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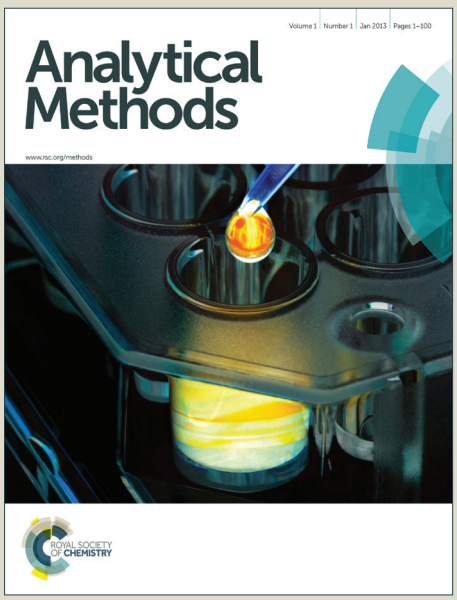

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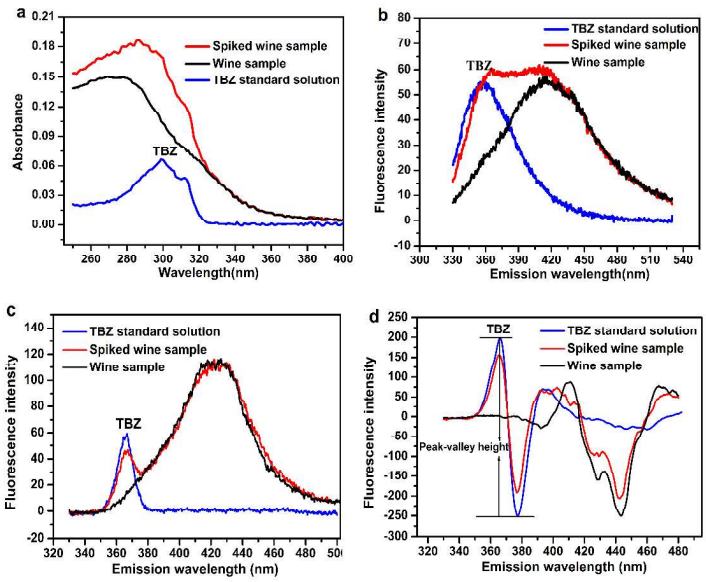
Graphical abstract



This method (d) has the advantages in improving spectral resolution and measurement sensitivity and eliminating the interference of spectral background from red wine compared with UV-Vis absorption spectroscopy (a) and conventional fluorescence spectroscopy (b).

Rapid fluorescence spectroscopic screening method for sensitive detection of thiabendazole in red wine

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Rapid fluorescence spectroscopic screening method for sensitive detection of thiabendazole in red wine

Abstract

In this work, a rapid, sensitive, reliable and cost-effective fluorescence spectroscopic method has been established for detection of thiabendazole (TBZ) in red wine based on second-derivative constant-wavelength synchronous fluorescence spectroscopy (DCWSFS) coupled with ultrasound-assisted liquid-liquid extraction technique. The constant wavelength difference ($\Delta\lambda$) was set at 50 nm between excitation and emission monochromators. Compared with UV-Vis absorption spectroscopy and conventional fluorescence spectroscopy, this method has the advantages in improving spectral resolution and eliminating the interference of spectral background from red wine. This method avoided the requirements of complicated purification and pretreatment procedures. The detection of TBZ is free from the interference of co-existence pesticides, demonstrating the high selectivity of this method. Satisfactory recoveries of the spiked red wine samples were obtained ranging from 85.9 to 102.8 %. The calibration curve showed good linearity in a range from 0.05 to 1.0 μg/mL for TBZ. The detection limit (S/N=3) was 7.2 ng/mL and the relative standard deviation (n=5) value was 3.9 %. The results obtained by this method for analyzing TBZ in red wine samples correlated well with those obtained by HPLC method. This method would be an attractive alternative for rapid screening of TBZ in large amounts of samples.

Keywords: synchronous fluorescence spectroscopy, derivative technique, thiabendazole, wine

Introduction

Red wine has been one of the most popularly consumed drinks in the world. It has shown good health-care effect on anti-aging, weight loss, beauty and its prevention and cure of different diseases such as cardiovascular disease, cerebral thrombosis and cancer [1, 2]. However, pesticides are commonly used to treat pests and diseases of grapes in vineyard, which may cause pesticide residues in grapes and thus may induce the residues in wine through the wine-making process. As a result, pesticide residue is quite a potential health threat to people through wine consumption [3, 4].

