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Salting-out assisted liquid–liquid extraction with the aid of experimental design for determination of benzimidazole fungicides in high salinity samples by high-performance liquid chromatography

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ABSTRACT

A novel method for the simultaneous separation and determination of four benzimidazole fungicides (*i.e.*, carbendazim, fuberidazole, thiophanate-methyl and thiophanate) in high salinity samples was developed by using salting-out assisted liquid–liquid extraction (SALLE) *via* water-miscible acetonitrile as the extractant coupled with high-performance liquid chromatography. Box–Behnken design and response surface were employed to assist the optimization of SALLE conditions, including volume of salting-out solvent, the pH of sample solution and salting-out solvent as variable factors. The optimal salting-out parameters were obtained as follows: 2 mL of acetonitrile was added to 2 mL of sample solution with pH=4 and then 2 mL salting-out solvent containing 5 mol L^{-1} sodium chloride at a pH of 7 was added to the solution for extraction. This procedure afforded a convenient and cost-saving operation with good cleanup ability for the benzimidazole fungicides, such as good linear relationships ($R > 0.996$) between peak area and concentration from 2.5 ng mL^{-1} to 500 ng mL^{-1}, low limits of detection between 0.14 ng mL^{-1} and 0.38 ng mL^{-1} and the intra-day precisions of retention time below 1.0%. The method recoveries obtained at fortified three concentrations for three seawater samples ranged from 60.4% to 99.1%. The simple, rapid and eco-benign SALLE based method proved potentially applicable for trace benzimidazole fungicides analysis in high salinity samples.

1. Introduction

Benzimidazole fungicides are used worldwide as broad spectrum pesticides against insects, fungi and weeds on a wide variety of fruits, vegetables and other crops [1,2]. Although many public benefits have been realized by utilizing benzimidazole fungicides, their massive use in the last years has led into their accumulation in the environment, thus contaminating the water streams which cannot be disregarded. European Water Framework Directive has established a maximum concentration level (MCL) of 0.1 μg L^{-1} for most benzimidazole fungicides present in natural waters, and a total concentration of all pesticides of 0.5 μg L^{-1} [3].

Because of widespread use and possible health effects, it is desirable to monitor benzimidazole fungicides in the environment. The low vapor pressure and the thermal instabililty of benzimidazole fungicides do not permit their direct analysis by gas chromatography unless they are derived into thermally stable derivatives. So the commonly used analytical techniques for benzimidazole fungicides are high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled with utraviolet (UV), fluorescence detection or mass spectrometry [4–9].

However, analytes extraction and sample pretreatment are the most challenging and time-consuming steps for a whole analytical process. In all the above work, many extraction methods such as molecularly imprinted solid phase extraction (MISPE) [4–6], dispersive liquid–liquid microextraction (DLLME) [7] and hollow fibre-based liquid phase microextraction (HF-LPME) [8] have been used. Most of these methods are often complicated and time spending,

Abbreviations: ACN, acetonitrile; CBZ, carbendazim; CE, capillary electrophoresis; DLLME, dispersive liquid–liquid microextraction; DMSO, dimethylsulfoxide; FBZ, fuberidazole; HF-LPME, hollow fiber-based liquid phase microextraction; HPLC, high-performance liquid chromatography; LOD, limit of detection; MCL, maximum concentration level; MISPE, molecularly imprinted solid phase extraction; RSD, relative standard deviation; SALLE, salting-out assisted liquid–liquid extraction; TP, thiophanate; TPM, thiophanate-methyl; UV, ultraviolet.

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e.g. the synthesis of the solid sorbent or fiber and the need of special instrument. Furthermore, the process of SPE is relatively expensive and a problem about batch-to-batch reproducibility of the SPE column cartridges must be concerned. More importantly, it is very hard to extract benzimidazole fungicides efficiently from high salt matrices by using these extraction methods.

Fortunately, salting-out assisted liquid–liquid extraction (SALLE) provides a feasible alternative for high salinity sample preparation which is a technique based on LLE in which an appropriate concentration of salt is added to achieve the separation of aqueous phase from the partially miscible organic phase [10] and simultaneously the target solutes are extracted into the separated organic phase. Some of the organic solvents used in SALLE are acetonitrile, acetone, ethyl acetate and isopropanol and the salts commonly used are magnesium sulfate, ammonium sulfate, calcium chloride, potassium carbonate, and calcium sulfate [11]. This method coupled sample clean-up (*e.g.* acetonitrile deproteinization) with enrichment (*via* salting-out extraction) has been reported for water [10,11], biological [12–14], food [15,16], swine muscle [17], and drug [18] sample extraction processes. Also its prospective use for high salinity sample preparation is booming.

Herein, we propose the use of SALLE for extraction of four benzimidazole fungicides from three high salinity samples of seawater. Box–Behnken design and response surface were employed to assist finding optimum extraction conditions, quickly and reliably. To the best of our knowledge, this is the first demonstration for the optimization of SALLE by virtue of experimental design for benzimidazole fungicides analysis. The SALLE coupled with HPLC with the aid of experimental design was developed, validated and successfully applied for simultaneous separation and determination of the several benzimidazole fungicides in seawater samples.

