Determination of benzimidazoles in fruits by open-tubular capillary electrochromatography based on ionic liquids grafted covalent organic frameworks

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Abstract
A novel capillary electrochromatography method has been developed for the simultaneous quantification of ten benzimidazole fungicides in fruits. Herein, covalent organic frameworks (COFs) and ionic liquids (ILs) were successfully introduced to prepare open-tubular capillary column to improve the loading capacity and separation performance. The parameters effecting the analytical performance including pH and concentration of running buffer, separation voltage and the addition of organic solvent were investigated systematically. Under the optimized conditions, the method allowed the baseline separation of ten benzimidazole fungicides, and showed a good linearity in the range of 3.5–200 μg kg⁻¹ with the detection limits between 1.0 and 2.8 μg kg⁻¹. The intraday and interday precisions for recoveries were lower than 7.9% and 12.2%, respectively. Intraday and interday precisions for their retention times were lower than 3.2% and 6.6%, respectively. Satisfactory recoveries for grape, pear and orange samples at two concentrations were obtained ranging from 85.0 to 95.9% with RSDs lower than 7.8%, demonstrating the potential applications of the open-tubular capillary electrochromatography method for trace benzimidazole fungicides analysis in fruits.

Keywords Benzimidazoles • Ionic liquids • Covalent organic framework • Open-tubular capillary electrochromatography

Introduction
Benzimidazoles (BZs) are broad-spectrum pesticides commonly used in agriculture for the prevention and treatment of parasitic and fungal infections [1, 2]. Despite many benefits to the public from the use of BZs, their large-scale use has caused some health alarms in the past few years. It is known that BZs have negative effects on the human health such as congenic malformations, teratogenicity, mutagenicity, and embryotoxicity [3–6]. European Union (EU) have recommended a maximum residue limits (MRLs) for many BZs in a variety of foods [1, 7]. Thus, the development of effective and sensitive detection methods is a key issue.

Currently, several analytical methods based on spectrophotometry, high-performance liquid chromatography (HPLC) coupled with UV/DAD detector and tandem mass (LC–Ms/Ms) have been developed for the determination of BZs [8–10]. Besides, capillary electrophoresis (CE) with several advantages including low solvent consumption and sample volume, high separation efficiency and rapid analysis [5] is an alternative method [11–13]. Open-tubular capillary electrochromatography (OT-CEC), as an alternative mode of CEC which is a hybrid technique combining the advantages of HPLC and CE, has attracted much attention because of its advantages, including easy preparation, good permeability, simple surface modification [14, 15]. However, OT-CEC suffers from the problems of low sample capacity and phase ratio. To overcome these limitations, various novel materials including gold nanoparticles [16], Fe₃O₄ nanoparticles [17], carbon nanotubes [18], graphene oxide [19] and metal–organic frameworks (MOFs) [20] have been used to prepare the open-tubular capillary column via physical coating and chemical bonding technologies.

Covalent organic frameworks (COFs), an exciting class of crystalline porous materials constructed from organic building units via covalent bonds [21], exhibit excellent characteristics such as extremely high surface area, tunable pore size, remarkably low density and good thermostability [15,
to the determination of trace BZs in fruit matrices. In addition, it has been always attracting much interest to evaluate the impact of the COFs on chromatographic separation. Yang et al. have successfully fabricated a spherical COF from 1,3,5-triformylphloroglucinol (Tp) and benzidine (BD) for baseline separation of various important industrial analytes by capillary gas chromatography [22]. The chiral COFs have been synthesized by Qian et al. via introducing chiral centers into organic ligands, and high-resolution chiral-bonded capillary columns were successfully prepared for enantiomeric resolution [21]. Niu et al. have used COF-LZU1 synthesized from benzene-1,3,5-tricarbaldehyde and benzene-1,4-diamine in OT-CEC for baseline separation of model analytes including polyaromatic hydrocarbons, alklylenzines and anilines based on the size selectivity of COF-LZU1 porous structure and hydrophobic interactions [15]. These results indicate that COFs effectively improve the chromatographic separation performance and load capacity for small organic molecules. Nevertheless, the lack of proper reaction sites of COFs with analytes limits their applications in chromatographic separation field.