Benzimidazole fungicides are effective pesticides and extensively applied for the pre- or post-harvest treatment of a great variety of crop diseases caused by various fungi. Thiabendazole (2-(4-thiazolyl) benzimidazole, TBZ) is one of the most widely used compounds to treat crop diseases such as mold, rot, blight and stain [5]. After treating crops and then entering the environment, pesticides can be transported in air-soil-water-plant systems and may be absorbed by plant tissues and then exist in fruits and vegetables or their processed products. Most of the fungicides cause severe damage to human health and their residues have been found to be very persistent in water, soil, crops and food for quite a long time [6-10]. In order to prevent consumers suffering from noxious pesticide damage, maximum residue levels (MRLs) have been established by the European Union (EU) and the Codex Alimentarius Commission (CAC) for a great quantity of pesticide residues in food [11-13]. Therefore, it is of great significance to determine pesticide residues in wine.

Common analytical methods used for the determination of benzimidazole fungicides include high-performance liquid chromatography with ultraviolet or fluorescence detection (HPLC-UV/FLD) [14] and liquid chromatography with mass spectrometry (LC-MS) [15]. For above analyses, the samples were first extracted with methanol, acetonitrile or dichloromethane, and then the extract was purified on different kinds of solid phase absorption column. Fractions eluted from the columns were subsequently measured by HPLC-UV/FLD or LC-MS. These techniques often consume large volumes of toxic organic solvent, especially in pre-separation and clean-up procedures and they are extremely time-costing and labor-consuming. Moreover, the maintenance of HPLC-UV/FLD or LC-MS is complicated and costly. These disadvantages restrict the development of rapid determination of benzimidazol fungicides in food samples. Therefore, it is indispensable to establish a rapid, reliable, and cost-effective method for evaluating and screening thiabendazole in food.

Since benzimidazol fungicides exhibits native fluorescence, highly sensitive fluorescence method coupled with sequential injection analysis or chemometrics has been proposed for determination of thiabendazole in mushroom sample [16] or environmental samples [17, 18]. Fluorescence method also has been used as the detection technique in HPLC method [19]. HPLC [20] and capillary electrophoresis [21] have already been proposed for determination of thiabendazole in grape or wine. However, to the best of our knowledge, there has been no report on a fluorescence spectroscopic method for direct detection of thiabendazole in red wine samples without resorting to chromatographic separation and purification procedures. Because the emission

spectrum obtained from the conventional fluorescence spectroscopy commonly suffers from light scattering interference and broad-band fluorescence features, it is still hard to utilize conventional fluorescence spectroscopy in this study.

Compared with the conventional fluorescence spectroscopy, synchronous fluorometric method can overcome the light scattering interference, narrow the broad band fluorescence features, simplify the spectrum and improve the selectivity and the spectral resolution of fluorescence spectroscopy [22-23]. Constant-wavelength synchronous fluorescence spectroscopy (CWSFS) is quite commonly used, which is achieved by maintaining a constant wavelength-difference $\Delta\lambda$ ($\Delta\lambda=\lambda_{em}-\lambda_{ex}$) between excitation and emission monochromators [24]. According to the scanning speeds and the wavelength differences between excitation and emission monochromators, several other synchronous fluorescence methods have been developed, including constant-energy synchronous spectroscopy (CESFS), variable-angle synchronous fluorescence spectroscopy (VASFS) and matrix-isopotential synchronous fluorescence spectroscopy (MISFS) [25-29]. Besides, based on the ability in narrowing band and amplifying minor spectral features, derivative technique has been extensively applied in analytical chemistry to effectively enhance the sensitivity and reduce the background or matrix interference [30, 31]. The incorporation of synchronous fluorescence spectroscopy and derivative technique shows synergetic effects and can further improve the sensitivity and the selectivity in contrast to the conventional fluorescence spectroscopy [32]. The incorporated technique can play a critical role in separating the overlapping spectrum without the request of complicated physical

pre-treatment procedures. It has been widely applied to determine analytes in complex matrices [33, 34].

The matrix of the red wine sample is extremely complex due to the existence of phenolic compounds, pigments, sugar, organic acids, vitamin, mineral element, amino acids and other unknown components, which has a great influence on the accurate analysis of TBZ. In general, a series of purification and pre-separation processes are required before instrumental analysis, which naturally make the entire analysis process more tedious and time-consuming. Based on the obvious synergistic effects between synchronous fluorescence spectroscopy and derivative technique, this method can effectually overcome matrices or background interference of red wine samples and largely simplify sample pretreatment process without resorting to complicated purification and chromatographic separation procedures.

The aim of this work was to develop a rapid, simple, reliable and sensitive fluorescence spectroscopic method for detection of thiabendazole in red wine. Based on second-order derivative constant-wavelength synchronous fluorescence spectroscopy (DCWSFS), high sensitivity and selectivity have been achieved. Complicated chromatographic separation and purification procedures were avoided in the sample preparation and the entire analysis time was greatly shortened to about half an hour. This approach has been used for detection of TBZ in different brands of red wine samples. Spiked recovery tests were carried out for validation, with average recovery values between 85.9 and 102.8 %. This method was compared with HPLC method to further confirm the analytical power of the proposed approach.

