Methyl Salicylate: an Alternative Extraction Solvent for Dispersive-Liquid-Liquid Microextraction of Benzimidazole Fungicides in Water Samples Followed by High-Performance Liquid Chromatographic Analysis

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Methyl salicylate based dispersive liquid-liquid microextraction method of benzimidazole fungicides (i.e., carbendazim, thiabendazole and fluberidazole) in water samples and analysis by high performance liquid chromatography has been firstly developed. The target fungicides in aqueous sample were extracted under the selected conditions of 250 μL of methyl salicylate without disperser solvent and 1.0% (m/v) sodium acetate without pH adjustment. The preconcentration factor and extraction recovery were obtained in the range of 24-38 and 54-85%, respectively. Limits of detection ranged from 0.03 to 0.05 μg L\(^{-1}\), while limits of quantification were in the range of 0.20-0.50 μg L\(^{-1}\). Recoveries at three spiked concentration levels of 5, 10, and 50 μg L\(^{-1}\) were obtained in the range of 74.1-118.4%, with the relative standard deviation (RSD) below 11%. The developed method was simple, rapid, low cost, and reliable for trace determination of the studied fungicides.

Keywords: carbendazim, thiabendazole, fluberidazole, preconcentration, environmental samples

Introduction

Nowadays, liquid-phase microextraction (LPME) such as dispersive liquid-liquid microextraction (DLLME) has been reviewed as an alternative powerful strategy to conventional sample preparation such as solid-phase extraction (SPE) and liquid-liquid extraction (LLE).\(^1\) The advantages of microextraction techniques over the conventional methods are simple, rapid, inexpensive, and low consumption of organic solvents. In typical DLLME, a mixture of extraction and disperser solvents was rapidly injected into the aqueous sample solution, resulting in forming a cloudy solution.\(^1\) The target analytes penetrate into the extraction solvent phase and are consequently concentrated. Although DLLME provides many good characteristics, high toxic halogenated organic solvents (e.g., chloroform, carbon tetrachloride and chlorobenzene) are normally required. To avoid these toxic extractant solvents, two possible ways can be instead employed using: lower toxic solvents such as dodecanol and hexane\(^2\) or based in ionic liquids (IL),\(^3\) as alternative extraction solvents. Nevertheless, the first way normally requires special devices for collecting the extracted-rich phase, while IL used for the later strategy is very expensive. Therefore, the other alternative solvents as extractants are very interesting to further study. Recently, the applications of methyl benzoate as alternative effective extraction solvent have been demonstrated for the analysis of inorganic and organic compounds in environmental water samples.\(^4,5\) The another interesting solvent is methyl salicylate. It seems to be possible to evaluate as an extraction solvent for the preconcentration of the selected target compounds because there are some interesting characteristics as follows:\(^6\) clear liquid solution at room temperature, high density (1.17 g mL\(^{-1}\) at 25 °C), low water solubility (700 mg L\(^{-1}\)), and low toxic and cheap cost. In addition, it provides the extract phase remaining at the bottom of the tube after complete phase separation, and can easily dissolve in chromatographic mobile phase. To our knowledge, this is the first application of methyl salicylate used as an extraction solvent in DLLME. The model target compounds in this purpose are benzimidazole fungicides.

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Benzimidazole fungicides are widely used in agriculture to control and kill fungi or fungal spores, in order to prevent the spoilage of crops.\textsuperscript{7,9} For instance, the effective benzimidazole fungicides normally used are benomyl (BN), carbendazim (CBZ), thiabendazole (TBZ), and fuberidazole (FuBZ). Since it is normally directly applied to soil or sprayed over the crop fields, it can release to and accumulate in food and environmental samples.\textsuperscript{7,10} The residues of these fungicides can cause several adverse effects to human health such as teratogenicity, congenic malformations, polyplody, diarrhea, anemia, pulmonary edemas, or necrotic lymphoadenopathy.\textsuperscript{9,11}

