Development of a Quinazoline-Based Chelating Ligand for Zinc Ion and Its Application to Validation of a Zinc-Ion-Coordinated Compound

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A novel fluorescent chelating ligand, 2,4-[bis-(2-hydroxy-3-methoxybenzylidene)]-dihydrazinoquinazoline (HBQZ), was synthesized, and the fluorescence characteristics of its complex with metal ions were investigated. Among the 36 different metal ions tested in this study, it was found that HBQZ emits intense fluorescence at 506 nm with an excitation wavelength of 414 nm in the presence of Zn^{2+} . The fluorescence intensity was almost constant in the pH range 3.5–10.5. Complexes of other metal ions with HBQZ did not show fluorescence, and the detection limit of Zn^{2+} was approximately 250 nM (16 ppb). The proposed method was applied to the validation test of a bioactive compound containing Zn^{2+} in its structure—an antibacterial and antifungal reagent, zinc pyrithione (ZnPT). In order to effectively release Zn^{2+} from ZnPT, a pretreatment procedure involving heating with H_3PO_4 at 100 °C for 60 min was adopted. Under these conditions, a linear calibration curve was obtained in the ZnPT concentration range of 0.79–15.7 μM (0.25–5.0 ppm); the correlation coefficient and the relative standard deviation were 0.996 and within 3.1% ($n=5$), respectively.

Key words metal-ion complex; quinazoline; fluorescence; zinc ion; zinc pyrithione

Metal ions can act as a Lewis acid, and due to this property, they have been artificially incorporated into drugs, pigments, or dietary supplements^{1–5)} in order to gain the intrinsic activity. Metal-ion-coordinated compounds are generally validated using atomic absorption spectrometry (AAS), because metal ions can be selectively quantified by AAS. However, a functionalized ligand that can chelate a metal ion selectively to emit fluorescence can also be used for such validation.

Recently, we developed a fluorescent chelating ligand, 2,4-[bis-(2,4-dihydroxybenzylidene)]-dihydrazinoquinazoline (DBHQ), which can selectively chelate with Ga^{3+} ions to emit fluorescence at 470 nm with an excitation wavelength of 405 nm.⁶⁾ The basic structure of DBHQ is a quinazoline skeleton with two Schiff base moieties that serve as chelating sites.

In DBHQ, two 2,4-dihydroxyphenyl groups are attached to the quinazoline moiety *via* Schiff bases, which are considered to play a role in chelation with Ga^{3+} . This moiety may be suitable for identifying the ionized atomic structure of Ga^{3+} . On the other hand, an *o*-vanillin moiety has recently been incorporated into fluorescent chelating ligands such as *N*-*o*-vanillidine-2-amino-*p*-cresol (OVAC),⁷⁾ *o*-vanillin furfuralhydrazone (OVFH),⁸⁾ and *o*-vanillin-8-aminoquinoline,⁹⁾ which can chelate Al^{3+} , Ga^{3+} , and Cr^{3+} , respectively. The benzaldehyde moiety of *o*-vanillin can be used to produce a Schiff base *via* a coupling reaction with an amino group, and the lone pair of electrons on the oxygen atom of the phenolic hydroxyl group may act as a Lewis base for chelation with metal ions. Similarly, *o*-vanillin has been used as a building block for functionalized chelating ligands.

In this study, we designed and synthesized a previously developed quinazoline-based fluorescent ligand as a structural analog of DBHQ. In this analog, the 2,4-dihydroxybenzene in DBHQ is replaced with the *o*-vanillin moiety, and its ability as a fluorescent chelating ligand was investigated. Con-

sequently, although 2,4-[bis-(2-hydroxy-3-methoxybenzylidene)]-dihydrazinoquinazoline (HBQZ, Fig. 1a) itself shows very weak fluorescence, it can selectively bind with Zn^{2+} to emit fluorescence at 506 nm with an excitation wavelength of 414 nm. Further, we exploited the fluorometric properties of HBQZ to detect Zn^{2+} for the validation of a Zn^{2+} -containing compound, because several compounds such as drugs or supplements contain Zn^{2+} in their chemical structure. In the present study, an antibacterial and antifungal reagent, zinc pyrithione (ZnPT),^{10–17)} in which a bidentate bis-(*N*-oxopyridine-2-thionato) ligand is coordinated with the zinc ion, was used as the Zn^{2+} -containing compound.