Experimental

Materials and reagents

Thiabendazole standard (purity ≥99.9 %) was obtained from J&K Scientific Ltd. (China). 100.0 mg/L thiabendazole-methanol stock solution was prepared in 25.0 mL brown volumetric flask. This solution was stored at 4 ℃ to avoid exposure to direct light. The solutions of lower concentrations were prepared by properly diluting the stock solution with methanol. Methanol (HPLC grade) and the other solvents (analytical grade) were purchased from Shanghai Reagent (China). Ultrapure water was acquired by a Millipore Milli-Q water purification system.

Apparatus

All synchronous fluorescence spectra were achieved on a laboratory-constructed, computer-controlled spectrofluorometer, which was provided with a 150 W xenon lamp. The slit band passes were set at 5 nm for both excitation and emission monochromators, which were commanded by a computer via a program package written in Turbo C 2.0 [35]. The data of synchronous fluorescence spectra were obtained by the computer through the program package. The second-order derivative synchronous fluorescence spectra were directly recorded by an electronic differentiator system attached to the spectrofluorometer. Besides the common fluorescent excitation and emission spectra, the instrument supplies all kinds of synchronous fluorescence spectra, including constant-wavelength, constant-energy, variable-angle and matrix-isopotential synchronous spectra. This instrument was applied successfully in previously reported work [36-38]. A UV-Visible

spectrophotometer (U-3900, Hitachi, Japan) was used to obtain the spectra and methanol was used for baseline measurement. HPLC analysis was completed on a Shimadzu LC-15C system equipped with a Shimadzu RF-20A fluorescence detector (Shimadzu (China) co. Ltd., Shanghai Branch, Shanghai, China). The ultrasonic extraction procedure was performed on an ultrasonic cleaner (Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China) and the rotary evaporator (YRE-52 C) was purchased from Gongyi Yuhua instrument Co., Ltd., Henan, China.

Sample preparation

The analyzed red wine samples were purchased in the supermarket and they were kept at 4 ℃ avoiding exposure to direct light. 1.0 mL of red wine was put into a 100 mL volumetric flask, to which 2.0 mL ultrapure water was added. This aqueous phase was extracted with 8.0 mL of dichloromethane in an ultrasonic cleaner for 8 min. The lower organic phase was separated and collected. This process was repeated in duplicate. All organic extracts were combined and then evaporated to dryness by a rotary evaporator at 35 ℃ water bath. The residue was re-dissolved with 5.0 mL of methanol.

Analysis of TBZ

2 mL of the extract was poured into a 1×1 cm quartz cuvette and then measured by spectrofluorometer. All synchronous fluorescence spectra were recorded by spectrofluorometer with a 50 nm wavelength-difference ($\Delta\lambda$) between excitation and emission monochromators at a scan speed of 240 nm min^{-1} and the emission

wavelength ranging from 330 to 480 nm, by which the second-order derivative fluorescence spectra were obtained within one minute. Second-order derivative synchronous spectra were recorded by the electronic differential system attached to the spectrofluorometer. The continuous standard addition method which could overcome the possible matrix effects was applied to calculate the concentration of TBZ in the red wine extracts. Calibration curve was obtained by continuous addition of TBZ standard solution to red wine extract. All experiments were completed at ambient temperature.

Method validation

In order to validate the accuracy of this method, the results obtained by DCWSFS were compared with those obtained by HPLC method. HPLC analysis was completed on a Shimadzu LC-15C system equipped with a Shimadzu RF-20A fluorescence detector. The excitation and emission wavelength for analyzing TBZ were set at 305 nm and 345 nm, respectively. A Diamonsil-C18 column (250 mm×4.6 mm ×5 μm, Dikma, China) was applied at room temperature. The isocratic mobile phase was composed of methanol-water (50:50, v/v) at a total flow rate of 0.8 mL/min. The elution conditions of HPLC method was adapted on the basis of reference method [39]. The injection volume was 20 μL.

