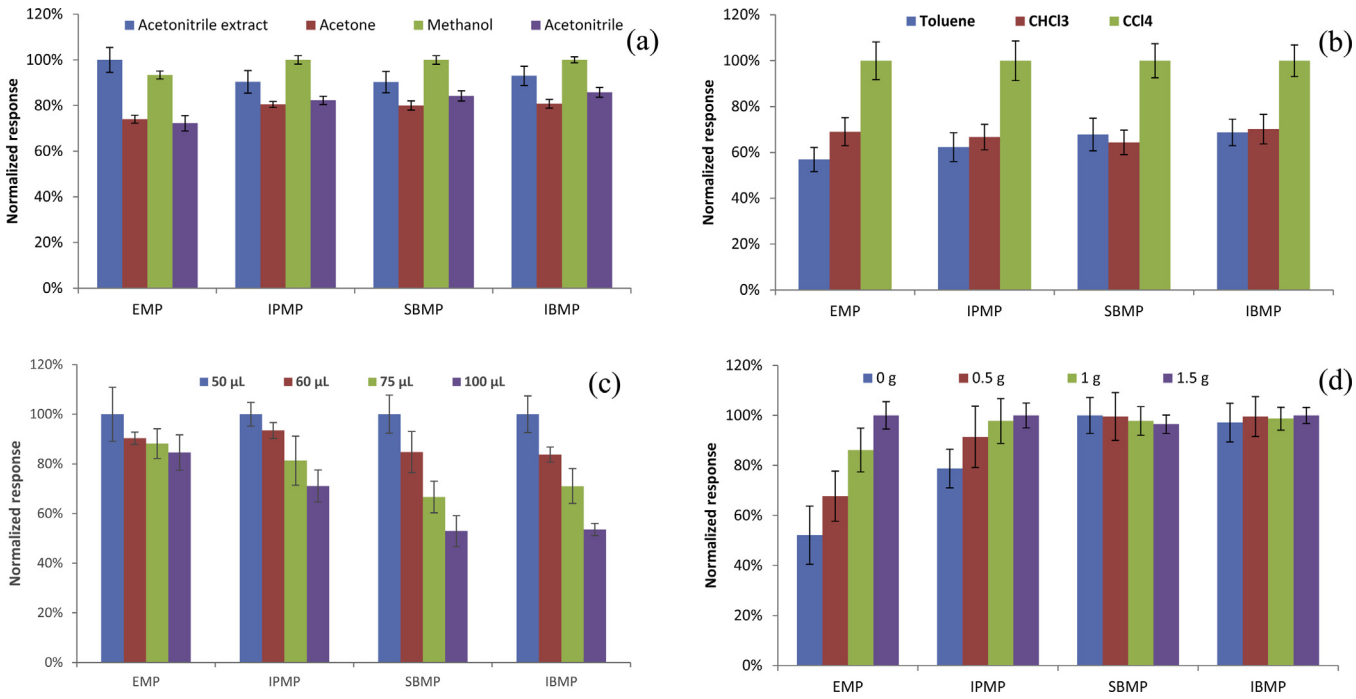

Fig. 1. (a) Effect of dispersant solvent on the response of target analytes. (b) Effect of extractant solvent on the response of studied MPs. (c) Effect of the type of extractant on the response of MPs, (d) Effect of NaCl in the efficiency of the DLLME process. Normalized responses to the maximum measured for each compound. Extraction conditions as described in text, $n = 3$ replicates.

also for IPMP for 40 mL samples (losses of 15%). The OASIS HLB cartridges permitted concentrating up to 40 mL of wine without analytes losses. Methanol and acetonitrile were compared as elution solvents. MPs could be eluted with just 1 mL acetonitrile. This solvent provided extracts with a much lower level of pigments than methanol. Under these conditions, the recoveries of SPE step varied from 81 to 94%, with standard deviations below 3% for red and white wines, Table S1.

3.2.2. DLLME parameters

3.2.2.1. Dispersant and extractant selection. The effects of both solvents (extractant and dispersant) in the responses of target compounds were investigated using the acetonitrile extracts obtained after SPE of red wine. Fig. 1a compares the responses obtained mixing the acetonitrile SPE extract with 0.1 mL of CCl_4 followed by dispersion on 8 mL of ultrapure water with those corresponding to dilution of the SPE extract with 8 mL of water followed by addition of a binary mixture containing 0.1 mL of CCl_4 and 1 mL of three different dispersants. The results are presented as normalized peak to those obtained for MeOH and reflected significant differences depending on the experimental conditions (Fig. 1a). With the single exception of EMP, aqueous dilution of the SPE extract followed by DLLME with methanol as dispersant resulted in the higher responses.