Experimental

Chemicals Anthranilic acid and hydrazine monohydrate were purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Urea, dimethyl sulfoxide (DMSO), phosphoryl chloride (POCl_3), ZnPT, phosphoric acid, and quinine sulfate were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). *o*-Vanillin was purchased from Kanto Kagaku Co., Ltd. (Tokyo, Japan). H_2O was used after purification using an Autopure WR600G (Yamato Scientific Co., Ltd., Tokyo, Japan). The cations and anions tested in this study, which were purchased in the form of salts containing NO_3^- , SO_4^{2-} , and Cl^- , were of atomic absorption grade and purchased from Wako Pure Chemical Industries.

Apparatus Excitation and fluorescence spectra were measured on a spectrofluorometer (FP-6300; Jasco Co., Tokyo, Japan) with slit widths of 10 nm. All fluorescence and absorption data were collected in a temperature-controlled room (25 °C). IR spectra were recorded on an FT/IR-4100 (Jasco) instrument. Mass spectra were measured on a JMS-600H (JEOL, Ltd.,

Fig. 1. Chemical Structures of (a) HBQZ and (b) Zinc Pyrithione (ZnPT)

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Tokyo, Japan). NMR spectra were measured by JEOL ECP1 (JEOL Ltd., Tokyo, Japan) (500 MHz for ^1H and ^{13}C) with tetramethylsilane as the internal standard. The refractive indices of the solvents were measured using an Abbe refractometer (model ER-1, ERMA Inc., Tokyo, Japan).

Synthesis and Characterization of HBQZ 2,4-Dihydrazinoquinazoline (**1**) was synthesized according to the method described in our previous paper.⁶⁾ Compound **1** (13.0 mmol) was mixed with *o*-vanillin (1.16 g, 0.40 mol) in EtOH (350 ml), and the obtained solution was refluxed at 80 °C for 3 h. After the solution was heated to dryness, the obtained brownish residue was recrystallized using MeOH to give HBQZ in the form of a yellow powder. The product was recrystallized using MeOH to obtain the final purified product (yield: 45.0%). FAB-MS: m/z 459 $[\text{M}+\text{H}]^+$; Elemental Anal. Calcd for $\text{C}_{24}\text{H}_{22}\text{O}_4\text{N}_6$: C, 62.87; H, 4.84; N, 18.33%. Found: C, 62.86; H, 4.95; N, 18.36%. FT-IR (solid phase) (cm^{-1}): 3394, 3304 ($\nu_{\text{O-H}}$), 1602 ($\nu_{\text{C=N}}$), 1266, 1250 ($\nu_{\text{Ar-O}}$). ^1H -NMR ($\text{CDCl}_3+\text{CF}_3\text{CO}_2\text{H}$) δ (ppm): 3.87 (3H, s), 3.93 (3H, s), 6.88–7.04 (6H, m), 7.46 (1H, dt, $J=2.0, 8.0$ Hz), 7.75–7.80 (2H, m), 8.09 (1H, d, $J=8.4$ Hz), 8.43 (1H, s), 8.55 (1H, s). ^{13}C -NMR ($\text{CDCl}_3+\text{CF}_3\text{CO}_2\text{H}$) δ (ppm): 56.0, 56.3, 109.5, 115.0, 115.3, 116.7, 117.0, 118.0, 121.4, 121.6, 122.2, 123.6, 123.9, 127.4, 137.0, 138.0, 145.2, 146.5, 147.5, 147.7, 150.2, 152.0, 154.1, 156.7. Melting point: 283–284 °C.

