Mixed-mode solid-phase extraction followed by acetylation and gas chromatography mass spectrometry for the reliable determination of *trans*-resveratrol in wine samples

R. Montes, M. García-López, I. Rodríguez*, R. Cela

Departamento de Química Analítica, Nutrición y Bromatología, Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain

This work presents advantageous analytical procedure for the accurate determination of free *trans*-resveratrol in red and white wines. The proposed method involves solid-phase extraction (SPE), acetylation of the analyte in aqueous media and further determination by gas chromatography (GC) with mass spectrometry detection (MS). The use of a mixed-mode SPE sorbent provides an improvement in the selectivity of the extraction step; moreover, the presence of several intense ions in the electron impact mass spectra of its acetyl derivative guarantees the unambiguous identification of *trans*-resveratrol. Considering a sample intake of 10 mL, the method provides a limit of quantification (LOQ) of 0.8 ng mL^{-1} and linear responses for concentrations up to 2.5 μg mL^{-1}, referred to wine samples. The average recovery, estimated with samples fortified at different concentrations in the above range, was 99.6% and the inter-day precision stayed below 8%. *Trans*-resveratrol levels in the analyzed wines varied from 3.4 to 1810 ng mL^{-1}. *Cis*-resveratrol was also found in all samples. In most cases, equal or higher responses were measured for this latter form than for the *trans*-isomer. The reduced form of resveratrol, dihydro-resveratrol, was systematically identified in red wines.

1. Introduction

Resveratrol (3,4*,5-trihydroxystilbene, CAS 501-36-0) is a phytochemical present in different vegetables, particularly in grapes. The natural production of the *trans*-isomer of this stilbenoid by *Vitis vinifera* is enhanced under stress conditions, such as fungal infections, adverse climate and exposure of grapes to UV light. Later, during the wine making process, *trans*-resveratrol is partially isomerized to the *cis*-form [1]. The strong antioxidant properties of *trans*-resveratrol have been correlated with several beneficial effects on human health. The most accepted ones are the prevention of coronary diseases [2,3] and, more recently, the inhibition of the proliferation of certain cancerous cells [4,5]. On the basis of this information, considering that wine ingestion constitutes one of the most important natural dietary sources of this compound, it is not surprising that the content of resveratrol in bottled wine is matter of interest for both, producers and consumers. The availability of simple and reliable analytical methodologies for the determination of resveratrol in this matrix is necessary to investigate the controllable variables, related to vine cultivation and wine elaboration, which increase the concentration of this antioxidant in commercialized wines.

Procedures for the determination of resveratrol in wine have to cope with (1) the complexity of the sample matrix, particularly in the case of red wines, and with (2) the wide range of concentrations (from low ng mL^{-1} up to several μg mL^{-1}) occurring in wines. In most applications, reversed-phase silica based solid-phase extraction (SPE) sorbents followed by liquid chromatography (LC) or gas chromatography (GC) determination are used [6,7]. In the latter case, derivatization of the phenolic moieties contained in the structure of resveratrol is highly advisable to improve the detectability of the compound and the quality of the separation process [1]. As regards the SPE step, in recent applications silica based sorbents have been replaced by co-polymeric materials, such as the OASIS HLB sorbent [8]. Moreover, solid-phase microextraction (SPME) followed by on-fibre derivatization has been also proposed for the determination of resveratrol in wine samples using GC with mass spectrometry (MS) detection [9–11]. Silylation is the most resorted derivatization reaction used for the GC-MS determination of resveratrol [1,9–11]. When combined with SPE, cartridges are normally eluted with ethyl acetate and then, the extract is mixed with bis(trimethylsilyl) trifluoroacetamide (BSTFA) before injection in the chromatographic system [1,9]. In the case of SPME based methods, resveratrol is first incorporated in the SPME coat-

* Corresponding author. Tel.: +00 34 981 563100x14387; fax: +00 34 981 595012.
E-mail address: isaac.rodriguez@usc.es (I. Rodríguez).

ing; after that, the fibre is exposed to the headspace of a vessel containing the silylation agent. Acetylation, using acetic anhydride under basic conditions in aqueous media, is another straightforward approach for the derivatization of phenolic moieties [12]. Despite its popularity in the field of environmental analysis, as far as we could trace, acetylation has been only applied to improve the extractability of resveratrol from wine using polydimethylsiloxane sorbents followed by LC separation with fluorescence detection [13].

