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Short communication

Stir bar sorptive extraction applied to the determination of dicarboximide fungicides in wine

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Abstract

The dicarboximide fungicides vinclozolin, iprodione and procymidone were analyzed in white wines using stir bar sorptive extraction (SBSE) in combination with thermal desorption–capillary GC–MS analysis (TD–cGC–MS). The method was optimized using spiked water samples in a concentration range between 0.5 and 100 μg/l. Iprodione was measured as its degradation product 3,5-dichlorophenyl hydantoin. Limits of quantification in the full scan MS mode are 0.5 μg/l for vinclozolin and procymidone and 5 μg/l for iprodione. In the ion monitoring mode, concentrations 100 times lower can be dosed. Because of wine matrix effects on the recoveries, quantification of the target fungicides in wine had to be carried out by standard addition. For the thermolabile iprodione, the accuracy of SBSE–TD–cGC–MS was verified using SBSE followed by liquid desorption and analysis by liquid chromatography–atmospheric pressure chemical ionization mass spectroscopy. Procymidone and iprodione were detected in wines in concentrations up to 65 μg/l while the highest concentration of vinclozolin detected was smaller than 3 μg/l.

Keywords: Stir bar sorptive extraction; Extraction methods; Wine; Food analysis; Pesticides; Vinclozolin; Procymidone; Iprodione

1. Introduction

Fungicides are intensively used in the wine industry and they are typically dosed close to or post harvest [1,2]. Iprodione, procymidone and vinclozolin are not fully removed or metabolized during the winemaking process and residues are distinctively present in wine and distillates [1–3]. Iprodione, procymidone and vinclozolin are found to act as androgen receptors and show xeno-endocrine disrupting properties in rats [4,5] and monkeys [6]. It was stated that it is very likely that humans would adversely be affected if the human fetus is exposed to sufficient levels during critical stages of neonatal life.

The analysis of dicarboximide fungicides has been described by many groups and is also incorporated in the US Food and Drug Administration (FDA) Pesticide Residue Monitoring Program of food samples. To the best of our knowledge, there are no regula-

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tions within the European Community. For the analysis of aqueous food products like wine and sherry, sample preparation mainly consists of liquid–liquid extraction (LLE) or solid-phase extraction (SPE). LLE with *n*-hexane [7] or acetone–dichloromethane (1:1) [8] followed by capillary GC analysis has been described. Selectivity and sensitivity is obtained by using electron-capture detection (ECD) or mass-selective detection (MS). Limits of detection are in the order of 1 μg/l for vinclozolin. A valid alternative is enrichment on SPE cartridges packed with C_{18} [9,10] or carbon black [11,12]. Using capillary GC–ion trap mass spectrometry (cGC–ITD) determinations in the ng/l range for vinclozolin and in the μg/l range for iprodione could be reached. The advantage of SPE is that the sample preparation can be fully automated [13]. Another very elegant and solvent-free enrichment technique for aqueous samples is solid-phase microextraction (SPME) [14,15]. In SPME, solutes are (ad)sorbed into a specific layer coated onto a fused-silica fiber. SPME has been applied to the analysis of fungicides in water and wine samples [16–18]. Fibers coated with 100 μm polydimethylsiloxane (PDMS) (approximately 0.5 μl) were used in combination with splitless thermal desorption of the fiber in the GC injector allowing quantitative transfer of the enriched analytes into the capillary column. On PDMS, solute enrichment is by partitioning between the polymer and the aqueous phase and the enrichment is controlled by the distribution coefficients. To increase recovery rates, modified fibers (PDMS–divinylbenzene 65 μm) or fibers with polar coatings (polyacrylate 85 μm and Carbowax–divinylbenzene 65 μm) were applied. Limits of detection were in the order of 50 ng/l with good linearity up to the 10-μg/l level.

Recently, a novel sorptive extraction technique for aqueous samples namely stir bar sorptive extraction (SBSE) was described [19]. In SBSE, a magnetic rod encapsulated in a glass jacket and coated with a relatively high amount (25–125 μl) of PDMS is placed in the aqueous sample and stirred for a given time. The stir bar is then thermally desorbed on-line with capillary GC–MS. The stir bars, commercialised under the name ''Twister'' (Gerstel, Mülheim a/d Ruhr, Germany) allow a 500-fold increase in enrichment, and thus sensitivity, compared to SPME with 100-μm PDMS fibers. SBSE was used for the analysis of contaminants in wine like phthalates, nonylphenols, organochloro pesticides [20] and 2,4,6-trichloroanisole [21], for the determination of benzoic acid in soft drinks [22], for the analysis of polychlorobiphenyls (PCBs) in sperm [23], etc.