Typical analytical techniques have been adopted for the determination of benzimidazole fungicides such as micellar electrokinetic chromatography (MEKC) mode,\textsuperscript{12} screening-based immunoassays,\textsuperscript{13,14} and high performance liquid chromatography (HPLC).\textsuperscript{15} Among these methods, HPLC is the most frequently used because it offers many advantages such as very simple and easy to operate, effective, reliable, and can be used for simultaneous determination of fungicide compounds. HPLC with ultraviolet (UV) detection,\textsuperscript{16,17,19} or mass spectrometry (MS) detection\textsuperscript{18} have been performed. MS and FL detectors are relatively expensive and complicated instrument, although they provide better sensitivity and selectivity than UV detector. The effective and simple strategies to improve detection sensitivity are the use of suitable sample preparations (including extraction and clean-up) in order to remove matrix interferences before instrumental analysis. The extraction and clean-up methods such as LLE,\textsuperscript{19,20} traditional DLLME,\textsuperscript{21} SPE,\textsuperscript{18,22,23} mix-mode SPE,\textsuperscript{24} SPE-DLLME,\textsuperscript{25} molecularly imprinted SPE (MISPE),\textsuperscript{8,9} magnetic SPE (MSPE),\textsuperscript{16} solid-phase microextraction (SPME),\textsuperscript{26} hollow fiber-liquid phase microextraction (HF-LPME)\textsuperscript{27} and salting-out assisted LLE (SALLE)\textsuperscript{15} have been developed and proposed for the analysis of the target fungicides. However, these techniques encounter at least one or more limitations such as tedious, time-consuming, and the use of large volumes of both samples and toxic organic solvents. Consequently, a green analytical method coupled with miniaturized preconcentration strategies to avoid the above limitations is of great interest to develop.

The aim of this work was to further develop and find a new green designated DLLME method using methyl salicylate as extraction solvent for the extraction and preconcentration of the studied benzimidazole fungicides (e.g., CBZ, TBZ, and FuBZ) in environmental water samples and analysis by HPLC. The parameters (e.g., volume of extraction solvent, type and volume of disperser solvent, salt amount and centrifugation) which affect the extraction recovery of the target fungicides were also investigated and optimized.

**Experimental**

**Chemicals and reagents**

All reagents were analytical reagent grade or higher. The analytical standards of benzimidazole fungicides were purchased from Sigma-Aldrich (Germany) for CBZ and FuBZ and Sigma-Aldrich (Italy) for TBZ. The stock solutions of each fungicide were prepared at 1,000 mg L\textsuperscript{-1} by dissolving an appropriate amount of them in formic acid (ca. 500 μL) to easily obtain a clear solution, and further diluted with methanol (MeOH). Methyl salicylate was purchased from Fluka (China). Formic acid, MeOH, ethanol (EtOH), and acetonitrile (ACN) were purchased from Merck (Germany). Sodium chloride (Ajax Finechem, New Zealand), anhydrous sodium sulphate (Carlo Erba, France), sodium carbonate (RFCL Limited, India), and anhydrous sodium acetate (Carlo Erba, France) was also used. All aqueous solutions were prepared in deionized water with resistivity of 18.2 MΩ cm from RiO\textsuperscript{2} Type I Simplicity 185 purifier (Millipore, USA).

**Instruments**

The HPLC system (Shimadzu Corporation, Japan) consisted of the DGU-20As in-line degasser, an LC 20AD pump, and a photo-diode array detector (PDA). LCSolution software (Shimadzu, Japan) was used to control the system and for the data analysis processing. An Inertsil C8 column (4.6 × 150 mm, 5.0 μm) and a guard C8 column (GL Sciences, Japan) were used for the separation of target fungicides. A centrifuge (NF200 model, Nüve Inc., Turkey) was used for complete phase separation.

**HPLC separation conditions**

The simultaneous analysis of the studied target benzimidazole fungicides was achieved using a reversed-phase HPLC system with the gradient elution mobile phase of acetonitrile (ACN) and 0.1% (v/v) of formic acid, and a flow rate of 1.0 mL min\textsuperscript{-1}. The gradient elution profile was performed as follows:\textsuperscript{5} 15% ACN (0-2.0 min), ramped to 45% ACN (2.0-4.0 min), then ramped to 75% ACN (4.0-6.0 min), and further kept constant at 75% ACN (6.0-9.0 min). After that, ACN was decreased to 15% and then equilibrated until the pressure reached the initial value (ca. 5 min). The chromatograms
were recorded at the maximum absorption wavelengths of 280 nm (for CBZ) and 296 nm (for TBZ and FuBZ).