SPME followed by GC-MS provides limits of quantification (LOQs) in the low ng L^{-1} level; however, sample preparation (extraction followed by on-fibre derivatization) is a relatively slow process (the original method developed by Luan and coworkers requires 50 min per sample [9]) and its selectivity is also limited, since extractions are carried out in the direct sampling mode. Thus, organic acids contained in wine are also concentrated in the coating (normally polyacrylate) of the SPME fibre and further derivatized during its exposure to the vapours of the silylation reagent. As a result, relatively complex chromatograms are obtained. Another potential limitation of SPME methods is the risk of cross-contamination problems, which is of particular relevance due to the variability of resveratrol concentrations occurring in wine. Finally, the yield of the microextraction process might change depending on the characteristics of wine samples [10] and/or the surface of the SPME coating which becomes irreversibly contaminated with sugars, salts and other non-volatile species contained in red wines [11]. Such changes can be only effectively compensated using isotopic labelled analogues of the target analyte are internal surrogates. Both factors (cross-contamination risk and variation in the yield of the SPME with the number of fibre uses) have a negative effect on the accuracy and the cost of SPME based procedures.

The aim of this research is the development of a simple and robust method for the determination of resveratrol in red and white wine samples. Due to its widespread use in control laboratories and limited risk of cross-contamination problems, SPE was chosen as extraction technique; moreover, the selectivity of the whole procedure was improved by using a mixed-mode (reversed-phase and anionic exchanger) sorbent. Acetylation was proposed as derivatization reaction on the basis of the additional selectivity achieved during liquid-liquid partition of acetyl derivatives between aqueous and organic phases, and the favourable features of the electron impact (EI) mass spectra of acetylated resveratrol, in comparison with the trimethyl silyl derivative. Parameters affecting the efficiency of derivatization and extraction steps were thoroughly investigated and the resulting method was characterized in terms of accuracy, precision, limit of quantification (LOQ) and linear response range.

2. Experimental

2.1. Solvents, standards, SPE sorbents and samples

HPLC-grade methanol, isooctane and ethyl acetate, trace analysis grade, were purchased from Aldrich (Milwaukee, WI, USA). Acetic anhydride, BSTFA and *trans*-resveratrol were also from the same supplier. Potassium carbonate (K_2CO_3) and di-potassium mono-hydrogen phosphate (K_2HPO_4) were provided by Merck (Darmstadt, Germany). Solutions of both salts were prepared using ultrapure (Milli-Q) water at different concentrations between 1 and 5%. The stock solution of *trans*-resveratrol was prepared in methanol, further dilutions were made in the same solvent and in ethyl acetate, depending on the considered derivatization reaction.

Oasis HLB and Oasis MAX SPE cartridges, both containing 60 mg of the respective sorbent, were obtained from Waters (Milford, MA, USA). The first polymer retains organic compounds on the basis of a reversed-phase mechanism, whereas the latter works also as anionic exchanger due to the presence of positively charged amino groups in its structure.

Wine samples were acquired from local markets. After arriving to the laboratory they were protected from light and stored at room temperature, for a maximum of 2 weeks, before being processed.

2.2. Sample preparation

Optimization of SPE conditions was carried out with commercial samples of red and white wines. Since the target species is already present in wine, spiked samples were used only to evaluate the yield of the extraction process, but not during the assessment of breakthrough and elution volumes of SPE cartridges. Wine samples were filtered using 0.45 μm pore size PVDF filters, fortified with a standard solution of *trans*-resveratrol in methanol when required, and diluted (1:1) with ultrapure water. After finishing the extraction step, SPE cartridges were rinsed with 10 mL of ultrapure water, dried using a gentle stream of nitrogen and eluted. Breakthrough studies were carried out by passing the samples through two cartridges connected in series. The elution volume was determined by collecting consecutive 1 mL fractions of a suitable solvent from the SPE cartridge. Under final working conditions, 10 mL of wine and 2 mL of methanol were selected as sample and elution solvent volumes, respectively. Extractions were accomplished using the Oasis MAX sorbent.

Trans-resveratrol standards in methanol and SPE extracts were diluted with an alkaline aqueous solution and further mixed with acetic anhydride followed by manual shaking. The highest yield of the derivatization step was achieved using 10 mL of a 5% K_2HPO_4 aqueous solution and 50 μL of acetic anhydride. The reaction was completed in 5 minutes. After that, the acetyl derivative was extracted with 2 mL of isooctane

In the earlier steps of this research silylation was also considered as derivatization approach. Trimethyl silyl derivatives of *trans*-resveratrol were obtained by adding 50 μL of BSTFA to 0.5 mL aliquots of standards prepared in ethyl acetate [9].