2. Experimental

2.1. Chemicals and materials

Four benzimidazole fungicides standards of carbendazim (CBZ), fuberidazole (FBZ), thiophanate-methyl (TPM) and thiophanate (TP), derived from carbamic acid with chemical structure R–O–C(O)–N(CH_3)–R′, where R is an alcohol, an oxime or a phenol, and R′ is a hydrogen or a methyl group [1], were purchased from Sigma-Aldrich (Shanghai, China), and their chemical structures are shown in Fig. 1. HPLC-grade acetonitrile (ACN) was provided by J&K Chemical (Beijing, China). Dimethylsulfoxide (DMSO), NaCl, NaH_2PO_4, H_3PO_4, NaOH, and other affiliated chemicals were all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solvents and chemicals were of analytical grade and used without further purification unless otherwise specified. HPLC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA), and was used throughout the work.

2.2. Apparatus and software

An HPLC instrument was provided by Skyray Instrument Inc. (Kunshan, Jiangsu, China), equipped with a UV detector. Separation was carried out on a Waters Arcus EP-C_{18} column (150 mm × 4.6 mm id, 5 μm particle size). Analytes were eluted by a mixture of ACN and water (70/30, v/v) at a flow rate of 1 mL min^{-1}. Benzimidazole fungicides were monitored at 230 nm. All the samples were passed through microporous nylon filters of 0.45 μm pore sizes in diameter (Pall Corporation, USA).

Lingo software (LINDO Systems Inc., USA) was used to assist obtaining the optimum SALLE conditions by computing theoretical recovery. Matlab 7.5.0.342 (Mathworks Corporation, USA) was employed to develop the response surface. The script was run in Windows XP on a personal computer.

2.3. Preparation of standard and sample

Standard stock solutions containing 1000 μg mL^{-1} of each benzimidazole fungicide were prepared by dissolving the required amounts of the standard in DMSO. They were stored in a refrigerator at 4 °C. Working solutions were prepared from the stock solutions by dilution with appropriate amounts of Milli-Q water.

Seawater was used as a model of high salt samples. They were collected from the coastal zone areas of Yantai City of China. Three surface water samples were from the junction of the Qinshui River and the Yellow sea (Seawater no. 1), the junction of the Xin′an River and the Yellow sea (Seawater no. 2), and Fisherman's Wharf of the Yellow sea (Seawater no. 3). The two junction water samples were taken near a sewage treatment plant of Yantai City. All the seawater samples were passed through microporous nylon filters with the pore sizes of 0.45 μm in diameter. The samples were kept under refrigeration at 4 °C in the dark. Several aliquots from 2 mL filtered water samples were spiked with the benzimidazole fungicide standard with different concentrations and followed by the SALLE procedure.

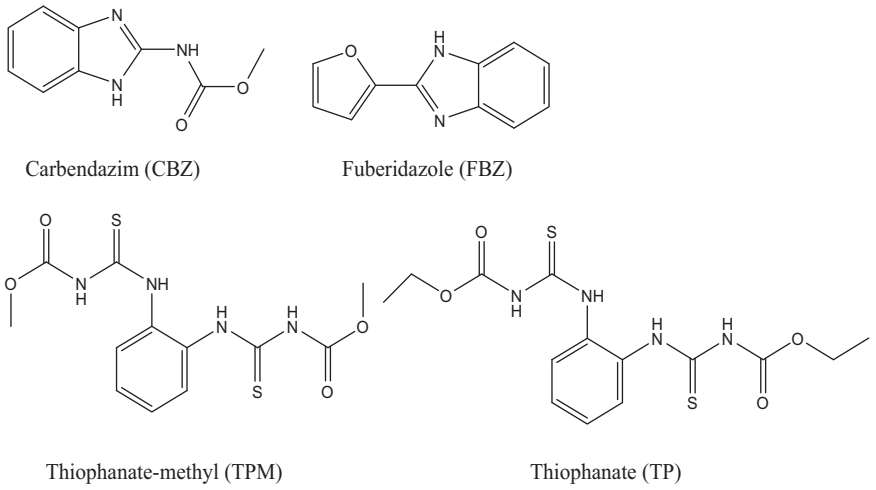

Fig. 1. Chemical structures of the benzimidazole fungicides analyzed.