Results and Discussion

Spectra analysis of thiabendazole and wine sample

Because red wine contains large amounts of pigment and other unknown fluorescence

compounds, serious matrix effect exists in the extract after simple extraction. In order to further eliminate the interference of spectral background from red wine and to propose a suitable method for direct accurate analysis of TBZ in wine extract, different spectroscopic methods were investigated, including UV-Vis absorption spectroscopy, conventional fluorescence spectroscopy and constant-wavelength synchronous fluorescence spectroscopy. As shown in Fig.1a, the absorption bands of TBZ and blank wine sample were extremely overlapped and high background signal was observed in red wine located at about 300 nm. Direct quantitative analysis of TBZ in wine extract was impossible by using UV-Vis absorption spectroscopy. Fig.1b showed the conventional fluorescence emission spectra of TBZ and blank wine sample; TBZ exhibits a broad emission peak located at about 355~365 nm in methanol with an excitation maxima located at 315 nm and the blank wine sample showed an extremely wide background interference peak located at about 390~450 nm. The emission spectrum of spiked wine sample indicated that the emission peak of TBZ was still dramatically interfered by the spectral background of red wine. It was still a challenge to eliminate the spectral background interference and achieve direct accurate quantitative analysis of TBZ by conventional fluorescence spectroscopy. Herein, in order to eliminate spectral background interference and obtain a narrower band of TBZ for accurate analysis, we proposed a constant-wavelength synchronous fluorescence spectroscopic approach in this work. To achieve the synchronous fluorescence spectra, a value of 50 nm was selected as the optimum wavelength difference ($\Delta\lambda$) between excitation and emission monochromators because this value

is close to the Stokes shift of TBZ and TBZ exhibits its highest fluorescence signal at this wavelength difference. In the case of synchronous fluorescence spectra (Fig.2a), the synchronous fluorescence peak of TBZ in spiked wine sample was obviously narrowed and more prominent in contrast to its conventional fluorescence emission peak (Fig.1b**)**. As shown in Fig.2b, the peak of TBZ was further narrowed accompanied with intensity greatly improved while synchronous fluorescence method was combined with second-order derivative technique owing to the obvious synergistic effect between them. The original small peak of TBZ was further enlarged by using second-order derivative technique, which was advantageous to quantification and could effectively improve the sensitivity of the measurement. Besides, this derivative synchronous technique reduced the interference from other fluorescent compounds existing in red wine sample and eliminated spectral background interference exclusively (Fig.2b, black line). Compared with UV-Vis absorbance spectroscopy and conventional fluorescence emission spectroscopy, second-order derivative constant-wavelength synchronous spectrofluorometry provided a method to measure directly TBZ in red wine samples without complicated pre-treatment processes.

The matrix of red wine sample is extremely complex due to the existence of pigments, phenolic compounds and other unknown components. As shown in Fig.2a, there was a wide interference peak of other fluorescence compounds located at about 430 nm in wine sample, whose shape and signal intensity almost remained consistent for different brands of red wine samples according to our study, which may produce a

similar matrix effect in different wine samples. The complicated matrices of red wine sample may create serious matrix effect to induce the fluorescence quenching of TBZ. As shown in Fig.3, due to the existence of the matrix effect, the slope of calibration equation of TBZ in wine sample (S=899.6) was smaller than that in pure methanol solvent (S=1310.3). Although the incorporation of derivative technique can eliminate the interference of spectral background signal, it can not eliminate the influence on measurement sensitivity. Besides, in order to further eliminate the matrix effect on the measurement sensitivity, the continuous standard addition method was used and performed by continuously spiking approximate concentration of TBZ standard solution into the red wine extract for four times to match matrices of red wine. And the quantification of TBZ was achieved by measuring its peak-valley height, which exactly matched the height between the maximum at 365 nm and the consecutive minimum at 376 nm at second-order derivative synchronous spectra (Fig.2b).

Selection of spectral analysis solvent

The influences of four solvents (methanol, dichloromethane, acetonitrile and ethyl acetate) on the fluorescence of TBZ were investigated in the corresponding optimum values of Δλ. As shown in Fig.4a，pure ethyl acetate exhibited its solvent interference peak located in about 350~390 nm, which overlapped seriously with the second-order derivative spectra of TBZ and influenced the fluorescence signal of TBZ. Therefore, ethyl acetate was not suitable for analyzing TBZ. The spectra of other three solvents had no significant influence on the spectra of TBZ. Fig.4b showed the second-order derivative synchronous spectra of the TBZ standard solution, the results indicated that

TBZ exhibited higher fluorescence intensity in methanol than that in other two solvents. So methanol was selected as spectral analysis solvent for TBZ to further improve measurement sensitivity since the fluorescence intensity of TBZ in methanol is the highest.

Optimization of extraction conditions

In order to obtain the optimal extraction efficiency of TBZ, major factors including the selection of extraction solvent, the volume of extraction solvent and extraction time were investigated. Experiments were performed with 1 mL red wine sample spiked with 0.5 μg/mL of TBZ. Parameters were maintained constant except the tested one and all experiments were completed at ambient temperature.