In this contribution the analysis of procymidone, vinclozolin and iprodione in spiked water samples and in several white wines by SBSE in combination with thermal desorption and on-line capillary GC–MS analysis is reported. Iprodione, a thermolabile fungicide, was measured through its degradation product (3,5-dichlorophenyl)hydantoin. To verify the accuracy of this method, SBSE followed by liquid desorption and analysis by liquid chromatography–atmospheric pressure chemical ionization mass spectroscopy (SBSE–LD–LC–APCI-MS) was also applied.

2. Experimental

2.1. Sample preparation

Ten ml ChromaSolv water (Riedel-de Haën, Seelze, Germany) or 10 ml undiluted wine were poured into a headspace vial of 20 ml. For recovery studies and standard addition quantification, an appropriate amount of a methanol solution of procymidone, vinclozolin and iprodione (Sigma–Aldrich, Bornem, Belgium) was added. A twister containing 25 μl PDMS was stirred in the sample for 40 min at a speed of 1400 rpm. After sampling, the twister was rinsed in distilled water and residual water droplets were removed with tissue paper. For thermal desorption (TD), the stir bar was put into a glass tube of 187 mm L, 6 mm O.D. and 4 mm I.D.). Blank runs of the stir bar were done before and after each analysis and no memory effects occurred for the target solutes. For liquid desorption (LD), the stir bar was extracted with 1 ml acetonitrile in an ultrasonic bath for 15 min. Five μl of the extract were injected for LC–MS analysis.

2.2. Instrumental set-up

2.2.1. Thermal desorption–capillary GC–MS (TD–cGC–MS)

A TDS-2 thermodesorption unit (Gerstel) was mounted on a 6890 Agilent GC (Agilent Tech-

nologies, Little Falls, DE, USA). The analytes were cryofocused in a programmed temperature vaporizing injector (PTV); (CIS-4, Gerstel) at −150°C with liquid nitrogen prior to injection. An empty baffled liner was used in the PTV. For splitless thermal desorption, the TDS-2 was ramped from 30 to 300°C at a rate of 60°C/min and the upper temperature was held for 10 min. Splitless injection (2.5 min) was performed by ramping the PTV from −150 to 300°C at a rate of 600°/min. Capillary GC analyses were performed on a 30 m×0.25 mm I.D., 0.25 μm d_f HP-5MS column (Agilent Technologies) with helium as carrier gas. The oven was sequentially programmed from 70°C (2.5 min) to 150°C at a rate of 25°C/min, to 200°C at a rate of 3°C/min and to 300°C at a rate of 8°C/min. The Agilent 5973 mass spectrometric detector was operated in the scan mode (*m*/*z* 50–300) or in the selected ion monitoring mode with a dwell time of 100 ms and 1.44 cycles/s.

2.2.2. Liquid chromatography–atmospheric pressure chemical ionization mass spectroscopy

LC–APCI–MS analyses were carried out on a benchtop HP1100 Series LC–mass-selective detection instrument (Agilent Technologies, Waldbronn, Germany). A Phenomenex Luna C_{18} column, 250 mm×4.6 mm I.D., 5 μm particle size (Bester, Amstelveen, The Netherlands) was used. The mobile phase consisted of water (solvent A) and 10% tetrahydrofuran in methanol (solvent B). A gradient from 70% B at 0 min to 80% B at 20 min was applied. The flow-rate was 1 ml/min and the analyses were performed at 22°C. The injection volume was 5 μl. APCI was carried out in the negative mode at a mass range between *m*/*z* 200–350. The fragmentor voltage was set to 70 V. The nitrogen drying gas was at 350°C with a flow-rate of 5 l/min. The nebulizer pressure was 60 p.s.i.g. (1 p.s.i.=6894.76 Pa). The capillary voltage was 4000 V and the corona current was 25 μA. Analyses in the selected-ion monitoring (SIM) mode for iprodione were carried out at *m*/*z* 242.9, 245.0 and 246.8.

3. Results and discussion

3.1. SBSE–TD–cGC–MS analysis of spiked water samples

Water samples were spiked in a concentration range between 0.5 and 100 μg/l for SBSE–TD–cGC–MS analysis. Fig. 1 shows the extracted ion