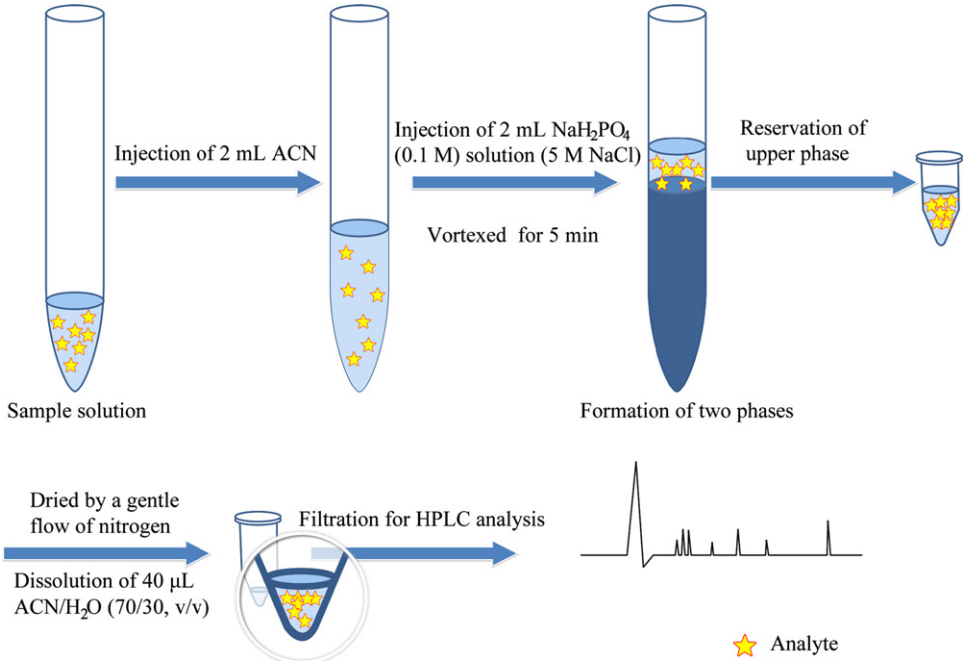

Fig. 2. Schematic illlustration of the SALLE procedure.

Table 1
Box–Behnken design chart including factors, levels and matrix with three factors.

Variable	Factor			Level			
				−1		0	+1
X1	pH1			4		5	6
X2	V (mL)			2		3	4
X3	pH2			5		6	7
Run	X1	X2	X3	Recovery CBZ (%)	Recovery FBZ (%)	Recovery TPM (%)	Recovery TP (%)
1	−1	−1	0	56	74	92	84
2	−1	1	0	46	58	84	74
3	1	−1	0	50	64	84	78
4	1	1	0	78	60	86	80
5	−1	0	−1	36	56	82	74
6	−1	0	1	44	78	86	78
7	1	0	−1	44	64	92	82
8	1	0	1	46	66	94	84
9	0	−1	−1	46	60	82	76
10	0	−1	1	50	60	82	74
11	0	1	−1	44	58	78	76
12	0	1	1	40	52	72	72
13	0	0	0	40	54	80	70
14	0	0	0	42	56	82	72
15	0	0	0	44	54	82	70

Table 2
Theoretical and experimental values for extraction recoveries under optimized conditions.

Optimized condition	CBZ			FBZ			TPM			TP		
	pH_1	V (mL)	pH_2	pH_1	V (mL)	pH_2	pH_1	V (mL)	pH_2	pH_1	V (mL)	pH_2
	4	2	7	4	2	7	4	2	7	4	2	5
Theoretical recovery (%)	60			80			92			84		
Experimental recovery (%)	58			85			98			95[a]		
RSD[b] (%)	0.024			0.042			0.045			0.084		

[a] pH_2=7.
[b] Relative standard deviation, n=3.

2.4. SALLE procedure

A salting-out solution of 5 mol L^{-1} NaCl was prepared by dissolving appropriate amounts of NaCl in 100 m mol L^{-1} phosphate buffer, and the pH was adjusted by 1 mol L^{-1} H_3PO_4 and 1 mol L^{-1} NaOH.