Ionic liquids (ILs) have recently attracted more and more interests due to their superior properties such as non-volatility, good thermal stability, non-flammability and high electrolytic conductivity [27, 28]. In particular, some “task-specific” ILs with alkyl groups, amino groups, vinyl groups, hydroxyl groups and carboxyl groups endow them with versatile abilities to interact with other molecules through hydrogen bonds, hydrophobic/hydrophilic interactions, π–π stacking, ion exchange and electrostatic interactions [29]. In the past few years, we have developed a series of ILs-based capillary electrochromatographic columns with good separation performance for the separation of protein macromolecules, demonstrating the potential of ILs in chromatographic separation [29–32].

In this study, we combined the superior performances of ILs and COFs, a novel OT-capillary column (ILs@COFs coated capillary) was prepared. Using the resulting OT-capillary as separation column, a OT-CEC method for the sensitive and accurate determination of BZs in fruits was established for the first time and further applied successfully to the determination of trace BZs in fruit matrices.

### Experimental

#### Chemicals

Aminopropyltrimethoxysilane (APTES), N,N'-dicyclohexylcarbodiimide (DCC) and glutaraldehyde were purchased from Sigma (St. Louis, MO, USA).

2,4,6-Trihydroxy-benzene-1,3,5-tricarbalddehyde (Tp), benzidine (BD), 1-aminopropyl-3-methylimidazole bromine (APMim“Br”), benomyl (BEN), carbendazim (MBC), thiabendazole (TBZ), 5-hydroxy thiabendazole (5-OH-TBZ), albendazole (ABZ), thiofanate-methyl (TPM), fuberidazole (FBZ), thiofanate (TP), cypendazole (CPZ) and 2-aminothiazole (2AB) were purchased from Aladdin’s reagent network (Shanghai, China). The chemical structures of these BZs are shown in Fig. 1. Anhydrous ethanol, methanol (MeOH), dimethyl sulfoxide (DMSO), acetonitrile (ACN), N,N-dimethylformamide (DMF), disodium hydrogen phosphate (Na2HPO4), citric acid (Cit), magnesium sulfate (MgSO4), sodium chloride (NaCl) and chloroform (CHCl3) of at least analytical grade were purchased from Concord Technology (Tianjin, China). The foodstuffs used in this study were purchased from local market. Doubly deionized water (DDW, > 18 MΩ cm) used throughout the work was supplied by a Milli-Q system (Millipore Corporation, USA). Other chemicals were at least of analytical grade.

#### Apparatus

Scanning electron microscopy images of the OT-capillary were obtained on a SU-1510 SEM (Hitachi, Japan). Vario MACRO cube (Elementar, German) was used to determine the contents of the elementals (C, O, N and H) in the prepared material. Nitrogen surface area analyzer (TriStar 3000, Micromeritics, Georgia, USA) was used to carry out adsorption/desorption analyses. F-120 ultrasonic cleaner purchased from Shenzhen Fuyang Technology Group Co., Ltd. was used to prepare the ILs@COFs coated capillary. The chromatographic performance of the resulting capillary column was evaluated on the P/ACE MDQ CE system (Beckman-Coulter, California, USA) with UV detector.

#### Preparation of ILs@COFs coated capillary column

The fused-silica capillary (150 μm i.d., 375 μm o.d.) was pretreated according to our previous report [30]. In detail, rinse sequentially with 1 mol L⁻¹ NaOH for 12 h, H2O for 0.5 h, 1 mol L⁻¹ HCl for 12 h, H2O for another 0.5 h and MeOH for 0.5 h to activate silanol groups. Then, the resulting capillary was further amino-modified with methanol solution of APTES (50%, v/v) at 40°C for 12 h, and dried by nitrogen gas.