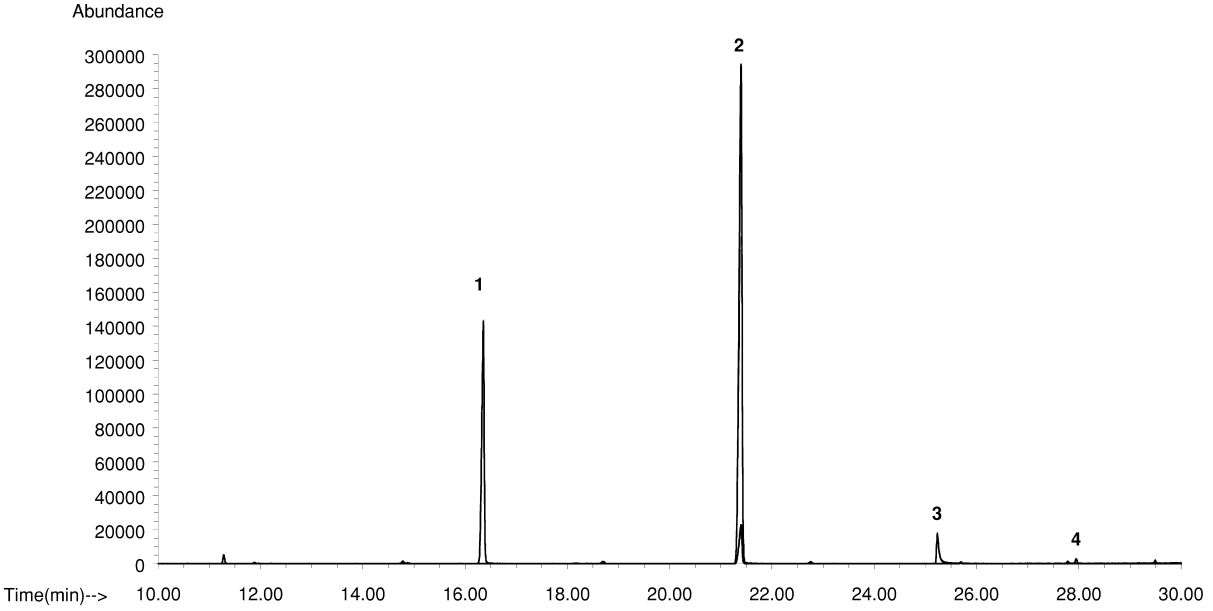

Fig. 1. Extracted ion chromatogram at *m*/*z* 187, 283 and 285 of a SBSE–TD–cGC–MS analysis of water spiked at 10 μg/l with vinclozolin (**1**), procymidone (**2**) and iprodione (**4**). Peak **3**, (3,5-dichlorophenyl)hydantoin, is the degradation product of iprodione.

chromatogram at *m/z* 187 (iprodione and degradation product), 283 (procymidone) and 285 (vinchlozolin) at the 10-μg/l level. The recorded spectra are shown in Fig. 2 together with the structures of the fungicides. Vinclozolin (**1**) and procymidone (**2**) can easily be identified, whereas iprodione (**4**) shows a relatively low abundance. It is known that iprodione [1-isopropylcarbamoyl-3-(3,5-dichlorophenyl)-hydantoin] shows sample decomposition in capillary GC at temperatures >200°C [24]. The carbamate-like compound is degraded for 90% to the more stable (3,5-dichlorophenyl)hydantoin (**3**). The degradation rate is expressed as the ratio of the peak areas (extracted ion at *m/z* 187) of the degradation product versus those of the sum of iprodione and (3,5-dichlorophenyl)hydantoin. Decomposition not only occurs during thermal desorption of the stir bar at 300°C and during transfer of the analyte in the hot transfer line (300°C), but additionally the solute only elutes at a temperature of 245°C (retention time, 28 min) and is therefore also degraded in the capillary column itself. The ratio of the peak areas of iprodione and its degradation product was constant for all SBSE–TD–cGC–MS analyses performed and quantification could be done on (3,5-dichlorophenyl)hydantoin.

Recoveries of the target solutes by SBSE were calculated by comparing the peak areas with those of a direct analysis of a standard solution spiked on glass wool placed in a thermal desorption tube (Table 1). Theoretical recoveries were calculated using the theory described by Baltussen et al. [19]. Octanol–water distribution coefficients ($K_{o/w}$) of the analyzed compounds were calculated with the *SRC-KOWWIN* software package (Syracuse Research, Syracuse, NY, USA) according to a ''fragment constant'' estimation methodology [25]. Theoretical recoveries are somewhat higher than the experimental recoveries. This indicates that equilibrium of the solutes between the PDMS coating and the sample is not yet attained after 40 min sampling. However, reaching equilibrium conditions is not stringent as long as the sampling conditions are kept constant for calibration. The difference between the theoretical and real recovery of iprodione is relatively high and this can be explained by its unequal degradation rate in thermal desorption from an inert PDMS stir bar (SBSE) or a plug of glass wool (injection standard). Repeatability of SBSE–cGC–MS analysis was verified by analyzing six samples spiked at the 10-μg/l level. Integration of the peaks was done in the extracted ion mode at *m/z* 187, 283 and 285. Relative standard deviations on the peak areas were 2 and 1% for vinclozolin and procymidone, respectively. For iprodione the precision was 7% RSD. Limits of detection (LODs) for full scan MS were 0.2 μg/l for vinclozolin and procymidone and 2 μg/l for iprodione. The limits of quantification were set at 0.5 and 5 μg/l, respectively. When operating the mass spectrometer in the ion monitoring mode the limits of detection were in the order of 2 ng/l for vinclozolin and procymidone and of 50 ng/l for iprodione. Linearity was tested in a concentration range between 0.5 and 100 μg/l using MS in the full scan mode and correlation coefficients were all above 0.997 (Fig. 3, full line).