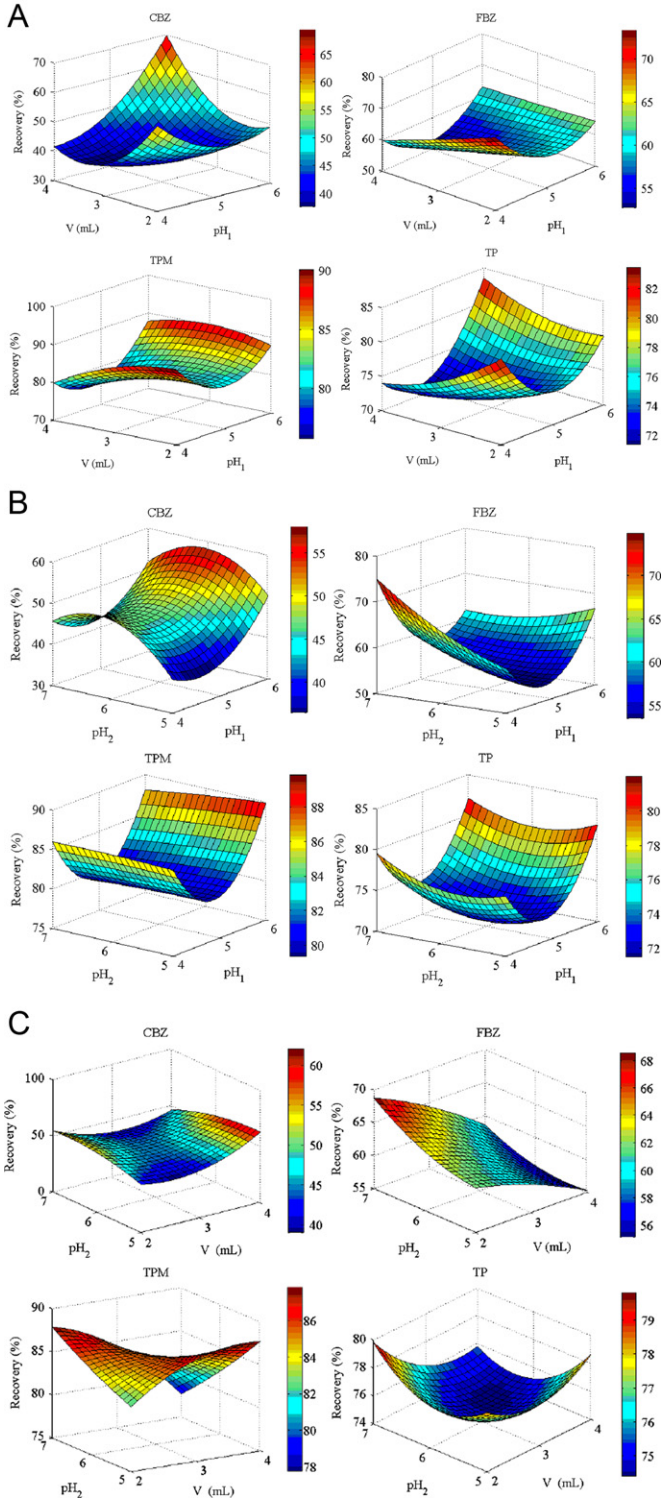

Fig. 3. Response surfaces (in recovery) for the Box–Behnken design of the analyzed benzimidazole fungicides. (A) pH_2 kept constant at 7; (B) V kept constant at 2 mL; and (C) pH_1 kept constant at 4.

Fig. 2 illustrates the SALLE steps. Briefly, 2 mL of water sample was placed in a 10 mL of screw-cap glass test tube and spiked with four benzimidazole fungicides individual at 1 μg mL^{-1}. ACN (2 mL) was added into the sample solution. After the mixture was gently shaken and then ultrasonicated for 1 min, 2 mL salting-out solution was added into the mixture and vortexed for 5 min. In this step, a two-phase solution was formed and the benzimidazole fungicides in the water samples were extracted into the upper organic phase (ACN phase). Then, the supernatant was collected and dried under a gentle flow of nitrogen. And the residue was dissolved using 40 μL of ACN/H_2O (70/30, v/v) and then 20 μL of the solution was injected for HPLC–UV analysis. In the UV detection, standard addition method was also employed for target peak identification in real water sample analysis, besides comparing with the migration time matching.

2.5. Computation procedure

All the data including the factor and the enrichment folds ($f=C_{\text{after enrichment}}/C_{\text{before enrichment}}$) were imported into the SPSS software to obtain the regression model with coefficients, as seen in Table S1. Then the recovery values were obtained, listed in Table 1, by calculation as follows:

Recovery $(\%)=f\times 40\ \mu L/2\ mL\times 100$, where 40 μL was the volume of the sample after enrichment, 2 mL was the volume of the sample. And the regression models for the four analytes were imported to the Lingo software to calculate the optimum extraction conditions. Later on, all the surface responses were made by the data of regression models in Matlab software.

3. Results and discussion

3.1. Box–Behnken design and response surface for SALLE condition optimization

Several parameters that may influence the SALLE performance, including extraction solvent type and volume, NaCl content, extraction solvent pH, vortex time and sample pH, should be investigated. After a one-factor analysis of variable preliminary experiment (data not shown), sample pH (pH_1: 4, 5, 6), salting-out solvent volume (V: 2, 3, 4 mL) and salting-out solvent pH (pH_2: 5, 6, 7) were considered in Box–Behnken design.

The employed Box–Behnken design involved 15 experimental runs, similar to our previous work [19], and the low, medium and high levels of each variable were coded as −1, 0 and +1, respectively. The Box–Behnken design matrix, *i.e.*, the extraction conditions for each of the 15 runs and the recovery values for four benzimidazole fungicides are listed in Table 1. As seen, their respective highest recovery values for the four analytes, *i.e.*, 78% for CBZ, 78% for FBZ, 94% for TPM and 84% for TP, could be obtained. However, different variable conditions are required. That is, it is not possible to simultaneously realize the above extraction. In order to attain the simultaneous extraction of the four analytes with ideal recovery, the variable values of $pH_1=X_1=4$, $V=X_2=2$ and $pH_2=X_2=7$ were utilized, and relatively higher recovery values for most of the analytes were attained, *i.e.*, 85% for FBZ, 98% for TPM and 95% for TP, except 58% for CBZ, lower, as listed in Table 2. Also, as shown in Table 2, relative standard deviation (RSD) values between the experimental recovery values and theoretical ones are very small, from 0.024% to 0.084%, indicating the present Box–Behnken design and the optimized computation are suitable and applicable for this work. Therefore, 2 mL of salting-out solvent with pH=7 and the sample pH=4, were finally chosen as the optimum extraction conditions.