The ILs@COFs coated capillary column was prepared as follows: 0.015 mmol Tp, 0.005 mmol BD, and 0.01 mmol APMim+Br⁻ were dissolved in 2 mL ethanol and 8 mL DMF and further sonicated for 3 min to obtain a homogeneous solution. Then the resulting mixture was injected manually into the APTES-modified capillary above to an appropriate length with a syringe. After both
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ends were sealed, the capillary along with pre-polymerization mixture was incubated under ultrasonication (40 W) for 6 h. The process was schematically illustrated in Fig. 2. To remove unreacted monomers and solvent, the resulting capillary was rinsed with MeOH. The resulting capillary with total length of 45 cm and effective length of 35 cm was tested under CEC mode.
For comparison, ILs coated capillary column were prepared in the same way, except for replacing 0.15 mmol Tp and 0.05 mmol BD with 0.2 mmol glutaraldehyde.

In order to systematically characterize the coating material ILs@COFs, 10 mL glass centrifuge tube was used as the reaction vessel instead of the fused-silica capillary via the same fabrication process.

**CEC conditions**

The resulting capillary column was first conditioned by running buffer for 0.5 h with a manual syringe, equilibrated at −5 kV until a steady electric current was obtained. Separations were performed at 25 °C using different voltages and running buffers. The samples were hydrodynamically injected for 5 s at 0.5 psi. All running buffers and samples should be sonicated and filtered with 0.45 μm syringe filters before use.

**Preparation of standards and samples**

Standard stock solution containing 250 μg mL⁻¹ of each analyte was confected with DMSO-ACN (2:3, v/v) and stored at −20 °C in the dark being stable for 6 months. Working standard solutions were freshly confected by the dilution of the standard stock solution with running buffer.

Pear, grape and orange were purchased from local supermarket. The sample was treated according to the previous report [5] as follows: representative homogenized sample of 1.0 g was placed in 15 mL centrifuge tube. After adding 2 mL of ACN, the mixture was agitated with swirler for 30 s. For ease of separation, 0.1 g of NaCl and 0.5 g of MgSO₄ were added. After vortexing for 5 min and centrifuging at 9000 rpm for 10 min, 1.7 mL supernatant was transferred into a 10 mL centrifuge tube. A mixture of 5 mL deionized water and 1 mL of CHCl₃ was rapidly added into the sample tube for dispersive liquid–liquid microextraction. The ternary system was vigorously shaken for 1 min and centrifuged for 5 min at 5000 rpm. The CHCl₃ phase was collected and dried under a stream of nitrogen gas. Finally, the residue was redissolve with 0.5 mL of running buffer and filtered before injection into the CEC system.

**Results and discussion**

**Characterization of ILs@COFs coated capillary column**

The morphologies of inner wall of ILs@COFs coated capillary and bare capillary were investigated by SEM. As can be seen in Fig. 3A(a), the inner walls of the bare capillary are quite smooth, while a layer of COFs on the inner surface of...
the ILs@COFs coated capillary can be seen in Fig. 3A(b). For further demonstrating the successful synthesis of ILs@COFs, the coating material synthesized in glass centrifuge tube was analyzed using XPS. The XPS spectrum (Fig. 3B) shows the presence of Br (68 eV), C (287 eV), N (400 eV) and O (532 eV) demonstrating the successful synthesis of ILs@COFs.

The specific surface area and average pore size of the coating material ILs@COFs were measured by nitrogen adsorption–desorption experiment. As shown in Fig. 3C, the specific surface area was determined to be 384 $\text{m}^2 \text{g}^{-1}$, and the pore size was 2.1 nm.

Electroosmotic flow

The electroosmotic flow (EOF), as driving power for EC, is a valid indicator for the evaluation of the prepared OT-capillary column, which was calculated using thiourea as EOF marker via the formula:

$$\mu_{\text{EOF}} = \frac{L_t - L_e}{V_{\text{EOF}}}$$

where $L_t$, $L_e$, $V$ and $t_{\text{EOF}}$ are the total length and effective length (cm) of the capillary, the separation voltage ($V$) and the retention time (s), respectively. Herein, the relationships between the EOF of the bare and coated capillaries and the pH of running buffer were investigated. Figure 4 shows that a pH-dependent EOF from anode to cathode for the bare capillary was obtained with pH ranging from 2 to 10. However, after coating with ILs and ILs@COFs, strong reversed EOFs (greater than $-4.1 \times 10^{-4} \text{cm}^2 \text{v}^{-1} \text{s}^{-1}$ for ILs coated capillary and $-4.7 \times 10^{-4} \text{cm}^2 \text{v}^{-1} \text{s}^{-1}$ for ILs@COFs coated capillary) were obtained, indicating that the inner wall was positively charged. Note that the EOFs for ILs and ILs@COFs coated capillaries reduced with pH increasing, which may be due to the ionization of the phenol hydroxyl groups and silanol groups. Besides, the EOF for ILs@COFs coated capillary was greater than that for ILs coated capillary. It may be due to the fact that the large specific surface area of the COFs gives rise to high grafting quantity of ILs onto the capillary.

In conclusion, these results suggest that both ILs and ILs@COFs coated capillaries were successfully fabricated.

Loading capacity

The loading capacity can be defined as the injection amount when the corresponding peak width at half-height ($w_{1/2}$) increased by 10% compared to the peak width at low injection amount [15, 33]. Thus, a series of 2-aminobenzimidazole standard solutions with different known concentrations (0.05–1 mg mL$^{-1}$) were injected and analyzed. As shown in Fig. 5, the $W_{1/2}$ of 2-aminobenzimidazole at 0.9 mg mL$^{-1}$ was 10% higher than that at 0.05 mg mL$^{-1}$. Thus, the loading capacity of the ILs@COFs coated capillary column for 2-aminobenzimidazole was 0.8 mg mL$^{-1}$, which was improved greatly comparing with that of the ILs coated capillary (0.1 mg mL$^{-1}$) as a result of the high surface area of COFs.

CEC separation

Several parameters affecting separation performance of the OT-CEC method were systematically investigated including pH and concentration of running buffer, separation voltage and organic solvent addition.

For BZs with two $pK_a$ values (pKa$_1$3–6 and pKa$_2$10–12) corresponding to two amino groups, they can be protonated or deprotonated at different pH, which implies that greater different electrophoretic mobilities can be obtained by varying pH of the running buffer [34]. Considering the ionization of the silanol groups and phenol hydroxyl groups at
Fig. 6 Optimization of the running buffer. A pH of Cit-Na₂HPO₄ (40 mM): pH 2.2 (a); pH 2.6 (b); pH 3.0 (c). B Concentration of Cit-Na₂HPO₄ (pH 2.6): 30 mM (a); 40 mM (b); 50 mM (c). C Mobile phase composition (ACN/Cit-Na₂HPO₄ (v/v), 40 mM, pH 2.6): 10% (a); 20% (b); 30% (c). Other CEC conditions are as in Fig. 5. Peak identification: 1, ABI; 2, 5-OH-TBZ; 3, TBZ; 4, MBC; 5, BEN; 6, ABZ; 7, FBZ; 8, TPM; 9, TP; 10, CPZ
The concentration of Cit-Na₂HPO₄ running buffer was investigated in the range of 30–50 mM at pH 2.6. It can be seen in Fig. 6B that baseline separation of these analytes could be achieved with a concentration of 40 mM. With the concentration above 40 mM, peak shapes of analytes were not improved, while their retention times prolonged. Thus, 40 mM Cit-Na₂HPO₄ was chosen.

As well as we known, the addition of organic solvent to the running buffer contributes to separate non-polar substances. Herein, the effect of ACN addition (v/v) in the running buffer on the separation performance was investigated. As shown in Fig. 6C, baseline separation can be realized
with ACN content reaching 10%(v/v). With ACN content increasing to 20%, their retention times decreased dramatically. However, with ACN content more than 20%, lower resolution of BZs was obtained. Therefore, 20% ACN was added in running buffer. These results indicate that hydrophobic interaction contributes to baseline separation of these BZs.

The effect of separation voltage on the chromatographic performance was also optimized from 15 to 25 kV, in which the increase of separation voltages led to decreased retention time but poor resolution with joule heat increasing. As a compromise between retention time and peak resolution, 20 kV was chosen.