2.3. GC-MS determination

Trans-resveratrol was determined by GC-MS, using a Varian (Walnut Creek, CA, USA) 450 GC instrument connected to an ion-trap Varian 240 mass spectrometer (MS), furnished with an electron impact (EI) source in the external configuration mode. Separations were carried out in a Varian Factor Four, BP-5 type column (30 m x 0.25 mm i.d., d_f: 0.25 μm) operated at a constant helium flow of 1.2 mL min^{-1}. The GC oven was programmed as follows: 90 °C (held for 1 min), rate at 15 °C min^{-1} to 280 °C (held for 15 min). The temperature of the injector was maintained at 280 °C. Standards and sample extracts were injected in the splitless mode (1-2 μL), with the solenoid valve switched to the split position after 1 min. Transfer line, electron impact ionization source and trap temperatures were set at 285, 180 and 120 °C, respectively. The helium dumping gas flow was fixed at 2.5 mL min^{-1}

The mass spectrometer was operated in the electron impact ionization mode (70 eV). MS spectra were acquired in the *m/z* range between 150 and 450 a.m.u., using a filament emission current of 50 μA. The three most intense ions in the spectra of the acetylated *trans*-resveratrol (m/z 228, 270 and 312) were used to monitor the chromatographic response of this species in calibration standards and sample extracts.

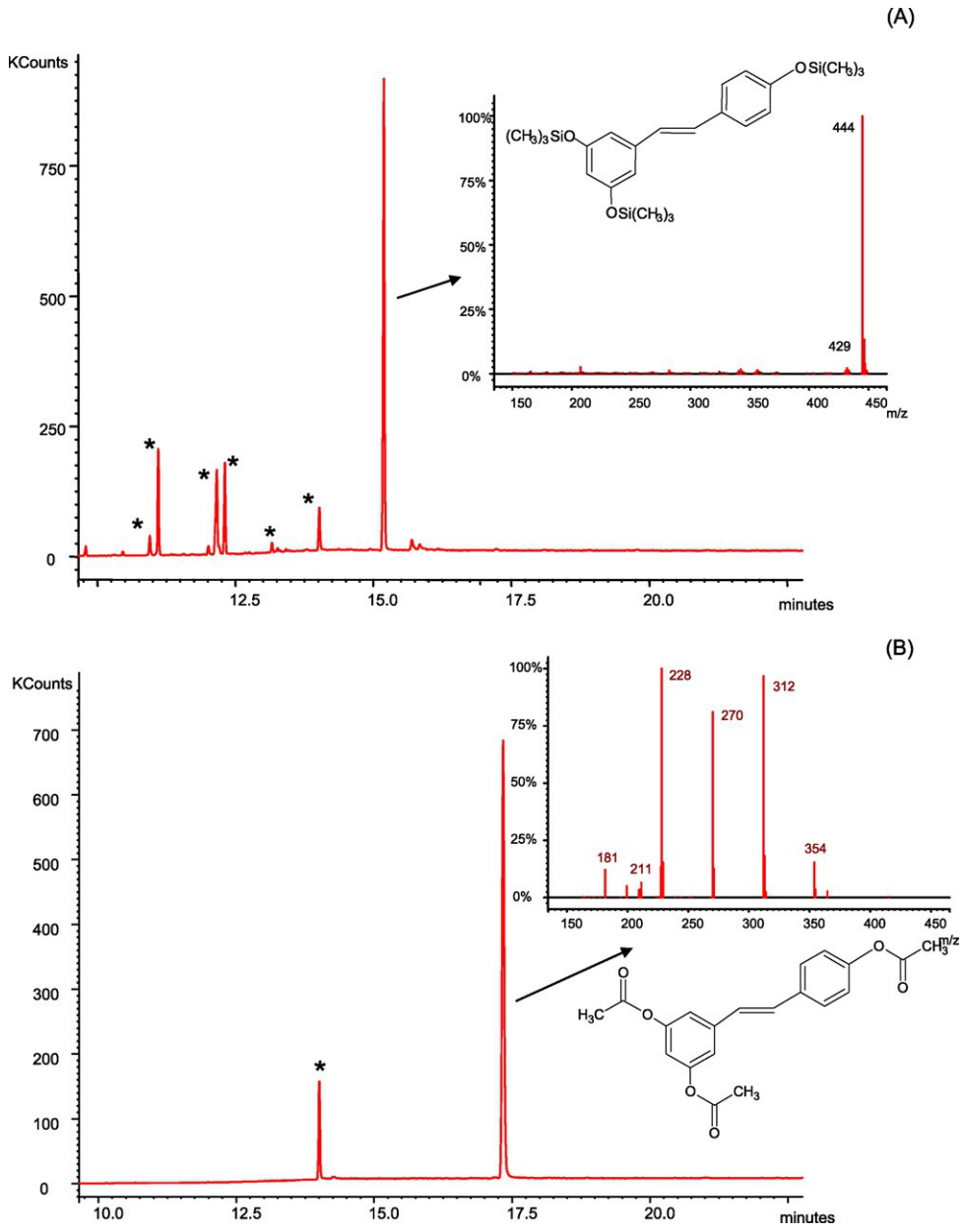

Fig. 1. Total ion current (TIC) chromatograms and MS spectra corresponding to a standard of *trans*-resveratrol (500 ng mL^{-1}). A, as trimethyl silyl derivative. B, as triacetylated specie. *derivatization by-products.