Fig. 3 shows the estimated response surfaces for each two ones of the above three factors. It is notable that high extraction recovery was achieved when pH_1 was 4 and V was 2 mL (Fig. 3A). Although when pH_1 was 4 and pH_2 was 7, the extraction recoveries were not very high for CBZ and TPM, they were still satisfactory and the recoveries for FBZ and TP were high under these conditions (Fig. 3B). Fig. 3C shows the similar trends of effect of each two factors; high extraction recovery was obtained when $pH_1=6$. Even so, regarding the factor interactions and balancing the high extraction recoveries for all the benzimidazole fungicides, the following conditions were finally selected as the optimum, *i.e.* $pH_1=4$, $V=2$ mL and $pH_2=7$. Although there are some differences in the ideal extraction conditions between the response surfaces deriving and that finally chosen, the response surfaces exhibited the consistent trends of the extraction recovery and in a sense testified the final choice.

The above optimal sample pH was consistent with the pK_a values reported for benzimidazole fungicides, which have values 4 in aqueous solutions (CBZ 4.2, FBZ 4.0, TPM 7.28 and TP was not obtained) [20]. When sample pH is <4.0, they will be ionized [21], however, the neutral species are still the major format which makes the analytes be extracted easily into the smaller volume organic solvent (ACN). So sample pH=4 was consistent with the theoretical rational. The salting-out theory has been described in Ref [22] in detail. When the extraction solvent (ACN) was added into the sample solution, ACN acted as a solute in the aqueous solution, and here ACN was a nonelectrolyte. After an electrolyte (salting-out solvent) was added to the solution of a nonelectrolyte (ACN), they would compete with each other for water molecules. As expected, the competition would be won by the electrolyte ions, while ACN would lose. This would cause preferential movement of the water molecules away from the ACN to those of the salt, which, in turn, decrease hydration and hence the solubility of ACN. As a result, the ACN phase would be separated from the solution. Since the analytes have higher solubility in ACN, they were also extracted by the ACN phase. However, the pH of the salting-out solvent could affect the octanol–water partition coefficient (Log P) of the analytes (CBZ 1.48, FBZ 2.71, TPM 1.45 and TP was not obtained) [18]. In the preliminary experiment, the extraction efficiencies of CBZ and FBZ were indeed decreased with the increase of pH values between 4 and 8. However, for the TPM and TP, the trend was opposite. This phenomenon may be caused by the effect of pH on the dissociation of the analytes. For CBZ and FBZ, when $pH>4$, the analytes began to dissociate, which would cause the Log P value decreased, resulting in the extraction efficiency decreased. With the same cause, the Log P value of TPM would decrease when $pH>7$. However, interestingly, through the experimental design, pH 7 was finally selected and the result was consistent with the experimental value that may be caused by the cross-correlation between the experimental parameters.

Data analysis permitted to obtain regressions of enrichment folds (f) to factors for each benzimidazole fungicide, as seen in Table S1. From the regression model (Table S1), for each analyte, at the optimum level of factors, 30 folds for CBZ, 40 folds for FBZ, 46 folds for TPM and 42 folds for TP were obtained, respectively, then the corresponding theoretical recovery values for CBZ, FBZ, TPM and TP were 60%, 80%, 92% and 84%, respectively. As shown in Table 2, the experimental results were close to the theoretical values and the RSDs were satisfactory. And from the statistic data of R and F in Table S1, it therefore concludes that the final models are considered to be satisfactory.

So, the optimized conditions for simultaneous extraction of benzimidazole fungicides with ideal SALLE efficiency were attained with the aid of experimental design and response surface as follows: 2 mL of salting-out solution containing both 5 mol L^{-1} of NaCl and 0.1 mol L^{-1} of phosphate buffer, pH=7; 2 mL of ACN as extraction solvent; 5 min as vortex time required; and pH 4 for sample solution.