Selection of extraction solvent

The extraction solvent is one of the major factors that affect the extraction efficiency of the target compound. In this work, the commonly used organic solvents in laboratory have been investigated, including acetonitrile, methanol, acetone, dimethylsulfoxide, tetrahydrofuran, ethyl acetate, dichloromethane and hexamethylene. When acetonitrile, methanol, acetone, dimethylsulfoxide and tetrahydrofuran were selected as extract solvent, wine-water phase and organic phase dissolved each other, the organic phase was difficult to be thoroughly separated from mixtures even with the help of centrifugal machine and the organic extract was so deeply red to cause serious matrix effects. However, when dichloromethane, ethyl acetate and hexamethylene were selected as extract solvent, organic phase can be

automatically separated from the wine-water phase and there was no red pigment dissolving in organic solvents to further reduce matrix interference. The results shown in Fig.5 indicated that dichloromethane showed the best extraction efficiency, and hexamethylene showed the worst, because hexamethylene showed bad solubility for TBZ and resulted in low extraction efficiency of TBZ. Therefore, dichloromethane was selected as the extraction solvent.

Effect of volume of dichloromethane

To achieve satisfactory recovery of TBZ, different volumes of dichloromethane (2, 4, 6, 8, 10 and 12 mL) were investigated and the results were shown in Fig.6a. The recovery increased as the volume of dichloromethane increased from 2 to 8 mL. When the volume of dichloromethane further increased over 8 mL, there was a clear decrease of extraction efficiency, which may be owing to the increasing of the matrix effect caused by the co-extraction from red wine sample with larger volume of extraction solvent. Thus, 8 mL of dichloromethane was selected as the optimal volume.

Effect of extraction time

The effect of the extraction time was investigated from 2 to 12 min (Fig.6b). The results indicated that the recovery increased as the extraction time increased from 2 to 12 min. And there was no district increase to the recovery of TBZ when extraction time further increased over 8 min. Therefore, 8 min was considered to be the optimal extraction time in the following work. And the extraction procedure satisfied the

requirement of rapid extraction.

Analytical features of the proposed method

The calibration curve was performed by plotting the peak-valley height of the second-order derivative synchronous fluorescence intensity of TBZ versus its corresponding concentration. As shown in Table 1, the calibration equation was calculated as I=48.4+908.6C in the range of 0.05-1.0 μg/mL with correlation coefficient higher than 0.99. The limits of detection and quantification of the proposed method were calculated via the analysis of a blank red wine sample (n=11). The blank red wine sample was found to have no detectable concentration of TBZ utilizing the proposed method. As shown in Table 1, the detection limit (LOD) and quantification limit (LOQ) of TBZ, defined as 3 SD/k and 10 SD/k, were 7.2 ng/mL and 23.9 ng/mL, respectively, where SD was the standard derivation of the 11 blank signals and k was the slope of the calibration equation. The precision of this method was further assessed by the application of the proposed method for analysing TBZ in a spiked red wine sample (spiked with 0.5 μg/mL of TBZ, n=5) and the relative standard deviation value was 3.9 %, demonstrating a good precision of the proposed method.

Method validation

The level of TBZ was not detected in real red wine samples by the proposed method. A spiked recovery experiment was performed to estimate the accuracy of the proposed method. The recovery tests were performed by utilizing the whole processes (extraction and measurement) to different brands of wine samples, to which different

amounts of TBZ had been added. The extract was then determined by both the proposed method and the HPLC method. As shown in Table 2, the recoveries of TBZ obtained by the proposed method ranged from 85.9 to 102.8 % with relative standard deviation (RSD, n=3) between 1.8 and 7.8 %. The results acquired by the HPLC method have also been shown in Table 2. The results obtained by these two methods correlated well, which further confirmed the accuracy and feasibility of the proposed method.

Interference study

In order to evaluate the availability of this method for the determination of TBZ in real samples, other interfering pesticides which could tend to coexist in real samples were added to 0.5 μg/mL thiabendazole standard solution and their effect on the fluorescence signal of TBZ was then tested. Common pesticides, which are widely used and may be formulated along with thiabendazole such as imazalil, were investigated. The results were shown in Table 3. The tolerance concentration was greater than 50 μg/mL for benomyl, carbendazim, imazalil, procymidone, and 10 μg/mL for thiophanate-methyl when the level of thiabendazole was 0.5 μg/mL in their mixture standard solution. It can be concluded that thiabendazole can be determined in the existence of higher concentrations of possibly interfering pesticides in real samples.

Conclusions

In this work, a rapid, simple, sensitive and economic fluorescence spectroscopic

method was developed for the detection of TBZ in red wine samples with simple pretreatment procedures. The entire analysis process including extraction and determination was simplified and accomplished in a short time (less than 0.5 h), which was in a contrast to complicated chromatographic separation and purification procedures. Based on the obvious synergistic effect between synchronous fluorescence spectroscopy and derivative technique, this method can effectively enhance the spectral resolution and eliminate the matrix interference of red wine. The selective analysis of TBZ was achieved even when possibly interfering pesticides in real samples are present with higher concentrations. The comparisons between this method and HPLC method in spiked red wine samples further validated the analytical power of the proposed approach. This method would be an attractive alternative for rapid screening of TBZ in large amounts of samples.