3. Results and discussion

3.1. Derivatization conditions and instrumental GC-MS performance

Fig. 1 shows the chromatograms (total ionic current, TIC) and mass spectra corresponding to a standard of *trans*-resveratrol (500 ng mL^{-1}) silylated with BSTFA in ethyl acetate (Fig. 1A), and acetylated using acetic anhydride in aqueous media followed by extraction into isooctane (Fig. 1B). The spectra of the silylated compound shows a single signal corresponding to the molecular ion (m/z 444) and a very weak transition, which reflects the loss of a methyl group (m/z 429). Moreover, several derivatization by-products were noticed in the corresponding chromatogram, Fig. 1A. On the other hand, the spectra corresponding to the acetyl derivative presents three intense ions at m/z ratios of 228, 270 and 312 units (Fig. 1B), which reflect stepwise replacements of acetyl moieties by hydrogen. These signals, in addition to the less intense one for the molecular ion (m/z 354), facilitate the unambiguous identification of resveratrol in real samples. In this case, the GC-MS chromatogram only shows a derivatization by-product (Fig. 1B). On the basis of these features, acetic anhydride was selected as the derivatization reagent.

Optimization of acetylation conditions was carried out with methanolic extracts obtained from wine, see section below, as well as using pure standards of *trans*-resveratrol in the same solvent. After completing the reaction, the acetylated derivative was extracted with 2 mL of isooctane and injected in the GC-MS system. In a first series of experiments the influence of the base (K_2CO_3 or K_2HPO_4) on the responses obtained for acetyl derivatives of *cis*- and *trans*-resveratrol in the extracts from red wine was assessed. In both cases, wine extracts (1 mL) were diluted with 10 mL of a 5% aqueous solution of the corresponding base. The volume of acetic anhydride and the reaction time were set at 200 μL and 5 min, respectively. For both isomers of resveratrol, around twice higher responses were attained for K_2HPO_4 (pH 9.3) than using K_2CO_3 (pH 11.4),

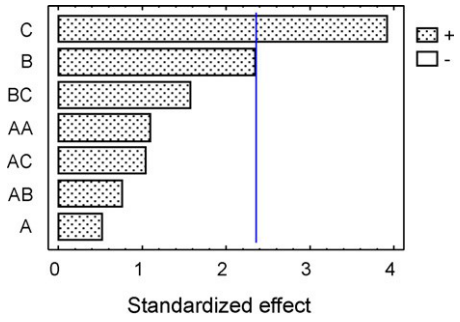

Fig. 2. Pareto chart with standardized values of main effects and two factor interactions corresponding to the optimization of *trans*-resveratrol acetylation in the extracts from red wine.

data not shown. Probably, under the more alkaline conditions provided by K_2CO_3, the hydrolysis of acetic anhydride is faster than the acetylation of the phenolic moieties contained in the structure of resveratrol. Another drawback of using K_2CO_3 was the generation of CO_2. The bubbles of this gas disturbed the separation of phases after addition of isooctane to the aqueous derivatization mixture. The effects of acetic anhydride volume, K_2HPO_4 concentration and reaction time on the efficiency of the acetylation were simultaneously evaluated using a $3^1 \times 2^2$ experimental factorial design, with 3 replicates of the central point. The volume of acetic anhydride was evaluated at three levels (50, 150 and 250 μL), whereas lower and upper values for the concentration of K_2HPO_4 and the reaction time were 1-5% and 1-5 min, respectively. In all cases, 1 mL of a pooled methanolic extract, corresponding to the SPE of non-spiked red wine samples, was used. Peak areas obtained for *trans*-resveratrol in the 15 experiments involved in the above design were processed using the Statgraphics Centurion XV software (Manugistics, Rockville, MD, USA) in order to evaluate the influence of each variable on the yield of the extraction. Normalized values corresponding to main effects, the quadratic term associated with the volume of acetic anhydride and two-factor interactions are graphically depicted in Fig. 2. The length of the bars is proportional to the influence of each variable on the performance of the reaction. The dotted line represents the statistically significance boundary at the 95% confidence level. All factors affected positively to the acetylation process, meaning that higher responses were attained when they were maintained in the upper level of the design. However, only the reaction time (code C), and in a minor extension the concentration of K_2HPO_4 (code B), exerted significant effects on the yield of the process. Thus, a reaction time of 5 min using 10 mL of a 5% K_2HPO_4 solution were adopted as working conditions for both factors. The volume of acetic anhydride (code A) was limited to 50 μL to save the consumption of this reagent. After that, 2 mL of isooctane were added to the derivatization tube, which was shaken again for other 5 min. An aliquot of the upper phase was transferred to an autosampler 1.5 mL volume vial and injected in the GC-MS system. Further derivatization assays, using pure standards of *trans*-resveratrol at the 5 μg mL^{-1} level, lead to chromatograms with a single peak corresponding to the tri-acetylated species. Consequently, the yield of the reaction was assumed to be quantitative. Another relevant aspect in connection with the acetylation reaction was to assess whether its efficiency changes between pure standards and extracts from wine samples. This question was addressed by comparing the differences between responses (peak areas) obtained for spiked and un-spiked extracts from wine versus those obtained for a pure standard, of the same concentration, in methanol. Table 1 summarizes the relative efficiencies achieved for red and white wine samples. Overall, values around 100% were noticed for both matrices, thus the yield of the acetylation remains the same for pure standards and sample extracts.