Table 4
Method precision for the SALLE–HPLC–UV determination of benzimidazole fungicides in spiked natural surface seawater samples.[a]

Spiking concentration (ng mL $^{-1}$)	Benzimidazole fungicides	RSD (%)			
		Intra-day ($n=6$)		Inter-day ($n=6$)	
		Retention time	Peak area	Retention time	Peak area
50	CBZ	0. 8	1.8	1.8	4.1
	FBZ	0.6	2.7	1.7	8.6
	TPM	0.7	3.6	1.8	7.2
	TP	0.4	5.6	2.5	7.7
100	CBZ	0.9	1.7	2.0	4.9
	FBZ	0.9	2.3	1.2	9.1
	TPM	0.7	1.6	1.6	3.3
	TP	0.3	3.7	1.9	7.8
200	CBZ	0.6	2.9	2.4	5.7
	FBZ	0.7	4.1	2.6	7.4
	TPM	0.6	5.8	1.7	10.0
	TP	0.3	3.2	2.3	6.3

[a] Seawater no. 2.

Table 3
Linear relationship parameters and LOD of benzimidazole fungicides for three natural surface seawater samples.

Source	Benzimidazole fungicides	Linear range (ng mL^{-1})	Slope (Mean ± SD)[a]	Intercept (Mean ± SD)	R	LOD (ng mL^{-1})
Seawater no. 1	CBZ	2.5–500	103000 ± 364.21	840 ± 19.68	0.9998	0.15
	FBZ	5.0–500	53000 ± 100.04	1870 ± 26.55	0.9988	0.38
	TPM	5.0–500	129000 ± 458.22	450 ± 18.21	0.9998	0.16
	TP	5.0–500	183000 ± 521.39	−710 ± 15.47	0.9998	0.26
Seawater no. 2	CBZ	2.5–500	109000 ± 398.72	770 ± 16.24	0.9967	0.14
	FBZ	5.0–500	109000 ± 287.16	−820 ± 19.45	0.9998	0.30
	TPM	5.0–500	115000 ± 422.68	−2680 ± 31.48	0.9996	0.18
	TP	5.0–500	152000 ± 411.28	−4700 ± 46.56	0.9991	0.27
Seawater no. 3	CBZ	2.5–500	82000 ± 124.57	1340 ± 22.18	0.9999	0.16
	FBZ	5.0–500	52000 ± 116.64	3560 ± 40.13	0.9957	0.36
	TPM	5.0–500	132000 ± 513.26	−2940 ± 30.59	0.9996	0.19
	TP	5.0–500	168000 ± 576.32	−3140 ± 37.46	0.9993	0.28

[a] Standard deviation, $n=3$.