3.2. SBSE–TD–cGC–MS analysis of wines

SBSE can be used to profile flavour compounds in wine [26], to dose μg/l amounts of contaminants [20] and ng/l concentrations of off-flavours [21]. This illustrates the very versatile and universal character of sorptive extraction.

White wines and sparkling wines of different origin (France, Italy, South Africa) were analyzed for the presence of vinclozolin, procymidone and iprodione. The relative large amount of PDMS (25 μl) allows, even for trace analysis, to use the mass spectrometer in the full scan mode. Fig. 4 shows the ion extracted chromatogram (*m/z* 187, 283 and 285) of an Italian sparkling wine. The three target fungicides can easily be detected. As for the water sample, the iprodione degradation product (**3**) is much larger than iprodione (**4**). Degradation rates were relatively constant and ranged between 89 and 91% for all white wines. Quantification of iprodione was thus done using the peak areas of the degradation product.

Repeatability of SBSE–TD–cGC–MS was tested by spiking six sub-samples of a South African blank white wine at the 10-μg/l level. Relative standard deviations of the peak areas never exceeded 5% (Table 1). Sorptive enrichment is equilibrium driven and is therefore subjected to changes in sampling conditions like sampling time and temperature but

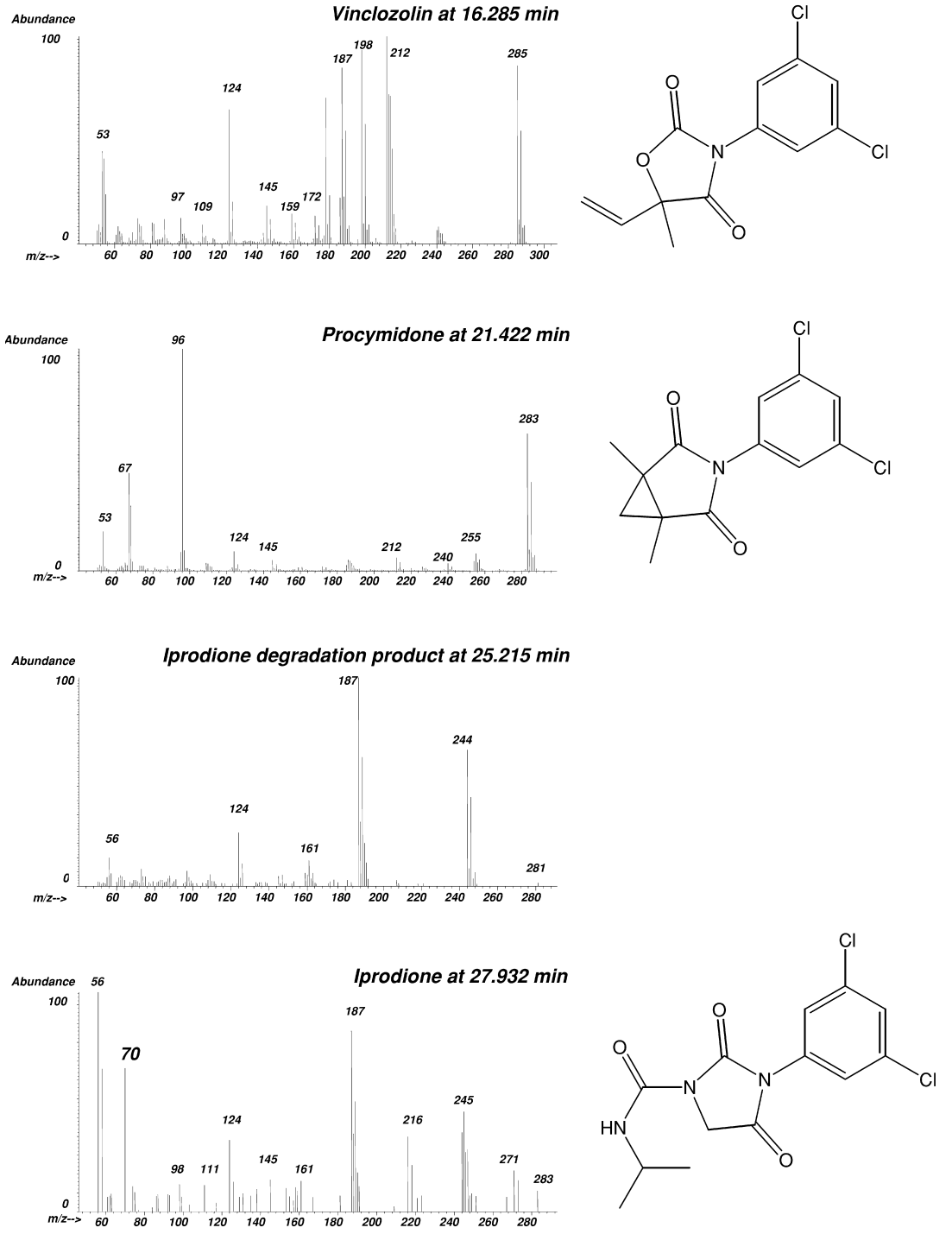

Fig. 2. Spectra recorded at 10 μg/l (Fig. 1) and structures of the fungicides.

Table 1
SBSE of fungicides: quantification ions, log $K_{o/w}$, recoveries (%) and repeatability ($n=6$) for vinclozolin, procymidone and iprodione in water and wine

Fungicide	Log $K_{o/w}$	Quant. ion (m/z)	Theoretical recovery (%)	Recovery water (%)	Repeatability water (RSD %)	Recovery wine (%)	Repeatability wine (RSD %)
Vinclozolin	3.03	285	76	51	2	35	2
Procymidone	2.59	283	53	41	1	15	3
Iprodione	2.85	187	67	31	7	7	5

Iprodion is measured as its degradation product (3,5-dichlorophenyl)hydantoin.

also to matrix effects [22]. In the case of SBSE sampling of wine the reduction in recovery compared to that in water was already demonstrated for organochloro pesticides [20]. Therefore, quantification of the target compounds was done using standard addition. The three pesticides were added to 10 ml of each wine sample in concentrations between 1 and 100 μg/l by spiking 10 μl of the corresponding standard solutions in methanol. Quantification of the three target fungicides was done in the extracted ion mode at m/z 187, 283 and 285. Linear regression was performed and correlation coefficients were higher than 0.99 for vinclozolin and procymidone and higher than 0.98 for iprodione. This is illustrated in Fig. 3 (dashed lines) showing the standard addition curves for the Italian sparkling wine. The