Table 1
Relative efficiencies of the derivatization reaction for SPE extracts from red and white wine. Normalized responses to those achieved for standards in methanol, n= 5 replicates.

Sample	Relative yield (%)	Standard deviation
Red wine	107.1	3.0
White wine	92.0	5.0

Table 2
Overall recoveries of the proposed method for spiked samples, n= 3 replicates.

Sample	Variety	Addition level (ng mL^{-1})	Recovery (%)	SD
White wine	*Palomino*	40	108.2	4.4
White wine	*Palomino*	200	92.5	3.2
Red wine	*Tempranillo*	300	93.9	5.0
Red wine	*Grenache*	1000	103.9	3.9

The linearity in the response of the GC-MS system for *trans*-resveratrol was evaluated with acetylated standards at nine different concentrations in the range between 5 ng mL^{-1} and 5 μg mL^{-1}. The plot of peak area (calculated using the sum of responses for m/z ratios 228, 270 and 314 m/z) versus concentration (ng mL^{-1}) fitted a linear model (y = ax + b) with a determination coefficient (R^2) of 0.997. Slope (a) and intercept (b) values were 1231 ± 15 and 98 ± 45, respectively. The limit of quantification (LOQ), defined as the concentration of *trans*-resveratrol providing a signal 10 times higher than the baseline noise, was 2 ng mL^{-1}.

3.2. Solid-phase extraction

Optimal SPE conditions were investigated using aliquots of red and white wines. Since the pH of wine (3.2-3.5 units) remains far below the pKa of resveratrol (9.15), samples were just diluted with ultrapure water (1:1), to limit the negative impact of ethanol in the yield of the retention [14], and passed through the SPE cartridge, without acidification, at an approximate flow rate of 2 mL min^{-1}. After the concentration step, the SPE sorbent was rinsed with 5 mL of ultrapure water to remove sugars, dried with a gentle stream of nitrogen and eluted with methanol. Using 10 mL of wine, breakthrough problems were not noticed either for HLB or for MAX cartridges, whereas traces of resveratrol appeared in the second cartridge when the volume of sample (wine) was increased to 20 mL. As regards the elution step, 2 mL of methanol also sufficed to recover the compound for both sorbents. The main difference between the reversed-phase (Oasis HLB) and the

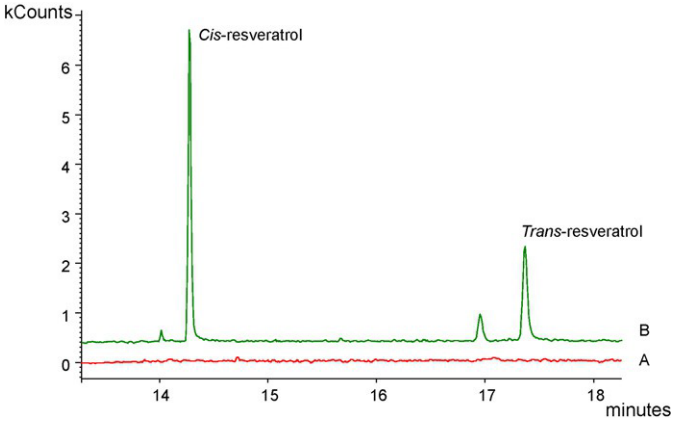

Fig. 3. Selected ion chromatograms (m/z 228 + 270 + 312) corresponding to a procedural blank (A) and a sample of white wine with a low level (3.4 ± 0.1 ng g^{-1}) of *trans*-resveratrol (B).

