Electronic Supplementary Information

"Organic solvent-free immunoassay for quantitative detection of neonicotinoid acetamiprid residues in agricultural products"

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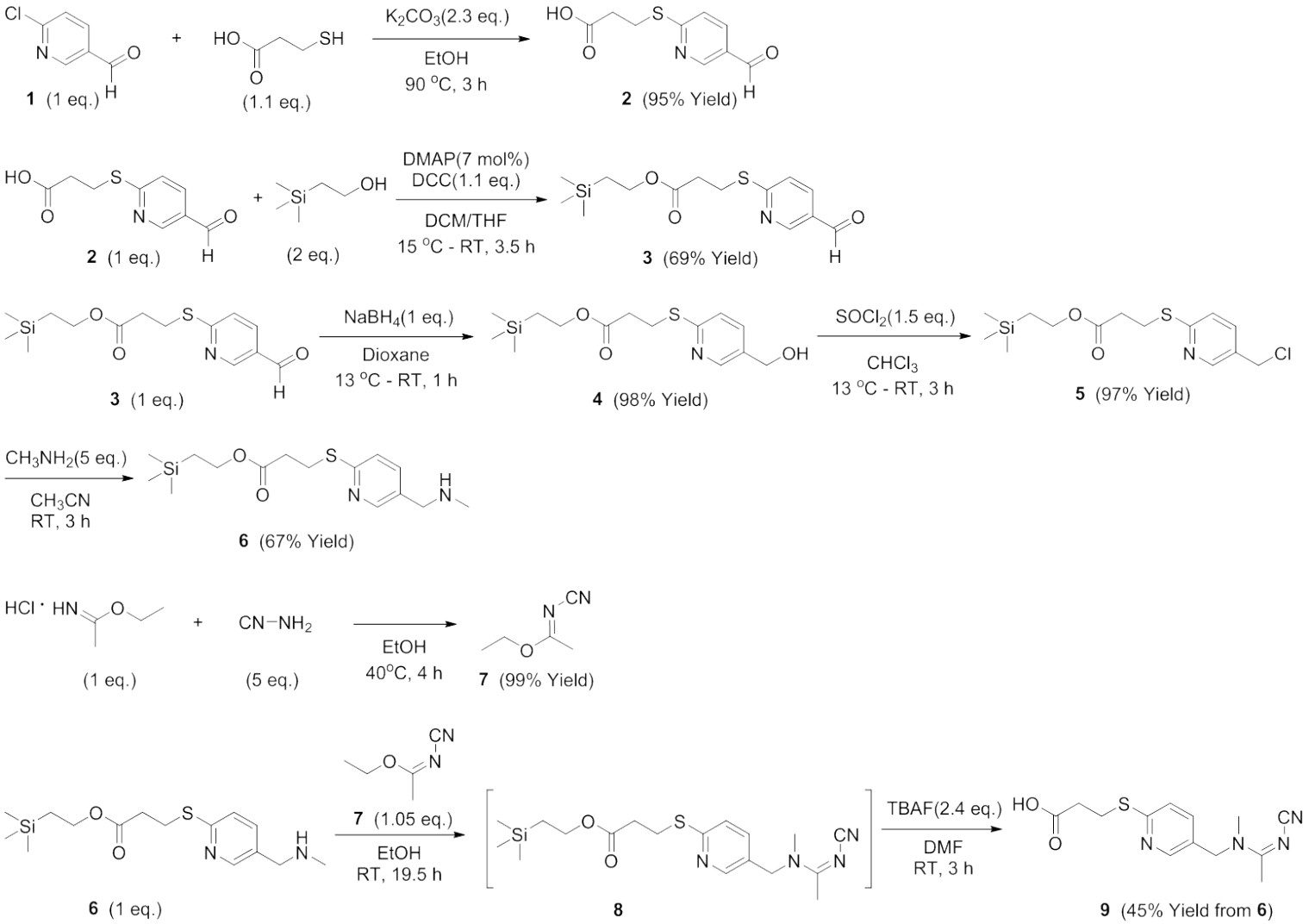

Figure 1S. Synthetic pathway used to prepare acetamiprid hapten.