fungicide recoveries were calculated for the South African blank white wine and are reduced to 35, 15 and 7% for vinclozolin, procymidone and iprodione, respectively (Table 1). The slopes of the standard addition curves, however, are relatively constant for all white wines. This means that calibration can be done by spiking a blank reference wine. However, attention should be paid when red wines or very sweet wines, containing rather high amounts of polyphenolic polymers or saccharides, respectively, are analysed. Matrix effects should be evaluated in this case before quantification is performed.

The carboximide fungicide concentrations in different positive white and sparkling wines were calculated using the standard addition curves and are listed in Table 2. Vinclozolin was only found in the Italian sparkling wine in low concentration (2.6 μg/l). Procymidone and iprodione are more abundant and their concentrations vary between 5 and 65.0 μg/l. In an Italian white wine, procymidone was present in higher concentration (61.3 μg/l) than iprodione (16.1 μg/l) while in the Italian sparkling wine iprodione (65.0 μg/l) was much higher in concentration than procymidone (10.7 μg/l).

3.3. SBSE–LD–LC–MS analysis of wine

The accuracy of the SBSE–TD–cGC–MS method for the iprodione determination via the degradation product was verified by analyzing the Italian sparkling wine with SBSE–LD–LC–MS. The LC separation of procymidone, vinclozolin and iprodione was optimised on a C_{18} column using a gradient of water (solvent A) and 10% tetrahydrofuran in methanol (solvent B) as mobile phase. The mass spectrometric detector was used in the negative APCI mode. Fig. 5 shows the total ion chromatogram and Fig. 6 the mass spectra of a 30-mg/l standard mixture of the target fungicides. Interesting to note is that under the LC conditions applied vinclozolin (M_r 285) and procymidone (M_r 283) give ions at $(M+CH_3OH-H)^-$ while iprodione (M_r 329) gives an $[M\text{-}CONHCH(CH_3)_2]^-$ ion. This illustrates the thermolabile character of iprodione because it decomposes under chemical ionization conditions. On the other hand, negative chemical ionization was giving much better ionization and robustness than positive chemical ionization and positive and negative electrospray ionization. For quantification of iprodione, MS was used in the SIM mode at m/z 242.9, 245.0 and 246.8. The linearity of LC–MS analysis for iprodione was tested in a concentration range between 10 μg/l and 10 mg/l and was excellent ($y = 562.69x + 3810.9 - R^2 = 0.999$). Iprodione in the Italian sparkling wine was quantified by standard addition in the range 20–100 μg/l to a 10-ml sample ($y = 1265.9x + 78541 - R^2 = 0.994$). After SBSE sampling, the stir bar was liquid de-