Table 3
Summary of data obtained for commercial wine samples.

Code	Type	Geographic denomination	Year	*Trans*-resveratrol Conc (ng mL^{-1} ± SD)	Ratio *cis*/*trans*
1	White	Albarin˜o	2008	150 ± 28	1.29
2		Bierzo	2006	92 ± 5	3.38
3		Mancha	2008	3.4 ± 0.1	1.91
4		Mancha	2008	74 ± 4	1.09
5		Ribeiro	2008	99 ± 5	1.00
6		Ribeiro	2008	70 ± 4	4.38
7	Red	Bierzo	2008	949 ± 9	1.25
8		Mancha	2008	688 ± 10	0.97
9		Mancha	2008	1300 ± 75	0.75
10		Ribeiro	2009	1800 ± 200	2.73
11		Rioja	2002	140 ± 8	1.18
12		Rioja	2004	137 ± 14	2.86
13		Rioja	2002	1810 ± 90	0.18
14		Rioja	2007	680 ± 14	1.19
15		Rioja	2003	575 ± 42	1.05

mixed-mode (Oasis MAX) polymers was the visual appearance of the extracts. In the first case, yellowish and intense red solutions were obtained after concentration of 10 mL of white (*Palomino*) and red (*Grenache*) wine, respectively. On the other hand, colourless and pink extracts were attained for same samples with the latter sorbent. Probably, pigments with acidic properties stayed retained in the MAX sorbent, due to electrostatic interactions with the positively charged groups existing in the surface of this polymer, resulting in cleaner extracts. After the acetylation reaction some problems were noticed to achieve a neat separation between aqueous and isooctane layers for red wine extracts provided by the HLB sorbent. This shortcoming was overcome with the MAX material.

3.3. Method performance

Table 2 summarizes the overall recoveries of the proposed method evaluated with samples spiked at different concentration levels. Depicted data correspond to triplicate extractions. Differences between peak areas measured for spiked and non-spiked aliquots (10 mL) of each sample were introduced in the equation of the calibration curve corresponding to acetylated standards of *trans*-resveratrol. Found concentrations were compared with the added ones. Obtained recoveries ranged from 92 to 108%, with associated standard deviations (SD) below 5. Procedural blanks did not reveal the existence of contamination problems, thus the LOQ of the method was controlled by sample and final extract volumes, as well as by the instrumental LOQ of the GC-MS system for acetylated *trans*-resveratrol (Fig. 3). The achieved LOQ (0.8 ng mL^{-1}) is low enough to guarantee the determination of *trans*-resveratrol in wine samples. This LOQ is fifty times lower than that achieved using GC-MS, but without analyte derivatization [7], around 20-fold below than that reported for SPE with LC-UV determination [8] and similar to those reported combining SPME and SBSE with LC using fluorescence detection [13]. LOQs reported using LC-MS and LC-MS/MS as determination techniques were 20 ng mL^{-1} [15] and 5 ng mL^{-1} [16], respectively. Although some SPME based methods, combined with GC-MS, provide two orders of magnitude lower LOQs [9], they render a lower sample throughput (extraction followed by on-fibre derivatization takes 50 min and samples are processed one-by-one) in addition to be prone to cross-contamination problems. The sample preparation methodology described in this work requires around 25 min per sample; moreover, several samples can be simultaneously processed and final extracts loaded in the tray of the GC autosampler. On the other hand, concentrations as high as 2.5 $\mu g\,mL^{-1}$ can be quantified without dilution, neither of the sample nor of the final extract. The inter-day precision of the overall method was evaluated by extracting two different samples with low (white wine) and high (red wine) *trans*-resveratrol contents, in duplicate during 5 consecutive days. The relative standard deviations (RSDs) were 4.1% and 7.8% for red and white wine samples, respectively.

3.4. Real samples analysis

The procedure described in this work has been applied to determine the free *trans*-resveratrol content in wine samples elaborated in different geographic areas of Spain. Found values are summarized in Table 3. Globally, the concentrations of *trans*-resveratrol were higher in red (from 137 to 1810 ng mL^{-1}) than in white (from 3.4 to 150 ng mL^{-1}) wines, which is in agreement with previously published data for Spanish wines [10] and also with the fact that, the skin of grapes is usually macerated together with must during the elaboration of red wines. The concentration of *cis*-resveratrol in wine could not be determined since this isomer is not commercially available. However, the ratio between peak areas of both isomers can provide an estimation of the levels of this latter species in wine. Obtained ratios (Table 3) pointed out to different grades of isomerisation, which might be related to the wine making process and/or with time and conditions occurring during storage of wine, first in the cave and then after being bottled during distribution and commercialization steps.