$CHCl_3$, CCl_4 and toluene were evaluated as extractant solvents. Fig. 1b shows the normalized responses measured for the DLLME extracts obtained with the above solvents (0.1 mL in all cases combined with 1 mL of methanol as dispersant). Depicted data correspond to peak areas normalized to those measured for CCl_4, without any correction for differences between the volumes of the final extract. CCl_4 rendered the highest responses for target MPs. Thus, it was selected as extractant.

3.2.2.2. Dispersant and extractant volume. The assays of dispersant volume were performed with increasing volumes of methanol (0.5, 1.0, 1.5 and 2.0 mL) combined with 0.1 mL CCl_4. The highest responses for all compounds were attained for the 1.0 mL of methanol (data not shown).

Four different volumes of CCl_4 between 0.05 and 0.1 mL were evaluated. A reduction in the volume from 0.1 to 0.05 mL improved the responses of IPMP, IBMP and SBMP, Fig. 1c. Such trends indicate that compounds are likely quantitatively extracted in all the cases. Regarding EMP, the obtained responses were practically independent of the volume of CCl_4. Under final conditions, 0.060 mL of CCl_4 were selected as a compromise between obtained responses and minimum volume of the settled phase (0.045 mL) to be handled with the autosampler, allowing re-injection if required.

3.2.2.3. Salting-out and buffering. Addition of different amounts of sodium chloride (0 g, 0.5 g, 1.0 g and 1.5 g) was considered in order to investigate the effect of the ionic strength in the DLLME efficiency (Fig. 1d). The achieved responses increased with the ionic strength of the solution in the DLLME extraction tube, particularly for EMP, the most polar compound. For 1.5 g of NaCl the responses for the four MPs were maximum, being the optimum condition selected for further studies.

The effect of different buffers was evaluated with the aim of eliminate some matrix interferences remaining after SPE step. Studies were performed by comparing the responses achieved without buffer and by conditioning the samples with K_2HPO_4 (pH 9.2) and sodium acetate (pH 4.9). No significant differences were observed neither on the responses of analytes (Fig. S1), nor in the complexity of GC-TOF-MS chromatograms for non-buffered and buffered samples. According to these results, no buffer addition was carried out in further experiments.

3.3. Performance of the method

The EEs (%) of the DLLME step are shown in Table 2. As expected from results displayed in Fig. 1c and d, propyl, butyl and sec-butyl pyrazines were extracted in a significant extend (above 77%) during the liquid-phase microextraction step, whereas DLLME efficiency

Table 2
Extraction efficiencies (EEs%) of the DLLME step and enrichment factors (EFs) of the overall procedure (SPE followed by DLLME) for the optimized method using red wine samples spiked at 2.5 ng mL^{-1}, $n = 3$ replicates.

Compound	EE (%)	EF
EMP	43 ± 5	379 ± 19
IPMP	77 ± 5	684 ± 34
SBMP	91 ± 4	807 ± 32
IBMP	87 ± 4	717 ± 29

remained below 50% for the more polar EMP. Assuming quantitative recoveries in the SPE process (see Table S1), the EFs of the overall procedure (calculated for 40 mL of wine and considering 0.045 mL as the final extract volume) varied between 379 and 807 times, Table 2.

The determination coefficients (R^2) obtained for red (Mencía variety) and white wine (Albariño variety) aliquots spiked at eight increasing concentration levels (1–100 ng L^{-1}) are compiled in Table 3. In most cases, R^2 values stayed above 0.99. Notice that, for EMP the lower level of calibration (1 ng L^{-1}) was not successfully detected, thus this compound showed a linearity range from 2.5 to 100 ng L^{-1}. The ratios between slopes of addition curves for red and white wines varied between 0.92 and 1.08, which points out to small changes in the efficiency of the overall method (including sample preparation and determination steps) for both matrices.

The accuracy of the method was evaluated with aliquots of two wine samples (Riesling and Loureiro varieties for white and red wine, respectively) fortified at two different concentration levels and quantified against the calibration curve obtained for spiked aliquots of a red wine (Mencía) sample. The recoveries (n = 3 replicates for spiked and non-spiked fractions) were in the range from 81 to 109%, with associated standard deviations remaining below 12%, Table 3. These data again support that the efficiency of the proposed sample preparation procedure is not affected by the characteristics of each wine sample, allowing the quantification of samples with a single calibration curve independently of the matrix (red or white wines).