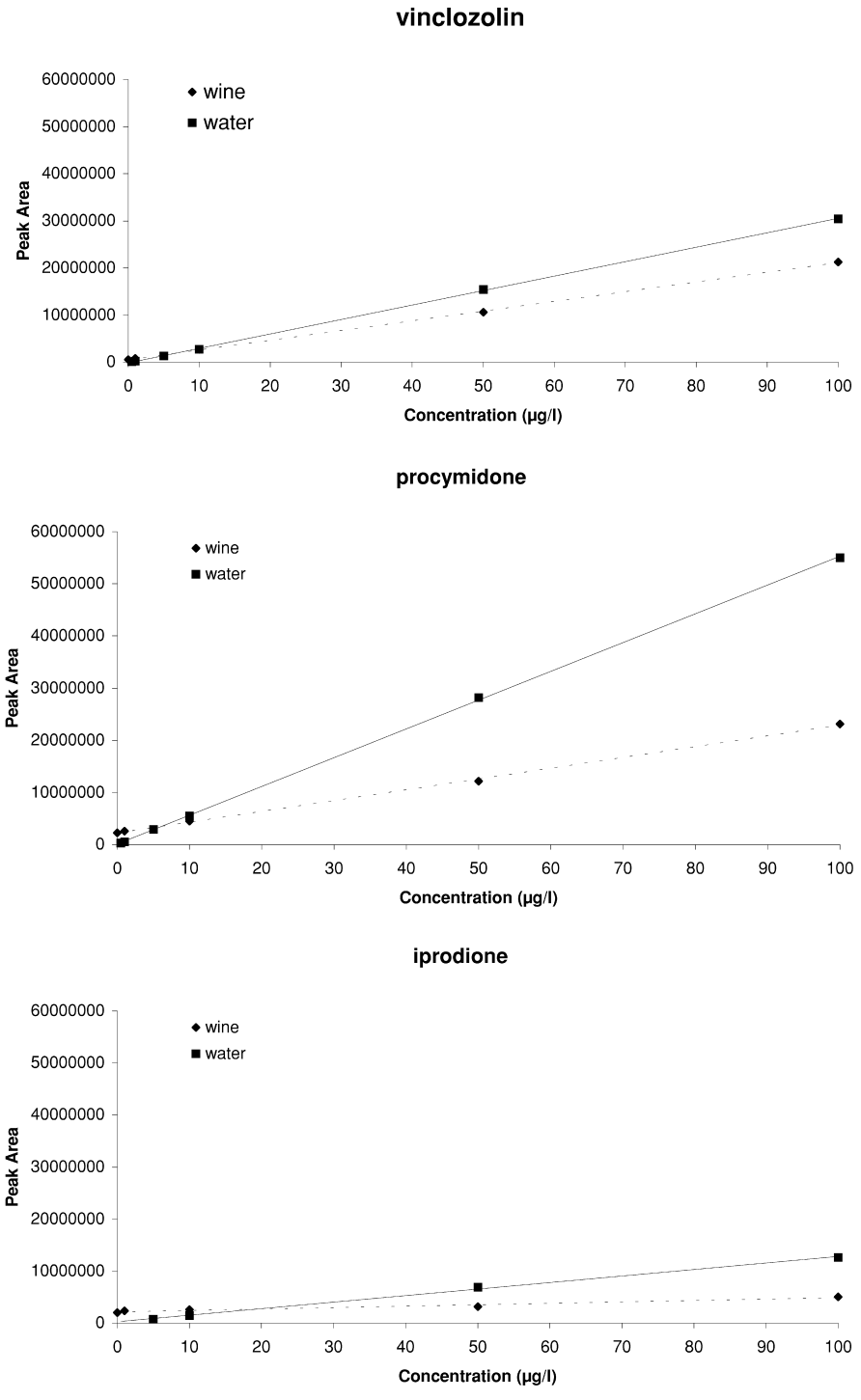

Fig. 3. Linearity of SBSE–TD–cGC–MS of vinclozolin, procymidone and iprodione spiked in water (full lines) and wine (dashed lines). Iprodione was quantified through its degradation product.