A noticeable difference between red and white wine samples was the systematic presence of an additional peak in the chromatograms of the former matrix. This peak showed (1) a retention time comprised between the acetylated forms of *cis*- and *trans*-resveratrol and (2) a MS spectra with three clusters of ions at m/z ratios two units higher than those reported (see Fig. 1B) for the acetylated isomers of resveratrol, Fig. 4A. On the basis of this spectral information the compound was tentatively identified as dihydro-resveratrol (CAS number 58436-28-5). The chromatograms and spectra obtained after dryness evaporation of the methanolic extract from the same sample (code 13, Table 3) followed by ethyl acetate re-constitution and addition of BSTFA are shown in Fig. 4B. The major ions in the spectra matched with those used by Ortuño and co-workers [17] for the identification of dihydro-resveratrol in biological samples. Dihydro-resveratrol is recognized as an urinary metabolite of *trans*-resveratrol [17,18]; moreover, its presence in wine has been also suggested in a recent research using overpressured layer chromatography as separation technique [19].

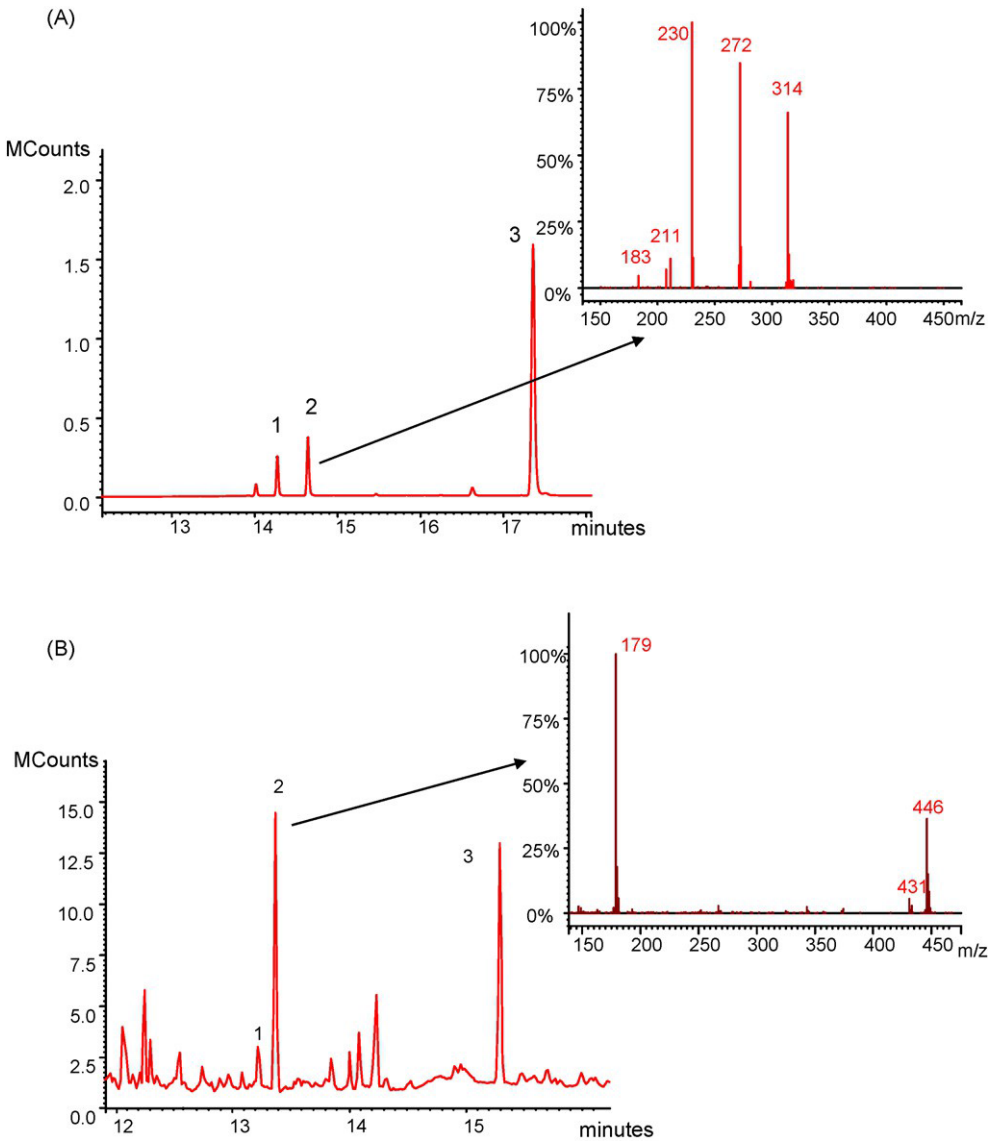

Fig. 4. TIC chromatograms for the methanolic extracts from the same red wine sample. A, after acetylation and re-extraction with isooctane. B, after dryness evaporation and silylation with BSTFA. Peak labels: 1, *cis*-resveratrol. 2, dihydro-resveratrol. 3, *trans*-resveratrol. Shown spectra correspond to acetyl (A) and silyl (B) derivatives of peak number 2.