The LOQs of the method were estimated from the signal to noise (S/N) values corresponding to chromatographic peaks in the lower levels of the linearity study for each compound. Values obtained for S/N = 10 stayed between 0.3 ng L^{-1} for IBMP and 2.1 ng L^{-1} for EMP. Recent methods for analysis of MPs in wine developed by Hjelmeland et al. [20], López et al. [11], Godelmann et al. [8] and Legrum et al. [10] all reported LOQs similar to those we presented here or higher. However, the majority of these methods require the application of HS-SPME or other sorptive extraction approaches, allowing processing one sample at the time resulting in lower throughput. In addition, higher sample preparation times for handling a single sample (c.a. 30 min) where reported. As well, some of these works reported RSD values as high as 20% for similar concentration levels as presented here.

3.4. Levels of target compounds in wine samples

The validated SPE-DLLME-GC-QTOF method was applied for the determination of MPs to a total of 12 samples of white and red wines from different grape varieties and countries. Eight red and 4 white specimens coming from different countries including Spain, France, Germany, South Africa, Chile and China were analyzed. The achieved results showed that IBMP was detected in six of the studied samples at concentrations above the LOQs of the method, Table 4. The rest of analytes remained under the LOQs of the method in all of them (data not shown). The levels found in the present work are in accordance with previously published literature and agree with the fact that IBMP is the most prevalent MP found in wine. As example, Hjelmeland et al. [20] have reported a maximum concentration of 6.2 ng L^{-1} of IBMP for Cabernet Sauvignon. As well, López et al. [11] reported levels ranging between 0.8 to 20.9 ng L^{-1}, with the highest concentrations corresponding to Sauvignon Blanc from Chile. A few studies have reported IPMP, SBMP and EMP levels in wines, in general with lower concentrations than IBMP. The levels strongly depend of the grape variety used for wine production and the place where wines are produced. Several factors including light exposure, crop level, and vintage among others can also affect

Table 3
Performance of the developed method for wine samples.

Compounds	Linearity evaluation		[a]Slopes ratio	Recoveries (%) ± SD ($n = 3$ replicates)				LOQs
			5 ng L^{-1}		25 ng L^{-1}			
	R^2, white wine	R^2, red wine		White wine	Red wine	White wine	Red wine	
EMP	0.989	0.987	1.08	85 ± 9	108 ± 11	87 ± 1	103 ± 8	2.1
IPMP	0.992	0.990	0.92	98 ± 3	109 ± 7	84 ± 1	105 ± 3	0.8
SBMP	0.984	0.994	1.05	81 ± 2	84 ±9	84 ± 6	91 ± 12	0.6
IBMP	0.995	0.992	1.02	97 ± 8	94 ± 2	98 ± 3	96 ± 1	0.3

[a] Ratios of white wine/red wine slopes.

Table 4
Levels of target MPs in non-spiked wine samples. Average concentrations (ng L^{-1}) with their standard deviations, $n = 3$ replicates.

Code	Grape variety	Sample origin	IBMP
W1	Verdejo	Valdepeñas, Castilla-La Mancha, Spain	n.d.
W2	Albariño	Rías Baixas, Galicia, Spain	n.d.
W3	Airén and Macabeo	Valdepeñas, Spain	n.d.
W4	Riesling	Rheinhessen, Germany	n.d.
R1	Mencía	Ourense, Galicia, Spain	n.d.
R2	n.i.	Bordeaux, France	3.7 ± 0.5
R3	Pinotage	Western Cape, South Africa	7.9 ± 0.7
R4	Cabernet Sauvignon	Central Valley, Chile	7.3 ± 0.9
R5	n.i.	Cotes Du Rhone, France	n.d.
R6	n.i.	Montagne Saint-Emilion, France	2.9 ± 0.4
R7	Shiraz and Cabernet Sauvignon	Victoria, Australia	2.2 ± 0.3
R8	Cabernet Gernischt, Cabernet Sauvignon and Cabernet Franc	Yantai, China	9.9 ± 1.1

n.i.: not informed; n.d.: not detected.
Codes W and R correspond to white and red wines, respectively.