**DLLME procedure**

Figure 1 shows the photographs of the solution during the DLLME procedure. A 10.00 mL aliquot of standard mixture or sample solution was mixed with sodium acetate (1.0%, m/v). After complete dissolve, the solution was transferred into a 15 mL conical tube (Figure 1, step 1). Then, 250 μL of methyl salicylate (as extraction solvent) was rapidly injected into the solution and the solution was shaken by hand for 1 min. Consequently, cloudy solution was obtained (Figure 1, step 2). The solution was subjected to centrifuge at 3500 rpm for 3 min. The methyl salicylate-rich phase was obtained at the bottom of the solution (Figure 1, step 3). Afterwards, the upper part of aqueous phase was removed by a syringe. Only the extract rich phase was dissolved with MeOH (100 μL). Finally, an aliquot of solution (20 μL) was then analyzed by HPLC.

![Figure 1. Photographs of the solution during the extraction procedure. Step 1: aqueous sample solution; step 2: cloudy solution obtained after the addition of methyl salicylate and salt and manual shaking; and step 3: the phase separation after centrifugation.](image)

**Sample analyzes**

Water samples were randomly collected from agricultural fields in Khon Kaen Province, Thailand. The water samples were firstly filtered through Whatman filter paper No. 42 and then passed through 0.45 μm membrane before analysis. A 10.00 mL water sample was subsequently subjected to the proposed DLLME followed by HPLC analysis. To evaluate accuracy (recovery), water samples were fortified with certain standard fungicide concentrations of 5, 10, and 50 μg L⁻¹ before subjecting it to DLLME. All analyses were performed in triplicate.

**Results and Discussion**

The parameters affecting the extraction recovery were firstly evaluated based on one-parameter-at-a-time method. The one-parameter-at-a-time method was done by varying each parameter, whereas the other variables were kept constant. The studied parameters included extraction solvent, disperser solvent, salt additive, and centrifugation. A preconcentration factor (PF) and extraction recovery (ER, in %) of each studied compounds were also calculated. PF and ER were evaluated by several determinations of certain concentration of target fungicide mixture (250 μg L⁻¹) in aqueous solutions and calculated (on average) using the following equations:

\[
PF = \frac{C_{\text{sed}}}{C_0} \quad (1)
\]
\[
ER = \frac{C_{\text{sed}} \times V_{\text{sed}}}{C_0 \times V_0} \times 100 = PF \times \frac{V_{\text{sed}}}{V_0} \times 100 \quad (2)
\]

where \(C_{\text{sed}}\) and \(C_0\) are concentration of target analytes in the sediment methyl salicylate-rich phase and initial analyte concentration, respectively. The \(C_{\text{sed}}\) was calculated from the calibration obtained from the direct analysis (before preconcentration). \(V_{\text{sed}}\) and \(V_0\) are the volume of the sediment phase (\(V_{\text{sed}}\) ca. 300 μL, on average) and initial aqueous sample solution (10 mL), respectively.

**Extraction solvent and its volume**

Methyl salicylate was chosen in this study to test the feasibility for the extraction of target fungicides. It is soluble in water (0.7 mg mL⁻¹ at 20 ºC) and other organic solvents (e.g., chloroform, EtOH, MeOH, and ether). It has a log Kow (correlates to hydrophobic property) of 2.55, which is close to their log Kow values of target fungicides including TBZ (2.47) and FuBZ (2.67), except CBZ (1.52), (values based in the Estimation Program Interface (EPI) Suite™, EPI WEB 4.1). Consequently, methyl salicylate is possible to be used as extraction solvent for concentrating target fungicides. In this study, it was further evaluated the effect of its volume in the range of 100-350 μL. The results are depicted in Figure 2. It was found that extraction recovery of target analytes increased with the increasing of methyl salicylate volumes up to 250 μL. Beyond this point, extraction recovery of most selected analytes decreased. This behavior may be explained by the dilution affecting from higher extraction solvent volume used. In this study, 250 μL of methyl salicylate was selected as the optimum value for the following experiments.

**Effect of type and volume of disperser solvent**

Generally, disperser solvent was also investigated because it can improve the emulsification phenomenon in DLLME, leading to enhance the extraction recovery. In the study,
EtOH, MeOH, and ACN were investigated in comparison with the case of not using disperser solvent. As can be seen in Figure 3, the microextraction without disperser solvent gave comparable results to those with the use of disperser solvents, e.g., ACN and EtOH. Meanwhile, MeOH provided the lowest extraction recovery of the studied fungicides. Thus, disperser solvents were not required in this study. It also indicated that the microextraction (emulsification) using methyl salicylate as extraction solvent without any disperser solvent could be easily carried out for the extraction and preconcentration of target fungicide compounds.