Screening Assay of Metal Ions Coordinated with HBQZ The probability of 36 different metal ions being coordinated with HBQZ to induce fluorescence emission was investigated. Solutions containing 100 μl of the metal ions (1000 ppm), 100 μl of a 100 μM solution of HBQZ in DMSO, and 100 μl of a buffer solution at various pH values (3.5–10.5) were taken in glass test tubes. Buffer solutions with pH 3.5–5.0, 5.5–7.0, and 7.5–10.5 were prepared from 100 mM $\text{CH}_3\text{CO}_2\text{Na}-\text{CH}_3\text{CO}_2\text{H}$, *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES)- NH_3 , and $\text{NH}_4\text{Cl}-\text{NH}_3$, respectively. Each test tube was irradiated by UV light from a D_2 lamp in a dark room. The sample emitting intense fluorescence was concluded to be the solution containing the fluorescent HBQZ complex. In this experiment, the fluorescence emission was evaluated by visual assessment.

Sample Preparation In the case of measuring fluorescence of HBQZ- Zn^{2+} complex, sample preparation was performed as follows. In a polypropylene test tube, 1.0 ml of 50 mM acetate buffer (pH 4.5) was added to the sample solution. This was followed by adding 200 μl of a 60 μM HBQZ in DMSO, and was left to stand for 10 min at room temperature. Then, the solution was diluted tenfold with distilled 2-propanol, and the spectrum of the diluted solution was measured in a 1.0 $\times$ 1.0 cm quartz cell.

Excitation and Emission Spectra For the measurement of spectra, 500 μl of a 30.8 μM (2 ppm Zn^{2+}) solution of $\text{Zn}(\text{NO}_3)_2$ in H_2O was treated as described above.

Effect of Interfering Ions on Fluorescence In experiments involving interfering ions, 400 μl of an aqueous solution of $\text{Zn}(\text{NO}_3)_2$ (7.69 μM , i.e., 0.5 ppm Zn^{2+}) and 100 μl of an aqueous solution of each metal ion or anion (31.0–15.0 $\times 10^3$ μM) were successively added to 1.0 ml of 50 mM acetate buffer (pH 4.5), and treated in a similar manner in sample preparation. If a cation or anion was found to affect the fluorescence, the procedure was repeated for an ion concentration diluted twice with H_2O . This process was repeated until the ion concentration at which the fluorescence emission of the HBQZ- Zn^{2+} complex did not change was obtained. An error within $\pm 5\%$ in the measured fluorescence intensity was considered tolerable.

Binding Ratio of Zn^{2+} to HBQZ Next, 500 μl of Zn^{2+} solutions of different concentrations (1.54–76.9 μM) were mixed with 200 μl of 60 μM HBQZ solution. This was followed by the addition of 1.0 ml of 100 mM acetate buffer (pH 4.5). The fluorescence intensity of each solution was measured at 506 nm with an excitation wavelength of 414 nm. Further, 500 μl of a 15.4 μM (1.0 ppm) Zn^{2+} solution was mixed with 200 μl of HBQZ solutions (5.0–150 μM), and treated in a similar manner. The binding ratio was determined using the following molar ratio method. The fluorescence intensities were plotted against the molar ratio of Zn^{2+} with HBQZ, and the molar ratio of HBQZ coordinated with Zn^{2+} was determined stoichiometrically from the plots.

The apparent binding constant of HBQZ with Zn^{2+} was calculated using the modified Benesi-Hildebrand Eq. 1 as reported by Roy *et al.*¹⁸⁾

$$\Delta F = \Delta F_{\text{max}} + (1/K[C])(\Delta F_{\text{max}}) \quad (1)$$

where ΔF and ΔF_{max} are equal to $F-F_0$ and $F_{\text{max}}-F_0$, respectively. F_0 , F , and F_{max} are the fluorescence intensities of HBQZ, HBQZ with test Zn^{2+} concentrations (7.69, 12.3, 15.4, 30.8 μM), and HBQZ with the maximum Zn^{2+} concentration (76.9 μM), respectively. K and $[C]$ are the apparent binding constant and the test concentration of Zn^{2+} , respectively. $\Delta F_{\text{max}}-\Delta F$ was plotted against $1/[C]$, and the value of K was obtained from the slope of the line.