4. Conclusions

An alternative method for the reliable determination of *trans*-resveratrol in wine samples is presented. The combination of mixed-mode SPE with further acetylation of the target compound increases the selectivity of the overall procedure allowing the unambiguous and accurate determination of *trans*-resveratrol, within the range of concentrations found in commercialized wine samples. To the best of our knowledge, this work constitutes the first application of a mixed-mode (anionic exchanger and reversed-phase) sorbent and acetylation for the GC-MS determination of *trans*-resveratrol in wine. The analytical features of the method make it suitable for the routine screening of the free levels of this antioxidant in wine samples and thus, to evaluate those parameters which affect the content of *trans*-resveratrol in bottled wine, as well as the ratio between *cis*- and *trans*-isomers. It is expected that it could be also used for the determination of other stilbenoids, e.g. dihydro-resveratrol, in wine providing that standards will be commercially available in the coming years.

Acknowledgments

This study has been supported by the Spanish Government and E.U. FEDER funds (projects CTQ2009-08377 and DE2009-0020). R.M. and M.G.L. thank their FPU contracts to the Spanish Ministry of Science and Innovation.

References

[1] G.J. Soleas, D.M. Goldberg, E.P. Diamandis, A. Karumanchiri, J. Yan, E. Ng, Am. J. Enol. Vitic 46 (1995) 346.
[2] G. Lippi, M. Franchini, G.C. Giudi, Inter. J. Wine Res 2 (2010) 1.
[3] A.A.A. Bertelli, D.K. Das, J. Cardiovasc. Pharmacol 54 (2009) 468.
[4] A. Puissant, G. Robert, N. Fenouille, F. Luciano, J.P. Cassuto, S. Raynand, P. Auberger, Cancer Res 70 (2010) 1042.
[5] A. Bishayee, T. Politis, A.S. Darvesh, Cancer Treat. Rev 36 (2010) 43.
[6] C. Domínguez, D.A. Guillen, C.G. Barroso, J. Chromatogr. A 918 (2001) 303.
[7] D.M. Goldberg, J. Yan, E. Ng, E.P. Diamandis, A. Karumanchiri, G. Soleas, A.L. Waterhouse, Anal. Chem 66 (1994) 3959.
[8] S. Perez-Magarino, M. Ortega-Heras, E. Cano-Mozo, J. Agric. Food Chem 56 (2008) 11560.
[9] T. Luan, G. Li, Z. Zhang, Anal. Chim. Acta 424 (2000) 19.

[10] P. Vin˜as, N. Campillo, N. Martínez-Castillo, M. Hernández-Córdoba, J. Chromatogr. A 1216 (2009) 1279.
[11] L. Cai, J.A. Koziel, M. Dharmadhikari, J. van Leeuwen, J. Chromatogr. A 1216 (2009) 281.
[12] I. Rodríguez, M.P. Llompart, R. Cela, J. Chromatogr. A 885 (2000) 291.
[13] P. Vin˜as, N. Campillo, M. Hernández-Pérez, M. Hernández-Córdoba, Anal. Chim. Acta 611 (2008) 119.
[14] R. Montes, I. Rodríguez, M. Ramil, E. Rubí, R. Cela, J. Chromatogr. A 1216 (2009) 5459.
[15] K. Gamoh, K. Nakashima, Rapid Commun. Mass Spectrom 13 (1999) 1112.
[16] L.D. Donna, F. Mazzotti, H. Benabdelkamel, B. Gabriele, P. Plastina, G. Sindona, Anal. Chem 81 (2009) 8603.
[17] J. Ortun˜o, M.I. Covas, M. Farre, M. Pujadas, M. Fito, O. Khymenets, C. Andes-Lacueva, P. Roset, J. Joglar, R.M. Lamuela-Raventós, R. de la Torre, Food Chem 120 (2010) 1123.
[18] D. Wang, T. Hang, C. Wu, W. Liu, J. Chromatogr. B 829 (2005) 97.
[19] Z. Kiraly-Veghely, A.M. Moricz, P.G. Ott, G. Katay, I. Belai, E. Tyihak, J. Liq. Chromatogr. Relat. Technol 32 (2009) 1259.