For comparison, bare capillary, ILs coated capillary and ILs@COFs coated capillary were also evaluated by the ten BZs. As shown in Fig. 7, neither the bare capillary nor the ILs coated capillary could baseline separate these BZs; however, ILs@COFs coated capillary could baseline separate them in 19 min, demonstrating that both ILs and COFs improved the chromatographic performance of the prepared ILs@COFs coated capillary column.

### Analytical performance

The applicability of the proposed OT-CEC analysis method for the quantification of ten BZs in grape, pear and orange samples was evaluated under the optimized conditions. The characteristics of the method including detection limits (LODs), linearity, quantification limits (LOQs), precision and recoveries were assessed.

Considering the matrix interference, matrix-matched calibration curves were performed by employing pear sample as a representative matrix, in which ten BZs at five different concentration levels (5, 20, 50, 100 and 200 μg kg⁻¹) were spiked. The determination of each concentration level was composed of two experimental and two instrumental repetitions (n=4). A blank sample was also analyzed, and no BZs were found. As shown in Table 1, calibration curves of the ten BZs using peak area showed good linearity of 3.5–200 μg kg⁻¹ with the regression coefficients ($R^2$) between 0.9942 and 0.9993.

LODs and LOQs of the OT-CEC method were calculated as the concentrations corresponding to signals which were 3 and 10 times the intensity of the signal-to-noise ratio, respectively. As shown in Table 1, the LODs and LOQs for the ten BZs in pear sample were 1.0–2.8 μg kg⁻¹ and 3.5–9.4 μg kg⁻¹, respectively, which are lower than the regulated MRL values of (EC) No 396/2005 demonstrating the potential of the proposed method for monitoring the BZs in fruits. Electropherograms corresponding to determination of the ten BZs in pear, orange and grape samples are shown in Fig. 8.

### Table 1 Analytical performance of the proposed method for pear sample

| Analyte | MRL (mg kg⁻¹) | LOD (μg kg⁻¹) | LOQ (μg kg⁻¹) | Linear dynamic range (μg kg⁻¹) | $R^2$ |
|---------|---------------|---------------|---------------|-------------------------------|-------|
| ABI     | Non-established | 1.3           | 4.3           | 4.3–200                       | 0.9942|
| 5-OH-TBZ | 5             | 1.5           | 4.8           | 4.8–200                       | 0.9976|
| TBZ     | 5             | 2.7           | 9.1           | 9.1–200                       | 0.9987|
| MBC     | 0.1           | 1.0           | 3.5           | 3.5–200                       | 0.9970|
| BEN     | 0.1           | 1.4           | 4.7           | 4.7–200                       | 0.9981|
| ABZ     | Non-established | 2.1           | 7.1           | 7.1–200                       | 0.9993|
| FBZ     | 5             | 2.6           | 8.6           | 8.6–200                       | 0.9992|
| TPM     | 0.05          | 2.6           | 8.5           | 8.5–200                       | 0.9981|
| TP      | Non-established | 2.6           | 8.7           | 8.7–200                       | 0.9979|
| CPZ     | Non-established | 2.8           | 9.4           | 9.4–200                       | 0.9977|