Electrospray Ionization (ESI) Mass Spectrometry Next, 200 μl of 1.0 mM Zn^{2+} solutions were mixed with 200 μl of 1.0 mM HBQZ solution. After the addition of 0.5 ml of 100 mM acetate buffer (pH 4.5), the solution mixture was diluted with 50% MeOH in H_2O containing 1% acetic acid to 10 ml. The samples were injected at a constant flow rate of 5 $\mu\text{l}/\text{min}$ using a syringe pump. All mass spectrometry (MS) data were acquired using an LCQ ion trap mass spectrometer (ThermoFisher Scientific, Yokohama, Japan) equipped with an electrospray ionization source, and the mass spectrometer was operated in the positive-ion mode. The experimental conditions were as follows: spray needle voltage, 5 kV; heated capillary temperature, 200 °C; and sheath gas flow rate, 40 (arbitrary units). Mass spectra were measured in the full ion scan mode for a mass-to-charge (m/z) ratio in the range of 200–2000.

Fluorescence Quantum Yield and Calibration Curve According to a previous method,¹⁹⁾ the fluorescence quantum yield (ϕ) of HBQZ in the presence or absence of Zn^{2+} was determined using quinine sulfate as a reference compound. In addition, the ϕ value of HBQZ- Zn^{2+} in the presence of 10 mM H_3PO_4 was also determined.

In brief, 1.0 ml of acetate buffer (pH 4.5) was added to 500 μl of 2.0 ppm Zn^{2+} and/or 500 μl of 20 mM H_3PO_4 . Finally, 200 μl of 60 μM HBQZ was added and treated as described above. In this case, a fluorescence integral with an excitation wavelength of 414 nm was obtained, and the absorbance at 414 nm was measured by using a Jasco V-650 spectrophotometer (Jasco, Tokyo, Japan). A calibration curve was drawn by plotting the values of $F-F_0$ against Zn^{2+} concentration (ppb). Then, 500 μl of a Zn^{2+} solution (50–1000 ppb) was used for the calibration curve ($n=5$). Detection limit (DL) was determined by the obtained calibration curve using the following Eq. 2.

$$\text{DL} = 3.3\sigma/s \quad (2)$$

where σ and s are the standard deviation of 0 ppm Zn^{2+} and slope of the calibration curve, respectively ($n=5$).

Release of Zn^{2+} from ZnPT ZnPT was dissolved in 10 mM HCl, HNO_3 , H_3PO_4 , and H_2SO_4 (5.0 ppm ZnPT), and was heated at 100 °C for 60 min. After the solution was cooled, 500 μl of the solution was sampled and was treated in a similar manner ($n=5$). ZnPT dissolved in 20 mM H_3PO_4 (5.0 ppm) was heated at 40, 60, 80, and 100 °C until 120 min, respectively. Then, 500 μl of the solution at each time point was treated ($n=5$). Next, 500 μl of 0.787–15.7 μM ZnPT in 20 mM H_3PO_4 were heated at 100 °C for 60 min and then used for obtaining the calibration curve for ZnPT ($n=5$).

Results and Discussion

Synthesis and Properties of HBQZ HBQZ was successfully synthesized in accordance with the procedure outlined in our previous paper,⁶⁾ judging from the data of the elemental analysis, FAB-MS, ^1H -NMR, ^{13}C -NMR, and FT-IR. Next, from among the 36 kinds of metal ions, the metal ion that could coordinate with HBQZ to afford a fluorescent complex was identified using different pH-buffered solutions. Consequently, in the presence of Zn^{2+} , intense fluorescence was observed upon irradiation from the D_2 lamp. Thus, Zn^{2+} -chelating and fluorescent property of HBQZ was mainly studied in this study.