Acknowledgements

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Table 1 Analytical parameters of the proposed method for TBZ

Linearity range (μg/mL)	Calibration Equation[a]	Correlation coefficient	LOD (ng/mL)	LOQ (ng/mL)	Precision (%)
0.05-1.0	I=48.4+908.6C	0.998	7.2	23.9	3.9

[a] I: fluorescence intensity; C: concentration (ng/mL)

Table 2 Recoveries of TBZ in spiked red wine samples

Sample	Spiked (μg/mL)	Found (μg/mL)		Recovery(%)[a]	
		Proposed method	HPLC method	Proposed method	HPLC method
Red wine 1	0.05	0.051	0.053	102(5.0)	106
	0.1	0.099	0.096	99(6.7)	96
	0.3	0.291	0.297	97(6.6)	99
Red wine 2	0.05	0.048	0.047	95(7.8)	94
	0.1	0.099	0.103	99(3.7)	103
	0.3	0.300	0.280	100(1.8)	94
Red wine 3	0.05	0.046	0.044	91(4.7)	88
	0.1	0.086	0.085	86(1.9)	85
	0.3	0.297	0.303	99(6.8)	101

[a] RSD (%) in parenthesis; n=3.

Table 3 Effect of foreign species on the determination of TBZ (0.5 μg/mL TBZ)

Foreign species	Toleranted[a] concentration (μg/mL)	Toleranted ratio (interference/analyte，c:c)
Benomyl	50[b]	100[b]
Carbendazim	50[b]	100[b]
Carbaryl	50[b]	100[b]
Imazalil	50[b]	100[b]
Procymidone	50[b]	100[b]
Thiophanate-methyl	10	20

[a] Tolerance concentration was defined as the amount of foreign specie that produced an error not exceeding ± 5% in the determination of thiabendazole.
[b] Maximum concentration tested

Figure Captions

Fig.1 (a) UV-Vis absorbance spectra of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample(black line); **(b)** fluorescence emission spectra of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample (black line) (λ_{ex}=315 nm)

Fig.2 Constant-wavelength synchronous fluorescence spectra (**a**) and second-derivative constant-wavelength synchronous spectra (**b**) of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample (black line) (Δλ=50 nm)

Fig.3 Calibration curve of TBZ in pure methanol solvent and in blank wine sample

Fig.4 The second-derivative constant-wavelength synchronous spectra of **(a)** blank pure solvents and **(b)** thiabendazole standard solution (0.3 μg/mL) in methanol (red line, Δλ=50 nm), in dichloromethane (blue line, Δλ=55 nm), and in acetonitrile (black line, Δλ=50 nm) (green line, Δλ=50 nm)

Fig.5 Effect of different extraction solvents on the recovery of TBZ

Fig.6 Effect of **(a)** the volume of dichloromethane and **(b)** extraction time on the recoveries of TBZ

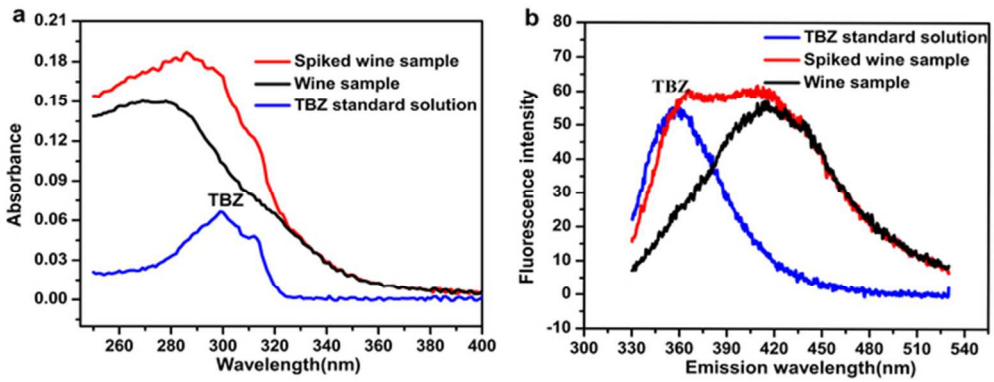

Fig.1 (a) UV-Vis absorbance spectra of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample(black line); (b) fluorescence emission spectra of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample (black line) (λex=315 nm)
60x22mm (300 x 300 DPI)