General procedure
All reactions were performed under an argon atmosphere unless otherwise noted. Dichloromethane (CH_2Cl_2) was purchased from Kanto Chemical Co., Inc. All reactions were monitored using thin layer chromatography (TLC), glass plates pre-coated with silica gel Merck KGaA 60 F_{254}, with 0.2 mm layer thickness. The products were visualized by irradiation with UV light or by treatment with a solution of phosphomolybdic acid or by treatment with a solution of *p*-anisaldehyde. Flash column chromatography was performed using silica gel (Art. No. 7734; Merck). IR spectra were measured using a spectrometer (FT/IR-4600; Jasco Corp.). ^{1}H NMR (500 MHz, 400 MHz) and ^{13}C NMR (125 MHz, 100 MHz) spectra were recorded on spectrometer (JNM-ECX500 or JNM-ECS400; JEOL). Chemical shifts are reported as δ values (ppm) relative to TMS (0 ppm) and DMSO-d_6 (2.50 ppm). DART mass (positive mode) analyses were performed on a LC–TOF JMS-T100LP.

Synthesis of 3-(5-formylpyridin-2-ylthio)propanoic acid (2)
A mixture of 6-chloropyridine-3-carbaldehyde **1 (**3.54 g, 25.0 mmol), 3-mercapto propionic acid (2.39 mL, *d* = 1.22, 27.5 mmol), and K_2CO_3 in ethanol (EtOH) (50 mL) was heated to 90 °C for 3 h with stirring. At the end of this period, the mixture was cooled to room temperature and was treated with 6 mol L^{-1} HCl solution (pH = ca. 3.0). The product was extracted using ethyl acetate. The organic phase was washed with water and was dried with Na_2SO_4. The solvent was removed. The desired crude product **1** (5.04 g) was obtained in 95% yield. Product **2** was used for next step with no further purification. **2:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H, -C*H*O), 8.32 (dd, 1H, *J* = 0.76, 2.29 Hz, H-6 Py), 7.94 (dd, 1H, *J* = 1.91, 8.41 Hz, H-4 Py), 7.30 (d, 1H, *J* = 8.41 Hz, H-3 Py), 3.51 (t, 2H, *J* = 6.88 Hz, -SC*H*$_2$CH$_2$COOH), 2.89 (t, 2H, *J* = 6.88 Hz, -SCH$_2$C*H*$_2$COOH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.1, 177.1, 166.0, 152.5, 134.5, 128.0, 122.3, 34.0, 24.6 ppm. HRMS (DART) *m/z*: Calcd for $C_9H_{10}NO_3S$ (M+1$^+$) 212.0381, Found 212.0381. IR (neat) 3046, 2951, 1730, 1700, 1586, 1464, 1350, 1109, 844 cm^{-1}.

Synthesis of 2-(trimethylsilyl)ethyl 3-(5-formylpyridin-2-ylthio)propanoate (3)
To a solution of 3-(5-formylpyridin-2-ylthio)propanoic acid **2** (1.61 g, 7.63 mmol) and dimethylaminopyridine (DMAP) (65.3 mg, 0.53 mmol) in dichloromethane (DCM) (9.0 mL) and THF (2.0 mL) was added trimethylsilylethanol (TMSE) (2.17 mL, *d* = 0.83, 15.25 mmol). The resulting mixture was cooled using ice-water (15 °C) followed by addition of a solid of dicyclohexylcarbodiimide (DCC) (1.73 g, 8.39 mmol). The resulting mixture was stirred at room temperature for 3.5 h. The product was then purified using column chromatography on silica gel (*n*-hexane/ethyl acetate (4:1, v/v)) to give the desired product **3** (1.63 g) in 69% yield. **3:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.99 (s, 1H, -C*H*O), 8.21 (d, 1H, *J* = 2.29 Hz, H-6 Py), 7.92 (dd, 1H, *J* = 2.29, 8.41 Hz, H-4 Py), 7.28 (d, 1H, *J* = 8.41 Hz, H-3 Py), 4.20–4.23 (m, 2H, -OC*H*$_2$CH$_2$Si-), 3.51 (t, 2H, *J* = 6.88 Hz, -SC*H*$_2$CH$_2$CO-), 2.79 (t, 2H, *J* = 6.88 Hz, -SCH$_2$C*H*$_2$CO-), 0.98–1.02 (m, 2H, -OCH$_2$C*H*$_2$Si-), 0.04 (s, 9H, -Si(C*H*$_3$)$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.9, 171.8, 166.2, 152.5, 134.3, 127.9, 122.2, 62.9, 34.4, 25.0, 17.2, -1.6 ppm. HRMS (DART) *m/z*: Calcd for $C_{14}H_{22}NO_3SSi$ (M+1$^+$) 312.1090, Found 312.1095. IR (neat) 2953, 1730, 1702, 1585, 1469, 1348, 1107 cm^{-1}.