Fluorescence Spectrum and Calibration Curve for Zn^{2+} Figure 2 shows the excitation and emission spectra of HBQZ with Zn^{2+} in 50 mM acetate buffer (pH 4.5). The optimum excitation and emission wavelengths were 414 and 506 nm, respectively. As shown in Fig. 2, the intensity of fluorescence emitted by the HBQZ- Zn^{2+} complex was approximately ten times that exhibited by HBQZ alone in the presence of 2 ppm Zn^{2+} . The fluorescence intensity was almost constant in the pH range 3.5–10.5 (data not shown), indicating that the HBQZ- Zn^{2+} complex is formed over a broad pH range, although most fluorescent ligands can chelate metal ions in neutral to alkaline solutions. Among the organic solvents used for the dilution of the HBQZ- Zn^{2+} complex and measurements of the fluorescence intensity, the most intense fluorescence was observed in 2-propanol, and therefore, 2-

propanol was used in further studies. A linear calibration curve was obtained in the Zn^{2+} ($r^2=0.994$) concentration range $0.77\text{--}15.4\text{ }\mu\text{M}$ ($50\text{--}1000\text{ ppb}$), and the precision (relative standard deviation (RSD)) at $12.3\text{ }\mu\text{M}$ Zn^{2+} was within 3.60% ($n=5$). The detection limit of Zn^{2+} was approximately 250 nM (16 ppb).

Effect of Additive Ions on Fluorescence As shown in Table 1, the coexistence of most metal cation or anion with Zn^{2+} did not affect the fluorescence emission of the HBQZ– Zn^{2+} complex. However, the addition of more than 2.0 eq of Co^{2+} , Cu^{2+} , Fe^{3+} , and Mn^{2+} to Zn^{2+} resulted in fluorescence quenching. It is suggested that these metal ions could also be chelated by HBQZ, but the complex may not emit fluorescence.

Binding Ratio of HBQZ to Zn^{2+} and Fluorescence Quantum Yield Figures 3a and b show the fluorescence intensity plotted against the molar ratio of Zn^{2+} to HBQZ

Fig. 2. Excitation and Emission Spectra of HBQZ in the Presence (Solid Line) or Absence (Dotted Line) of Zn^{2+}

Table 1. Effect of Interfering Ions on the Detection of $7.69\text{ }\mu\text{M}$ (0.5 ppm) Zn^{2+} Using $60\text{ }\mu\text{M}$ HBQZ (Tolerable Error: $\pm 5\%$)

Tolerance ratio (m/m)	
>500	K^+ , CH_3CO_2^- , NO_3^- , SO_4^{2-} , Cl^- , Br^- , I^- , PO_4^{3-} , Na^+
250	Ag^+ , Ge^{4+}
100	Mg^{2+}
50	Be^{2+} , Ba^{2+} , Sr^{2+} , Ca^{2+} , Pb^{2+} , Cd^{2+}
25	Hg^{2+} , ZrO^{2+}
10	Ti^{4+}
5	Sn^{2+} , Al^{3+} , Ni^{2+} , In^{3+} , $\text{Cr}_2\text{O}_7^{2-}$
2	Co^{2+} , Cu^{2+} , Fe^{3+} , Mn^{2+}

Tolerance ratio (m/m) was expressed as a ratio of the tested ion (mol) to Zn^{2+} ion (mol).

and that of HBQZ to Zn^{2+} , respectively. Both plots indicate that HBQZ binds to Zn^{2+} in a $1:1$ molar ratio. In addition, the ESI-MS spectrum of the solution of the HBQZ– Zn^{2+} complex showed the presence of ions with m/z of 520.1 (data not shown); these are molecular ions of the HBQZ– Zn^{2+} complex $[(\text{HBQZ}-2\text{H}+\text{Zn})^+]$. This result confirms that the binding ratio of the HBQZ– Zn^{2+} complex was $1:1$. The binding association constant K was determined by using the Benesi–Hildebrand method, and $\log K$ was approximately 5.6 . The ϕ value of the HBQZ– Zn^{2+} complex was 0.349 , while that of HBQZ alone was 0.021 . These $\log K$ and ϕ values were considered to be sufficient for the fluorescence detection of Zn^{2+} .