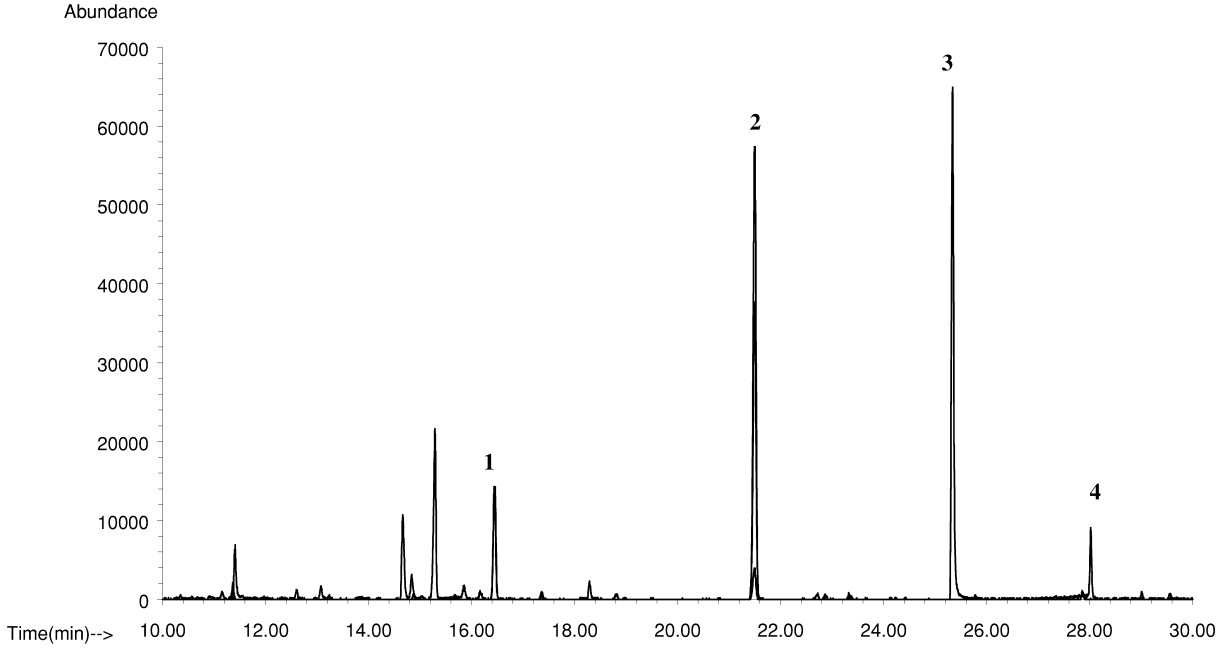

Fig. 4. Extracted ion chromatogram at m/z 187, 283 and 285 of the SBSE–TD–cGC–MS analysis of Italian sparkling wine; vinclozolin (**1**), procymidone (**2**), (3,5-dichlorophenyl)hydantoin (**3**) and iprodione (**4**).

Table 2
Different white wines and blank wine used for spiking

Wine	Vinclozolin (μg/l)	Procymidone (μg/l)	Iprodione (μg/l)
White wine, 1997, France	<0.5	10.8	5.6
Champagne, 2000, France	<0.5	5.5	3.7
Sparkling wine, 1995, Italy	2.6	10.7	65.0
White wine, 2000, Italy	<0.5	61.3	16.1
Sparkling wine, 1998, South Africa	<0.5	<0.5	<0.5

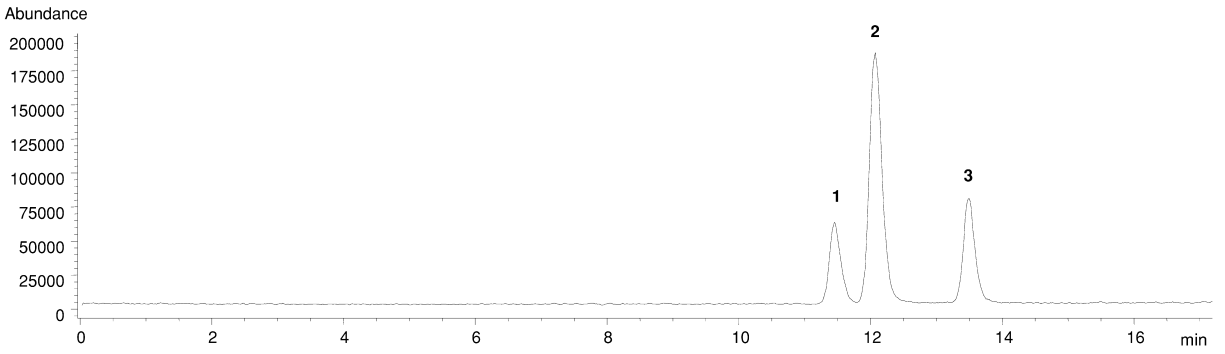

Fig. 5. LC–APCI-MS chromatogram of a 30-mg/l standard mixture.