### Table 2 The method precisions (RSDs, n=3) for different concentrations of the ten BZs in pear samples

| Analyte   | Recovery (%) | Retention time (%) |
|-----------|--------------|--------------------|
|           | Intraday     | Interday           | Intraday     | Interday |
|           | 20 (μg/kg)   | 100 (μg/kg)       | 20 (μg/kg)  | 100 (μg/kg) |
| ABI       | 6.3          | 7.4                | 7.9          | 6.2       | 3.2       | 5.5        |
| 5-OH-TBZ  | 7.1          | 4.3                | 12.1         | 4.1       | 3.1       | 5.0        |
| ABZ-NH₂-SO₂| 7.7          | 3.9                | 7.6          | 6.5       | 2.3       | 5.1        |
| TBZ       | 9            | 4.7                | 11.5         | 4.8       | 2.5       | 5.5        |
| MBC       | 7.8          | 4.9                | 9.4          | 6.3       | 3.0       | 6.6        |
| ABZ       | 6.3          | 5.1                | 7.2          | 5.0       | 2.5       | 5.8        |
| MBZ       | 2.5          | 4.2                | 3.0          | 7.0       | 3.1       | 6.4        |
| FBZ       | 7.2          | 5.7                | 9.3          | 6.3       | 2.1       | 5.1        |
| OFZ       | 7.6          | 7.9                | 12.1         | 9.4       | 2.6       | 6.4        |
| FBT       | 7.7          | 6.8                | 12.2         | 9.0       | 3.2       | 5.0        |
In addition, the precisions of the OT-CEC method were evaluated according to intraday and interday precisions by applying the proposed method to determining the BZs at two concentration levels (20 and 100 μg kg\(^{-1}\)) in pear samples. Intraday precisions were evaluated by duplicate injections of three samples on the same day. Interday precisions were evaluated by duplicate injections of two samples for 3 continuous days. As can be seen in Table 2, satisfactory recoveries were achieved in all cases with RSDs less than 12.2%. Intraday and interday precisions for their retention times were calculated as 2.1–3.2% and 5.0–6.6%, respectively.

### Applications to food samples

The proposed OT-CEC method was subsequently applied to determine the ten BZs in grape, pear and orange samples, which were verified to not contain BZs. The analytical performance was assessed by spiking matrix samples with the target analytes at two different concentration levels (10 and 50 μg L\(^{-1}\)). As shown in Table 3, the satisfactory recoveries of the ten BZs ranging from 85.0 to 95.9% with RSDs (\(n = 3\)) less than 7.8% in three matrices were obtained. The results demonstrate that the proposed method is reliable for the quantification of the BZs in real samples.

### Comparison with other analysis methods

The comparison with other analysis methods is made in Table 4. The proposed method can simultaneously detect ten BZs, which is more than what were detected in DLLME-CEC-UV [34]. The LODs and LOQs of the proposed method for BZs were lower than those obtained by other methods including DLLME-CEC-UV [34], CE-UV [12], CE-ESI–MS [35] and DLLME-CZE-MS/MS [5]. The retention time of the proposed method is shorter than CE-UV [12], CE-ESI–MS [35] and DLLME-CZE-MS/MS [5]. Although the species of the BZs is less than that obtained by DLLME-CZE-MS/MS [5], the cost for this method is much lower.

### Conclusions

A novel OT-CEC method was successfully established for the first time to simultaneously detect ten BZs in grape, pear and orange samples. The introduction of COFs and ILs greatly improved the loading capacity and separation performance of capillary. Under the optimized conditions, the proposed method could baseline separate ten BZs in 19 min. Good linearity, precision and high sensitivity, satisfactory recoveries were obtained, showing the potential of the proposed method for monitoring of the ten BZs in fruits at trace level.
Table 4 Comparison of the proposed method with others for the determination of BZs

| Method                | DLLME-CEC-UV | CE-UV | CE–ESI–MS | DLLME-CZE-MS/MS | CEC-UV |
|-----------------------|--------------|-------|-----------|------------------|--------|
| Number of BZs         | 7            | 10    | 10        | 10               | 10     |
| Real samples          | Fish farm water | Swine muscle | Egg | Poultry muscle | Pear |
| LODs (μg kg⁻¹)        | 1.7–2.8      | 1.05–10.42 | 3–51 | 1–4               | 1.0–2.8 |
| LOQs (μg kg⁻¹)        | 5.7–9.3      | 3.49–34.72 | 50   | 4–16              | 3.5–9.4 |
| Linear range (μg kg⁻¹) | 5.7–100  | 50–2000 | 50–400 | 4–500          | 3.5–200 |
| Retention time (min)  | 15           | 27    | 52        | 32.0             | 19     |
| Ref                   | [34]         | [12]  | [35]      | [5]              | This work |

Author contributions CL: conceptualization and writing original draft. BZ: optimization of chromatographic separation, method characterization, and writing original draft. XL: preparation of ILs@COFs coated capillary column, optimization of chromatographic conditions, and writing original draft. AZ: preparation of standards and samples, and writing original draft.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare there are no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Informed consent Not applicable.

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