Fluorescence Detection of Zn^{2+} from ZnPT Next, HBQZ was applied to the validation of a bioactive compound containing coordinated Zn^{2+} in its structure. Many Zn^{2+} -coordinated compounds are used as drugs, nutrients, or supplements. For example, *N*-(3-aminopropionyl)-L-histidinato zinc (Z-103) shows anti-ulcer activity,¹⁾ while bis(pyrrole-2-carboxylato)zinc shows insulinomimetic activity.²⁾ Such Zn^{2+} -containing drugs are usually validated using AAS, because AAS can achieve selective quantification of a metal ion. In AAS measurement, however, a hollow cathode lamp is required for each metal ion; the flame from the lamp is supplemented with combustible acetylene gas for atomization of the sample. Fluorescence detection using a functionalized ligand, which can selectively chelate the metal ion, is preferred over AAS measurement due to its inherent simplicity and safety. Therefore, in order to exploit the usefulness of the validation test of a Zn^{2+} -coordinated compound, in the present study, we attempted to validate an antibacterial and antifungal reagent ZnPT, which has Zn^{2+} ions coordinated bilaterally with electrons on the sulfur and oxygen atoms of 1-hydroxypyridine-2-thione (pyrithione) (Fig. 1b).^{10,11)}

In our preliminary experiments, in which HBQZ was directly reacted with ZnPT in 50 mM acetate buffer at pH 4.0, the observed fluorescence intensity of the $15\text{--}63\text{ }\mu\text{M}$ ZnPT solution was considerably low—one-tenth of that of the solution containing $15\text{--}63\text{ }\mu\text{M}$ free Zn^{2+} . This result suggests that insufficient Zn^{2+} ions were released from ZnPT when the acetate buffer with pH 4.0 was used. Therefore, it was necessary to release free Zn^{2+} from the chelated Zn^{2+} in ZnPT. On the basis of the fact that $\text{p}K_a$ of pyrithione is 4.6 , Zn^{2+} could be completely released from ZnPT in the region with acidic pH. Thus, HCl , HNO_3 , H_3PO_4 , and H_2SO_4 were

Fig. 3. Fluorescence Intensity of HBQZ– Zn^{2+} Complex (a) at a Binding Ratio of Zn^{2+} to HBQZ and (b) at a Binding Ratio of HBQZ to Zn^{2+}

Fig. 4. (a) Effect of 10 mM Inorganic Acids on Fluorescence Intensity of HBQZ- Zn^{2+} Complex Produced from ZnPT (Mean $\pm$ S.D., $n=5$); (b) Time Course of Change in Fluorescence Intensity of HBQZ- Zn^{2+} Complex Produced from ZnPT, Plotted as a Function of Heating Time in 20 mM H_3PO_4 (Mean $\pm$ S.D., $n=5$)

Closed circle: 100 °C, diamond: 80 °C, square: 60 °C, and triangle: 40 °C.

tested for identifying the type of inorganic acid required for the release of Zn^{2+} from ZnPT. The highest fluorescence intensity of the HBQZ- Zn^{2+} complex was observed in the presence of H_3PO_4 (Fig. 4a). Therefore, H_3PO_4 was utilized for the release of free Zn^{2+} from ZnPT. It is still not clear why H_3PO_4 was effective for the detection of free Zn^{2+} in ZnPT. The cooperative coordination between the phosphoryl group in H_3PO_4 and Zn^{2+} was thought to have enhanced the fluorescence intensity of the HBQZ- Zn^{2+} complex. Certainly, the ϕ value of the HBQZ- Zn^{2+} complex in the presence of 10 mM H_3PO_4 was 0.712, whereas the ϕ value of the HBQZ- Zn^{2+} complex alone was 0.349. Similar phenomena were observed in our previous study, in which a quinazoline-type ligand, 2,4-bis-(2,4-dihydroxybenzylidene)-dihydrazinoquinazoline (DBHQ),^{6,20} was used. It was recently reported that the structural analog, DBHQ, with Ga^{3+} was also enhanced by the addition of phosphoryl compounds or dihydrophosphate ions.²⁰