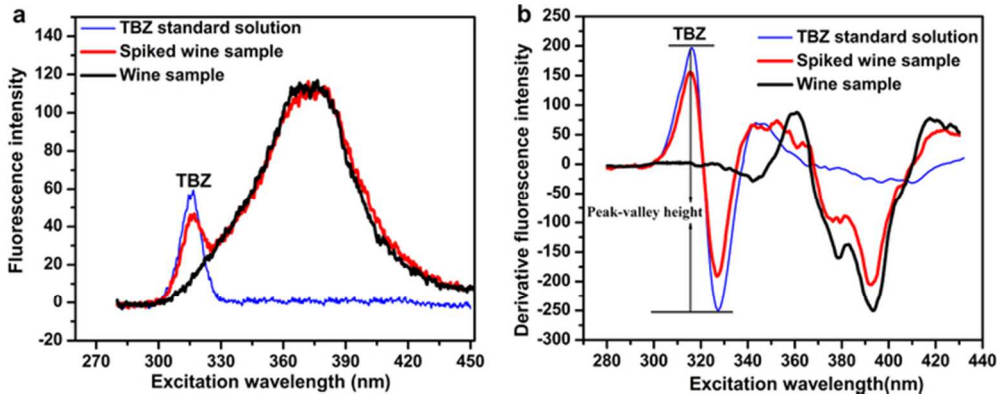

Fig.2 Constant-wavelength synchronous fluorescence spectra (a) and second-derivative constant-wavelength synchronous spectra (b) of spiked wine sample (red line, 0.5 μg/mL), TBZ-methanol standard solution (blue line, 0.5 μg/mL) and wine sample (black line) (Δλ=50 nm)
62x24mm (300 x 300 DPI)

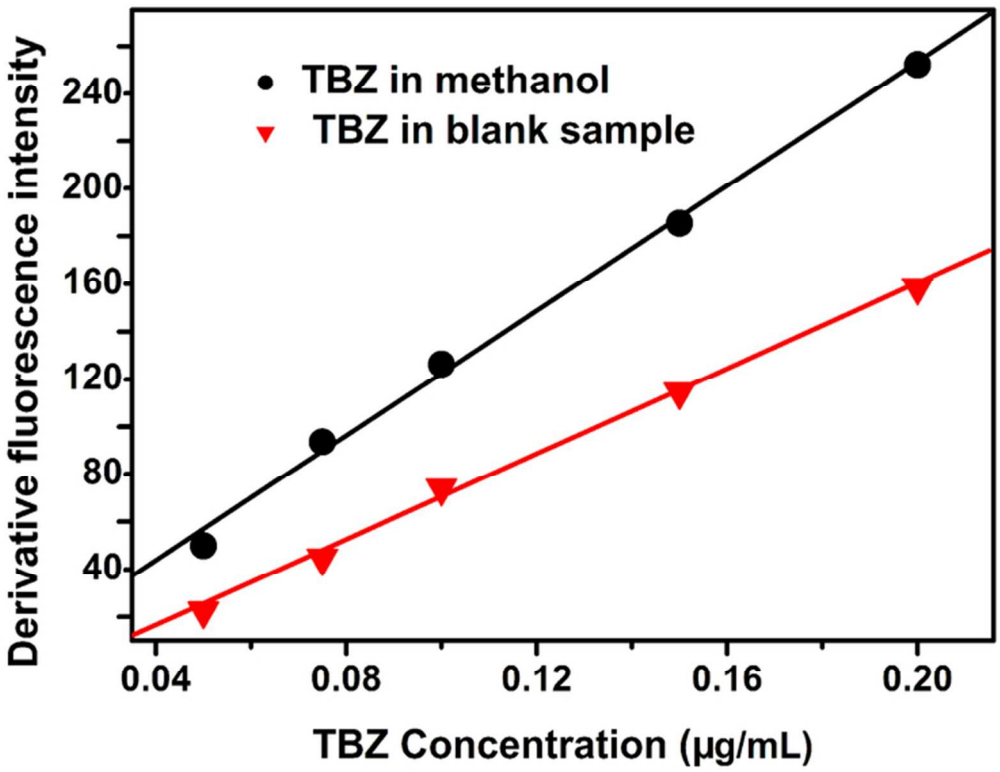

Fig.3 Calibration curve of TBZ in pure methanol solvent and in blank wine sample
62x48mm (300 x 300 DPI)