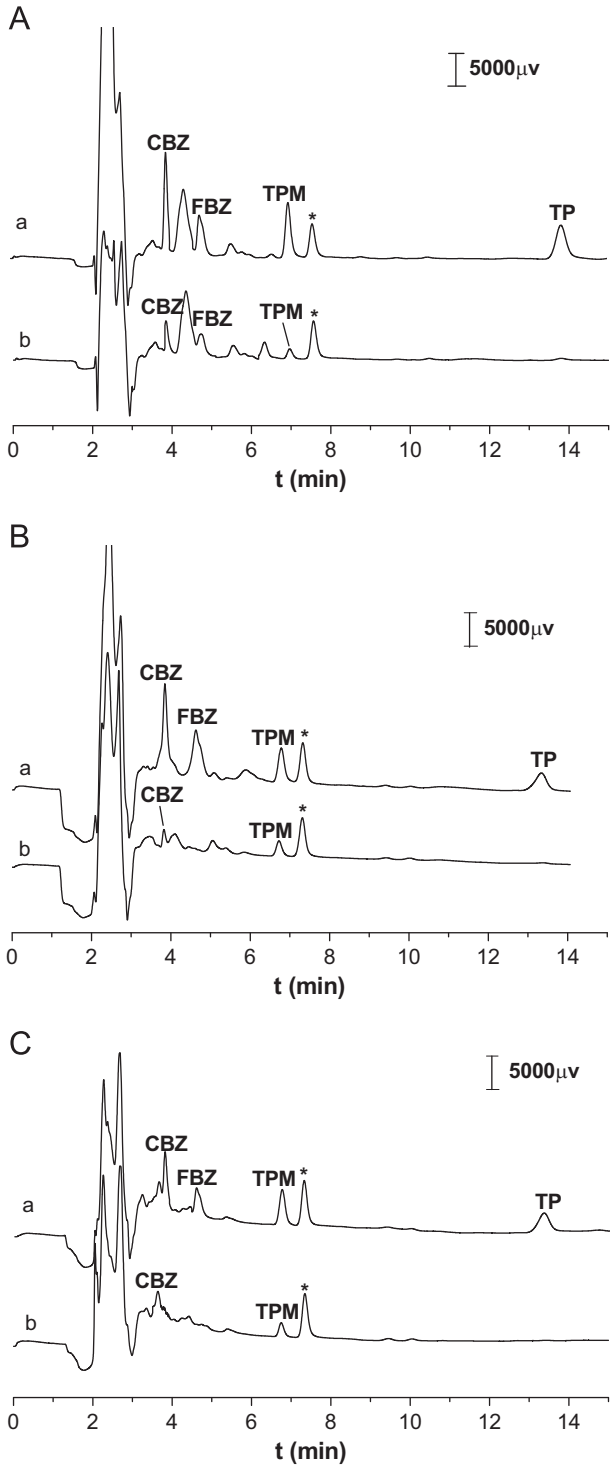

Fig. 4. Typical HPLC–UV chromatograms of (a) spiked and (b) no spiking high salinity samples of (A) Seawater no. 1, (B) Seawater no. 2 and (C) Seawater no. 3 after SALLE. The spiked concentration of benzimidazole fungicides standards was 100 ng mL^{-1} for Seawater no. 1 and 50 ng mL^{-1} for Seawater nos. 2 and 3. SALLE conditions: sample volume, 2 mL and pH 4; salting-out solution, 5 mol L^{-1} NaCl, 2 mL and pH 7; extraction solvent, ACN, 2 mL; vortex time, 5 . HPLC–UV conditions: mobile phase, ACN and water (70/30, v/v); flow rate, 1 mL min^{-1}; sample injection, 20 μL of ACN/H_2O (70/30, v/v) solution containing benzimidazole fungicides; detection wavelength, 230 nm.

3.2. Analytical figures of merit of the SALLE–HPLC–UV method

The aforementioned optimal extraction conditions for SALLE were employed in subsequent work. Calibration curves were obtained from the analysis of the spiked seawater matrix with benzimidazole fungicides standards at various concentrations. Table 3 lists the linear range, slope, intercept, correlation coefficients, and limit of detection (LOD) for the benzimidazole fungicides determination in three different seawater samples. As seen, good linearity assessed using samples fortified at six different concentration levels was obtained between peak-area and the corresponding concentrations of benzimidazole fungicides in the range over 2.5–500 ng mL^{-1} or 5.0–500 ng mL^{-1}. LOD for all the four benzimidazole fungicides, calculated as the analyte concentration for which the peak height was three times the background noise (3*S*/*N*), were attained within 0.14 ng mL^{-1} and 0.38 ng mL^{-1} for the HPLC–UV analysis. And this method was satisfactory considering a MCL of 0.5 ng mL^{-1} established by European Water Framework Directive.

The method precision was determined by analyzing spiked seawater samples at three different concentrations of benzimidazole fungicides. As shown in Table 4, the precision obtained under intra-day repeatability ($n=6$) in terms of retention time and peak area were less than 1.0% and 5.8%, respectively, while precision under inter-day reproducibility (6 different days) remained under 2.6% and 10.0%, respectively. The relatively high RSD results obtained may be caused in part by the adsorption of the hydrophobic analytes onto the surfaces of the sample vials and/or the evaporation of ACN over a period of ca. 24 h. In addition, the uncertainties introduced by the various individual steps within the salting-out extraction processes were not negligible, possibly resulting in high RSD. Still, the method was demonstrated stable and potentially applicable for the simultaneous separation and accurate determination of four benzimidazole fungicides in such high salt matrices.

3.3. Determination of benzimidazole fungicides in seawater samples

To further evaluate the feasibility of the developed method, three natural surface seawater samples without spiking and spiked with the mixture standards of CBZ, FBZ, TPM and TP individual at 100 ng mL^{-1} or 50 ng mL^{-1}, respectively, were pretreated using the SALLE. In order to ensure the accurate target peak identification for the real water sample analysis in the UV detection, standard addition method was also employed besides comparing the migration time matching with the standard chromatograms. As shown in Fig. 4a, three benzimidazole fungicides were remarkably detected, which was attributed to the fact that the SALLE has high cleanup ability, and thereby the matrix effects could be significantly reduced. It was observed from Fig. 4b that endogenous FBZ was not detected in seawater no. 2 and no. 3, as well as TP was not found in seawater nos. 1–3. As seen in Table 5, the endogenous FBZ was detected at 10.10 ng mL^{-1} in Seawater no. 1; CBZ and TPM were found in the range from 5.0 ng mL^{-1} to 8.0 ng mL^{-1} and from 12.2 ng mL^{-1} to 16.3 ng mL^{-1} in all the analyzed three seawater samples, respectively. These results suggested that the analyzed seawater samples had been polluted by benzimidazole fungicides to some degree, which might be from the effluents of a wastewater treatment plant nearby. Also, as seen from Fig. 4, there was an unknown peak beside TPM at the retention time of 7.5 min for the three seawater samples with and without spiking benzimidazole fungicides standards. It is very likely to be a matrix peak of an interfering substance contained in the seawater samples. Recoveries were calculated for the spiked seawater samples with 50, 100 and 200 ng mL^{-1} standards, respectively. The results are listed in Table 5. Satisfactory recoveries were obtained, such as 62.0%–97.9% at 100 ng mL^{-1} in the three samples.

All the above experimental results validated the developed SALLE–HPLC–UV greatly applicable for high efficient matrix cleanup and targeted compounds enrichment, and simultaneous separation and accurate quantitation of trace benzimidazole fungicides in high salinity samples.