Effect of salt type and its concentration

To improve the extraction recovery of target organic compounds, the addition of suitable salt is one effective way and easy to perform. For the preliminary investigation, Na₂SO₄, Na₂CO₃, and CH₃COONa were studied at the chosen appropriate amount of 3.0% (m/v) by trial and error method. It was found (Figure 4) that the presence of salts can lead to enhance the extraction recovery for almost target compounds in comparison to without salt addition. Based on the results obtained (Figure 4), the effective order of the studied salt for salting out was CH₃COONa > Na₂CO₃ > Na₂SO₄ >> without salt added. Thus, effect of CH₃COONa contents in the range of 0-5% (m/v) was then chosen to examine. According to results in Figure 5, it is clearly seen that extraction recovery of the studied target compounds rapidly increased until the addition of salt content up to 1.0% (m/v) and further increased up to 5.0% (m/v). A proper amount of salt added in aqueous solution alters the hydrophobicity of target fungicides and facilitated the target analytes transfer into the extraction solvent phase, while high excess salt content causes viscosity of solution increased and leads to inhibit the mass transfer of target analytes into the extraction solvent-rich phase. Therefore, sodium acetate of 1.0% (m/v) was selected in this experiment.

Figure 2. The effect of volume of methyl salicylate on the extraction of target benzimidazole fungicides (250 μg L⁻¹). Conditions: 3.0% (m/v) sodium acetate and centrifugation at 3500 rpm for 3 min.

Figure 3. The effect of type of disperser solvents. Conditions: 250 μg L⁻¹ fungicide each, 200 μL disperser, 250 μL methyl salicylate, 3% (m/v) sodium acetate, and centrifuged at 3500 rpm for 3 min.

Figure 4. The influence of salt type on the extraction efficiency of benzimidazole fungicide (250 μg L⁻¹ each). Conditions: 250 μL methyl salicylate, 3.0% (m/v) salt added, and centrifuged at 3500 rpm for 3 min.

Effect of centrifugation

Centrifugation was also investigated in this study. The suitable time and speed used for the centrifugation can reduce time to separate the phase completely. It was found (Figure S1 of the Supplementary Information) that the centrifugation speeds (0-3500 rpm) did not affect the extraction recovery. However, speeds used lower than 2500 rpm could not help to separate the phase completely. Meanwhile, the extraction recovery decreased when the
centrifugation speed used was higher than 3500 rpm (i.e., 4000 rpm). Therefore, the centrifugation speed at 3500 rpm was chosen. Centrifugation times (0-10 min) at 3500 rpm was then evaluated. It was found (Figure S2 of the Supplementary Information) that the highest extraction recovery of the studied fungicides was found at 3 min. Prolonged centrifugation times reduced the extraction recovery of the compounds. Thus, the centrifugation at 3500 rpm for 3 min was used throughout the experiments.

Analytical performance and method validation

The performance of the proposed method was evaluated by the study of linearity, limits of detection (LOD), limits of quantitation (LOQ), preconcentration factor (PF), extraction recovery (ER), and precision (intra-day and inter-day). These evaluations were done in deionized water medium. Calibration graphs were plotted using different concentrations against peak area of analyzed target compounds. The results were summarized in Table 1. Linearity was obtained in the range of 0.20-200 μg L\(^{-1}\), with the coefficient of determination (R\(^2\)) greater than 0.997. LOD were determined base on the concentration providing signal-to-noise of 3 (S/N = 3), while LOQs were defined as S/N = 10. The LODs ranged between 0.03 and 0.05 μg L\(^{-1}\), whereas the without preconcentration gave LODs of 5 μg L\(^{-1}\). The LOQs were obtained in the range of 0.20-0.50 μg L\(^{-1}\).

Intra-day precision (n = 6) and inter-day precision (n = 6 × 3 days) were evaluated by the replicate injections of spiking deionized waters at concentration of 100 μg L\(^{-1}\) each, expressed in terms of relative standard deviations (RSD) of retention time (t\(_R\)) and peak area. It was found (Table 1) that a good RSD was below 1.9 and 6.3% for t\(_R\) and peak area, respectively. In addition, the RSDs of concentration calculated were lower than 3.2% for intra-day and inter-day precisions.