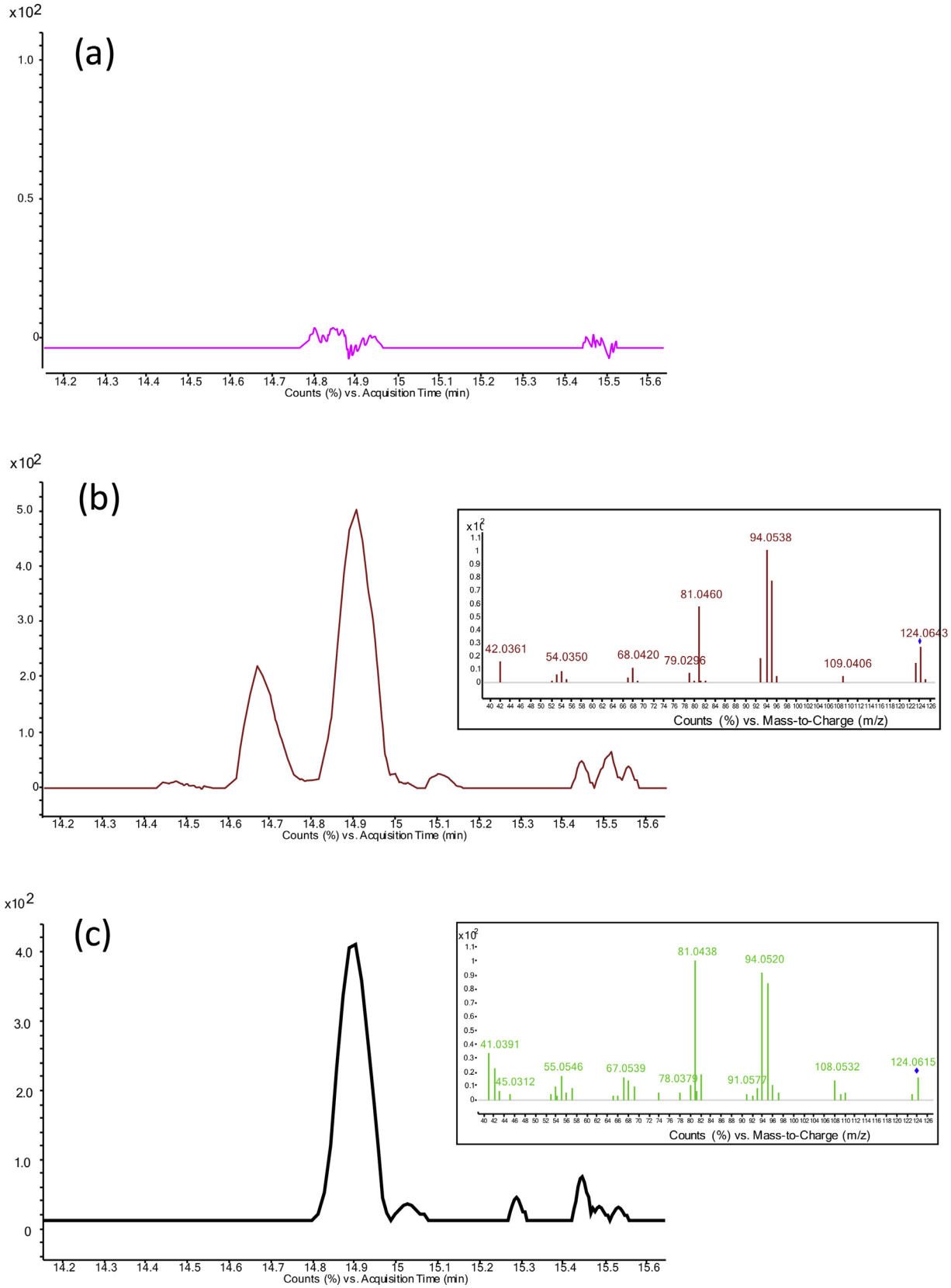

Fig. 2. Extracted chromatograms and product ion spectra for quantification product ion of IBMP in (a) blank red wine sample, (b) same red wine after addition of MPs at 10 ng L^{-1}, (c) a non-spiked wine containing 7.3 ng L^{-1} of IBMP (code R4, Table 4).

the levels of MPs [21–24]. Fig. 2 shows the chromatographic plots for IBMP (most intense MS/MS transitions) in a procedural blank, the same sample spiked with MPs and a positive sample together with product ion scan spectra for IBMP. The accurate measurement of the *m*/*z* ratios for fragment ions guaranteed the unambiguous identification of target analytes.

4. Conclusions

A high throughput methodology combining SPE as pretreatment technique with the high concentration capabilities of DLLME and selectivity associated to accurate mass spectrometry (MS/MS mode) is presented for the first time for the determination of MPs in wines. Exploiting the advantages of both techniques allowed to achieve high EFs and clean-up of samples, which are critical points for MPs quantification because their expected concentrations are at the low ng L^{-1} level. In this sense, the method achieved LOQs low enough to guarantee the applicability of the method in different wine samples with concentrations at or below reported sensory detection thresholds in wine. Moreover, the QTOF product ion spectra permitted the unambiguous identification and quantification of target compounds based on the accurate mass of fragment ions. The sample preparation method also provided quantitative recoveries of MPs by using a single matrix-matched calibration as quantification technique for white and red wines.

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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.chroma.2017.07.085.

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