Table 5
Contents and recoveries of benzimidazole fungicides in three natural surface seawater samples determined by SALLE–HPLC–UV.

Source found	Benzimidazole fungicides	Endogenous	Recovery (%)[a]		
			50 ng mL^{-1} spiked	100 ng mL^{-1} spiked	200 ng mL^{-1} spiked
Seawater no. 1	CBZ	8.0[b] ± 0.3[c]	77 ± 1	68 ± 2	66 ± 1
	FBZ	10.1 ± 0.5	86 ± 2	98 ± 4	78 ± 1
	TPM	16.3 ± 0.6	91 ± 4	73 ± 2	78 ± 2
	TP	ND[d]	61 ± 2	65 ± 2	78 ± 2
Seawater no. 2	CBZ	5.5 ± 0.2	89 ± 3	88 ± 2	69 ± 2
	FBZ	ND	89 ± 3	84 ± 2	98 ± 3
	TPM	14.6 ± 0.5	60 ± 1	70 ± 2	68 ± 3
	TP	ND	65 ± 2	76 ± 2	64 ± 3
Seawater no. 3	CBZ	5.0 ± 0.1	68 ± 2	87 ± 3	98 ± 1
	FBZ	ND	99 ± 3	93 ± 4	70 ± 2
	TPM	12.2 ± 0.5	67 ± 2	90 ± 3	72 ± 1
	TP	ND	62 ± 2	62 ± 1	70 ± 2

[a] Recovery (%)=[(total amount of detected − amount of endogenous benzimidazole fungicides)/amount of added] × 100; data are expressed as the mean ± RSD determined from triplicate independent experiments.
[b] Average value from triplicate independent experiments.
[c] Standard deviation (SD) from triplicate independent experiments.
[d] Not detected.

Table 6
Performance comparisons for benzimidazole fungicides determination with other reported analytical methods.

Detection technique	Pretreatment method	Sample	LOD (ng mL^{-1})	Linear range (μg L^{-1})	Recovery (%)	Ref.
HPLC–UV	Off-line MISPE	Tap, river, well water	0.03–0.07	–	89–106	[3]
HPLC–UV	On-line MISPE	Tap, river, well water	2.6 ng L^{-1}	2.3–500 ng L^{-1}	89–95	[4]
CE–UV	In-capillary SPE	Tap water	0.01	0.1–5	–	[5]
CE–UV	In-capillary microextraction	–	7	–	–	[6]
HPLC–FD	DLLME	Soil	13.92–15.76ng g^{-1}	50–3000 ng g^{-1}	92–119	[7]
HPLC–FD	HF–LPME	Apple juice	0.8	2.5–500	86.3–102.0	[8]
HPLC–MS	Magnetic nanoparticles–SPE	Soft drink	0.012	0.5–100	–	[9]
HPLC–UV	SALLE	Seawater	0.14–0.38	2.5–500	60.4–99.1	This work

3.4. Method performance comparison

Analytical performances of the developed SALLE–HPLC–UV toward benzimidazole fungicides were mainly compared with the most commonly used HPLC hyphenated techniques, and some CE-hyphenated technique was also given for comparison. As can be seen from Table 6, some of the reported methods provide higher sensitivity for benzimidazole fungicides than the present method [3–6]. Nevertheless, in a sense, they are also involved in complex enrichment procedures, long time-consuming, rigorous detection media, or high cost. For example, although there is an in-tube SPE method [5] for the extraction of the benzimidazole fungicides, the procedure for the whole process is complicated and needs a long time for the preparation of the SPE procedure. And most of all, no high salinity water samples were analyzed [3–9], which is very likely because the salinity will disrupt seriously the extraction process and decrease efficiency. And our newly developed method was satisfactory with MCL established by European Water Framework Directive. Also, it was demonstrated to be a simple, fast, cost-effective and eco-benign option for simultaneous determination of benzimidazole fungicides in high salinity water samples.

4. Conclusions

A simple, fast and sensitive SALLE–HPLC method was developed with the aid of Box–Behnken design and response surface for the determination of benzimidazole fungicides in seawater samples. It avoided the need for the elimination of salinity in the sample matrix, as well as clean-up of the SALLE extractant. LODs at low ng mL^{-1} were achieved for all the compounds, enabling use of the method for the determination of benzimidazole fungicides in high salinity matrices. Also, compared with the classic extraction methods based on LLE and SPE, which often required large volume of sample and organic solvent, this method is an excellent cost-effective alternative for sample preparation. The developed SALLE procedure might well become a greatly practical option in terms of rapidity and automatization.

The developed SALLE–HPLC with simple UV detector offered a number of features including good quantitative ability, wide linear range, high recovery, simple operation process and short analysis time, as well as low cost and environmental benignity, especially high salt matrix applicability. Given the advantages, further research focusing on SALLE will be significantly promising for trace analysis of various contaminants in high salinity samples.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.12.011.

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