From the evaluation, the PF and ER of the analytes under the optimum condition were obtained in the range of 24-38 and 54-85%, respectively.

Application to real water samples

The proposed method was applied to extract and analyze target fungicides in different sources of field water samples (assigned to sample #1 to sample #4). It was found (Table 2) that the analyzed water samples were free of target fungicides contamination. Validation of the method was modified based on SANCO guideline. Accuracy in terms of recovery of the target compounds in real water sample matrices was investigated by spiking of known different concentrations (5, 10, and 50 μg L\(^{-1}\) before extraction and analysis. According to the results in Table 2, it shows that the average recoveries of all studied fungicides in water samples were in the range of 74.1-118.4% with good RSDs of less than 11%. The obtained recovery and RSDs (%) were in good agreement with the acceptable range of 70-120% and RSDs < 20%, respectively, at the evaluated concentrations in the range of 10-100 μg L\(^{-1}\). In addition, it was also found that the different physicochemical properties (e.g., pH and conductivity) of the studied samples have less effect in the analysis. Repeatability (n = 6) and intermediate precision (n = 3 × 3 days) of the method was also evaluated in spiked water sample #2 (as a representative sample) at the concentration of 10 μg L\(^{-1}\) each. The obtained relative recoveries were in the range of 97.8-104.1% (repeatability)

![Figure 5. Effect of sodium acetate on the extraction efficiency of benzimidazole fungicides (250 μg L\(^{-1}\) each). Other conditions are as the same as in Figure 4.](image)

### Table 1. Analytical characteristics of the proposed method for the determination of the benzimidazole fungicides

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity / (μg L(^{-1}))</th>
<th>Linear equation</th>
<th>R(^2)</th>
<th>LOD / (μg L(^{-1}))</th>
<th>LOQ / (μg L(^{-1}))</th>
<th>Precision(^a) / %</th>
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<tr>
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<td></td>
<td></td>
<td>intra-day (n = 6)</td>
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<td></td>
<td>Inter-day (n = 3 days)</td>
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<td></td>
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<td></td>
<td></td>
<td>t(_R) / min</td>
<td>Peak area</td>
<td>t(_R) / min</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peak area</td>
</tr>
<tr>
<td>CBZ</td>
<td>0.50-200</td>
<td>y = 521.2x + 311.8</td>
<td>0.9974</td>
<td>0.05</td>
<td>0.50</td>
<td>1.3</td>
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<td>1.6</td>
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<td>1.9</td>
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<td>6.3</td>
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<tr>
<td>TBZ</td>
<td>0.20-200</td>
<td>y = 3215.0x - 974.8</td>
<td>0.9996</td>
<td>0.03</td>
<td>0.20</td>
<td>1.4</td>
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<td></td>
<td></td>
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<td>2.8</td>
</tr>
<tr>
<td>FuBZ</td>
<td>0.20-200</td>
<td>y = 2161.9x + 71.6</td>
<td>0.9999</td>
<td>0.03</td>
<td>0.20</td>
<td>1.3</td>
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<td>2.9</td>
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<td>2.2</td>
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<td>5.4</td>
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</table>

\(^a\)Precisions (%RSD) were evaluated at the concentration of 100 μg L\(^{-1}\) each; t\(_R\): retention time.
and 100.2-101.8% (intermediate precision) with RSDs lower than 10.1%. It indicated that the developed method was effective and reliable for monitoring target fungicide contaminants in real water sample matrices. Typical chromatograms obtained applying the proposed method to analyze water samples is demonstrated in Figure 6. It is clearly seen that no interference peaks at the retention times of target analytes were observed.