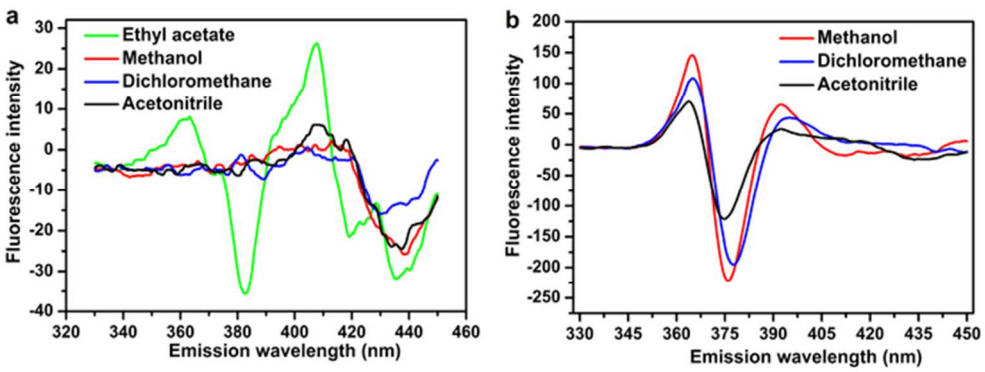

Fig.4 The second-derivative constant-wavelength synchronous spectra of (a) blank pure solvents and (b) thiabendazole standard solution (0.3 μg/mL) in methanol (red line, Δλ=50 nm), in dichloromethane (blue line, Δλ=55 nm), and in acetonitrile (black line, Δλ=50 nm) (green line, Δλ=50 nm)
59x22mm (300 x 300 DPI)

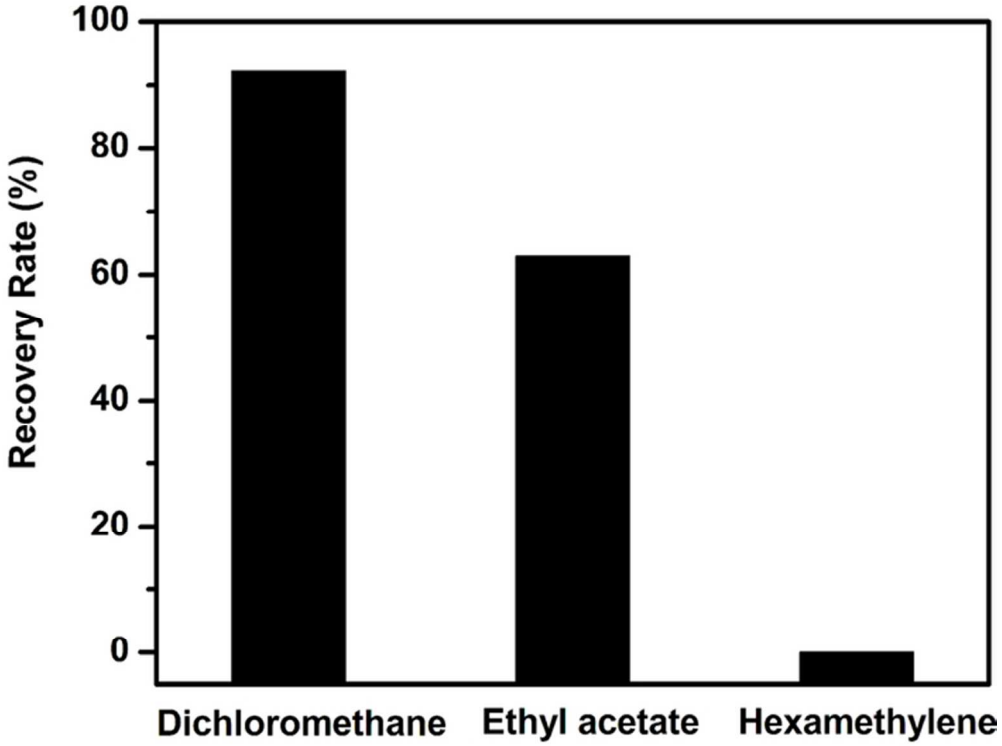

Fig.5 Effect of different extraction solvents on the recovery of TBZ
59x44mm (300 x 300 DPI)

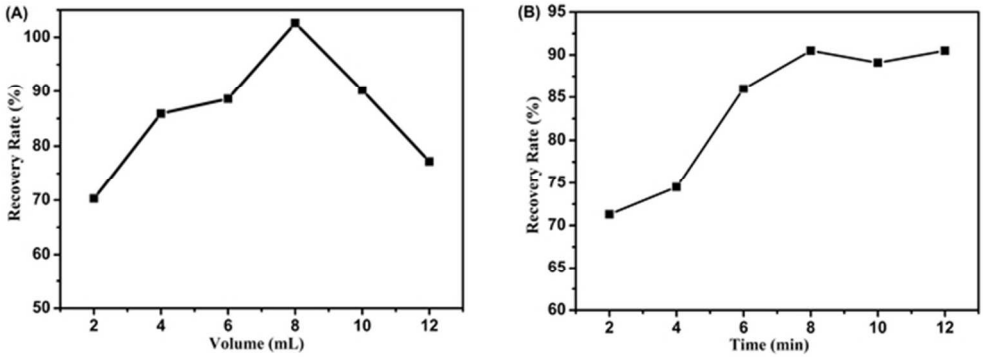

Fig.6 Effect of (a) the volume of dichloromethane and (b) extraction time on the recoveries of TBZ
58x21mm (300 x 300 DPI)