Figure 4b shows the time course of the change in the fluorescence intensity as a function of heating time. As shown in Fig. 4b, heating at 100 °C resulted in high fluorescence intensity after the onset of the reaction, indicating that the release of free Zn^{2+} from ZnPT was accelerated at 100 °C. Therefore, in this study, heating was carried out at 100 °C for 60 min. Under these conditions, a linear calibration curve was obtained for the ZnPT concentration range 0.79–15.7 μM (0.25–5.0 ppm); the correlation coefficient (r^2) and RSD were 0.996 and within 3.1% ($n=5$), respectively. These data indicate that the fluorescence method involving the use of HBQZ is suitable for the validation of ZnPT. The detection sensitivity of Zn^{2+} by using HBQZ was not superior to that by using AAS; however, fluorescence detection of Zn^{2+} has the advantage of simplicity. Therefore, HBQZ can be used for the validation of a compound containing Zn^{2+} , owing to its capacity to capture Zn^{2+} ions.

Conclusion

A novel quinazoline-based chelating ligand, HBQZ, with

an *o*-vanillin moiety was newly developed. It was shown that HBQZ fluoresced selectively with Zn^{2+} and that HBQZ can be used for the validation of ZnPT after a pretreatment procedure. The proposed fluorescent method using HBQZ will be useful for the validation test for a bioactive Zn^{2+} -containing compound.

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References

- 1) Nishide M., Yoshikawa Y., Yoshikawa E. U., Matsumoto K., Sakurai H., Kajiwarra N. M., *Chem. Pharm. Bull.*, **56**, 1181–1183 (2008).
- 2) Ito M., Tanaka T., Suzuki Y., *Jpn. J. Pharmacol.*, **52**, 513–521 (1990).
- 3) Ferruzzi M. G., Failla M. L., Schwartz S. J., *J. Agric. Food Chem.*, **50**, 2173–2179 (2002).
- 4) Chen J. H., Jiang S. J., *J. Agric. Food Chem.*, **56**, 1210–1215 (2008).
- 5) Nève J., Hanocq M., Peretz A., Khalil F. A., Pelen F., *Biol. Trace Elem. Res.*, **32**, 201–212 (1992).
- 6) Kimura J., Yamada H., Ogura H., Yajima T., Fukushima T., *Anal. Chim. Acta*, **635**, 207–213 (2009).
- 7) Kara D., Fisher A., Hill S. J., *J. Environ. Monit.*, **9**, 994–1000 (2007).
- 8) Tang B., Chen Z. Z., Zhang N., Zhang J., Wang Y., *Talanta*, **68**, 575–580 (2006).
- 9) Tang B., Yue T. X., Wu J. S., Dong Y. M., Ding Y., Wang H. J., *Talanta*, **64**, 955–960 (2004).
- 10) Doose C. A., Ranke J., Stock F., Bottin-Weber U., Jastorff B., *Green Chem.*, **6**, 259–266 (2004).
- 11) Bao V. W. W., Leung K. M. Y., Kwok K. W. H., Zhang A. Q., Lui G. C. S., *Mar. Pollut. Bull.*, **57**, 616–623 (2008).
- 12) Kondoh Y., Takano S., *J. Chromatogr.*, **408**, 255–262 (1987).
- 13) Shih Y., Zen J. M., Kumar A. S., Chen P. Y., *Talanta*, **62**, 912–917 (2004).
- 14) Thomas K. V., *J. Chromatogr. A*, **833**, 105–109 (1999).
- 15) Doose C. A., Szaleniec M., Behrend P., Muller A., Jastorff B., *J. Chromatogr. A*, **1052**, 103–110 (2004).
- 16) Yamaguchi Y., Kumakura A., Sugawara S., Harino H., Yamada Y., Shibata K., Senda T., *Int. J. Environ. Anal. Chem.*, **86**, 83–89 (2006).
- 17) Bones J., Thomas K. V., Paull B., *J. Chromatogr. A*, **1132**, 157–164 (2006).
- 18) Roy P., Dhara K., Manassero M., Ratha J., Banerjee P., *Inorg. Chem.*, **46**, 6405–6412 (2007).
- 19) Bag B., Bharadwaj P. K., *J. Lumin.*, **126**, 27–36 (2007).
- 20) Kimura J., Yamada H., Yajima T., Fukushima T., *J. Lumin.*, **129**, 1362–1365 (2009).