**Evaluation of the proposed method compared to other strategies**

Table 3 presents the reported linearity, LOD, and recovery obtained from the other related works for the analysis in water sample matrices such as micellar extraction, SPME, MISPE, conventional DLLME, and SALLE. It is found that the proposed method gave comparable analytical results of linearity and recovery with those of other reported methods. LODs are comparable with those obtained from SPME and MISPE methods and seem to be better than those of micellar extraction, conventional DLLME, and SALLE. In comparison to the previous our work with vortex-assisted liquid-liquid microextraction (VA-DLLME) using methyl benzoate as extraction solvent, the proposed method provided comparable results. Although the LOD for TBZ and FuBZ were higher, the main different point of the method was not required disperser solvent. The developed method could improve the sensitivity than the method without preconcentration. The developed method is superior to other reported methods in the points of simplicity, short extraction time, short analysis time, and environmentally friendly. It also reveals that the developed method is powerful, effective, reliable, and can be used as an alternative technique for trace analysis of fungicides in water samples.

**Conclusions**

In this work, a simple miniaturized method using methyl salicylate-based-DLLME followed by HPLC analysis has been successfully developed for the determination of benzimidazole fungicides in environmental water samples. The target compounds were simply extracted by methyl salicylate (ca. 250 μL) without disperser, which is
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less toxic than the solvents normally used in the typical conventionally DLLME. Under the optimized conditions, LODs were obtained in the range of 0.03-0.05 μg L⁻¹. Good recovery obtained in the acceptable range (i.e., 70-120%) with RSDs < 10% demonstrated that the developed method is capable to determine target fungicides in real water samples with adequate accuracy and high reproducibility. It can be also concluded that methyl salicylate is feasible to be used as an alternative extraction solvent. To extend the capable of methyl salicylate, more investigation into other different analytes as well as complicated sample matrices (e.g., food samples) will be further applied.

**Supplementary Information**

Supplementary data (Figures S1 and S2) are available free of charge at http://jbcs.sbq.org.br as PDF file.

**Acknowledgements**

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**References**


**Table 3. Comparison of the proposed method with other techniques used for the determination of benzimidazole fungicides in water sample matrices**

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Analysis technique</th>
<th>LOD (μg L⁻¹)</th>
<th>Recovery (%)</th>
<th>Extraction condition and comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPME</td>
<td>HPLC-FL</td>
<td>0.03-1.30</td>
<td>80.9-119.6</td>
<td>carboxen-polydimethylsiloxane (expensive fiber) long incubated at 60 °C for 40 min desorbed by MeOH for 10 min required high NaCl (15% m/v) and cleaning procedure for removing high salt was needed</td>
<td>7</td>
</tr>
<tr>
<td>Micellar extraction</td>
<td>HPLC-FL</td>
<td>0.004-6.4</td>
<td>72.0-92.0</td>
<td>genapol-X-080 or POLE (4% v/v) + 4% m/v NaCl and incubated at 90 °C for 20 min long extraction time and required high temperature</td>
<td>30</td>
</tr>
<tr>
<td>Conventional DLLME</td>
<td>HPLC-FL</td>
<td>0.5-1.0</td>
<td>84.0-94.0</td>
<td>chloroform + tetrahydrofuran + 10% m/v NaCl used very toxic chlorinated solvent chloroform needed disperser solvent</td>
<td>21</td>
</tr>
<tr>
<td>MISPE</td>
<td>HPLC-PDA</td>
<td>0.002-0.012</td>
<td>90.0-106.0</td>
<td>50:50 v/v MeOH/acetic acid (eluent) required long synthesis of molecularly imprinted polymer-divinylbenzene (sorbent) tedious procedure</td>
<td>9</td>
</tr>
<tr>
<td>SALLE</td>
<td>HPLC-UV</td>
<td>0.14-0.38</td>
<td>60.4-99.1</td>
<td>2 mL ACN + 0.1 mol L⁻¹ NaH₂PO₄ + 5.0 mol L⁻¹ NaCl difficult to withdraw the upper rich phase and remove aqueous lower phase</td>
<td>15</td>
</tr>
<tr>
<td>VA-DLLME</td>
<td>HPLC-PDA</td>
<td>0.01-0.05</td>
<td>77.4-110.9</td>
<td>250 μL methyl benzoate + 300 μL EtOH + NaOAC (1.0% m/v) required disperser solvent and salt to improve the extraction efficiency</td>
<td>5</td>
</tr>
<tr>
<td>Proposed DLLME</td>
<td>HPLC-PDA</td>
<td>0.03-0.05</td>
<td>74.1-118.4</td>
<td>250 μL methyl salicylate + NaOAC (1.0% m/v) not required disperser solvent Proposed method</td>
<td></td>
</tr>
</tbody>
</table>