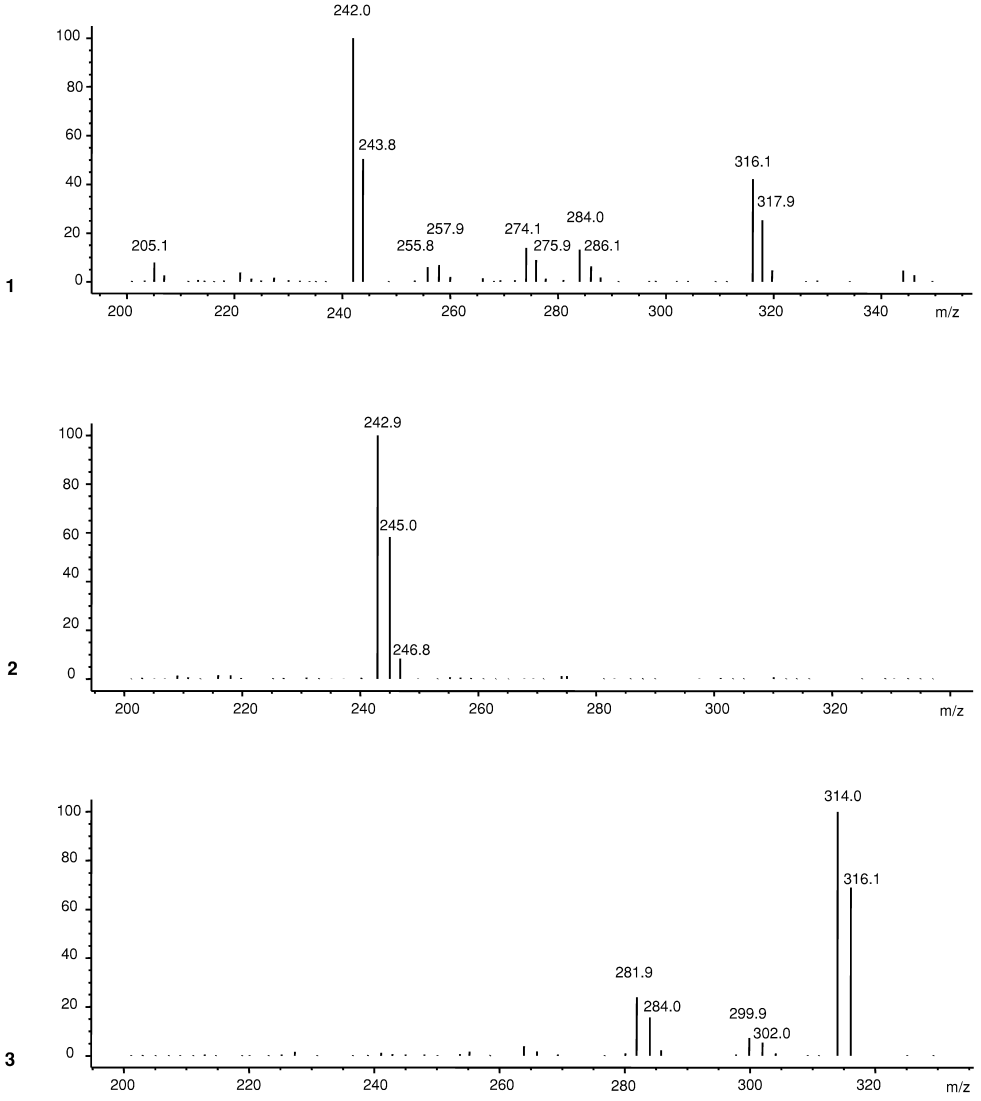

Fig. 6. Mass spectra, LC–negative APCI-MS of procymidone (**1**), iprodione (**2**) and vinclozolin (**3**).

sorbed in 1 ml acetonitrile and the extract was injected for LC–MS analysis. This is the first application of SBSE followed by liquid desorption and this principle broadens the applicability of sorptive extraction on PDMS to non-volatile solutes. The calculated concentration of iprodione in the Italian sparkling wine was 66 μg/l (RSD 4.6% for triplicate analysis). This proves that SBSE–TD–cGC–MS and SBSE–LD–LC–MS gives comparable data for iprodione.

4. Conclusions

SBSE in combination with TD–cGC–MS is a simple, fast and sensitive method for the analysis of vinclozolin, procymidone and iprodione in wine samples. Iprodione was measured as its degradation product (3,5-dichlorophenyl)hydantoin. The accuracy of SBSE–TD–capillary GC–MS for iprodione was verified using SBSE–LD–LC–APCI-MS. Both techniques gave comparable data. Liquid desorption

of the stir bars broadens the applicability of sorptive extraction on PDMS to non-volatile solutes.

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