Synthesis of 2-(trimethylsilyl)ethyl 3-((5-(hydroxymethyl)pyridin-2-yl)sulfanyl) propanoate (4)

To a solution of 2-(trimethylsilyl)ethyl 3-(5-formylpyridin-2-ylthio)propanoate **3 (**1.56 g, 5.02 mmol) in 1,4-dioxane (15.2 mL) was added a solution of $NaBH_4$ (189.9 mg, 5.02 mmol) in water (2.0 mL) at 13 °C (ice-water). The resulting mixture was stirred at room temperature for 1 h. The solvent was then removed under reduced pressure followed by addition of water. The product was extracted with ethyl acetate and dried with Na_2SO_4. The solvent was removed. Desired product **4** (1.55 g) was obtained in 98% yield. Product **4** was used for the next step with no purification. **4:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H, H-6 Py), 7.54 (dd, 1H, *J* = 2.29, 8.03 Hz, H-4 Py), 7.18 (d, 1H, *J* = 8.03 Hz, H-3 Py), 4.66 (s, 2H, -C*H*$_2$OH), 4.18–4.22 (m, 2H, -OC*H*$_2$CH$_2$Si-), 3.44 (t, 2H, *J* = 6.88 Hz, -SC*H*$_2$CH$_2$CO-), 2.75 (t, 2H, *J* = 6.88 Hz, -SCH$_2$C*H*$_2$CO-), 0.98–1.01 (m, 2H, -OCH$_2$C*H*$_2$Si-), 0.04 (s, 9H, -Si(C*H*$_3$)$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 157.3, 148.2, 135.3, 132.1, 122.0, 62.9, 62.1, 34.7, 25.0, 17.2, -1.6 ppm. HRMS (DART) *m/z*: Calcd for $C_{14}H_{24}NO_3SSi$ ($M+1^+$) 314.1246, Found 314.1250. IR (neat) 3416, 2952, 1730, 1593, 1464, 1346, 1109 cm^{-1}.

Synthesis of 2-(trimethylsilyl)ethyl 3-(5-(chloromethyl)pyridin-2-ylthio)propanoate (5)

To a solution of 2-(trimethylsilyl)ethyl 3-((5-(hydroxymethyl)pyridin-2-yl)sulfanyl) propanoate **4 (**1.46 g, 4.64 mmol) in $CHCl_3$ (4.6 mL) was added a neat $SOCl_2$ (505 µL, *d* = 1.64, 7.0 mmol) at 13 °C (ice-water). The mixture was stirred for 3 h at room temperature. Then the solvent was removed. The residue was treated with 5% $NaHCO_3$ solution (20 mL). The product was extracted with ethyl acetate and dried with Na_2SO_4. The solvent was removed. Desired product **5** (1.49 g) was obtained in 97% yield. Product **5** was used for next step with no purification. **5:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (d, 1H, *J* = 2.29 Hz, H-6 Py), 7.52 (dd, 1H, *J* = 2.29, 8.41 Hz, H-4 Py), 7.17 (d, 1H, *J* = 8.41 Hz, H-3 Py), 4.54 (s, 2H, -C*H*$_2$Cl), 4.18–4.22 (m, 2H, -OC*H*$_2$CH$_2$Si-), 3.43 (t, 2H, *J* = 6.88 Hz, -SC*H*$_2$CH$_2$CO-), 2.76 (t, 2H, *J* = 6.88 Hz, -SCH$_2$C*H*$_2$CO-), 0.98-1.01 (m, 2H, -OCH$_2$C*H*$_2$Si-), 0.04 (s, 9H, -Si(C*H*$_3$)$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 158.7, 149.0, 136.1, 128.8, 122.1, 62.8, 43.1, 34.6, 24.9, 17.2, -1.6 ppm. HRMS (DART) *m/z*: Calcd for $C_{14}H_{23}ClNO_2SSi$ ($M+1^+$) 332.0907, Found 332.0910. IR (neat) 2953, 2898, 1731, 1593, 1467, 1379, 1250, 1113 cm^{-1}.

Synthesis of 2-(trimethylsilyl)ethyl 3-(5-((methylamino)methyl)pyridin-2-ylthio) propanoate (6)

To a solution of CH_3NH_2 (40% H_2O solution, 0.56 mL, *d* = 0.9, 6.5 mmol) was slowly added a solution of 2-(trimethylsilyl)ethyl 3-(5-(chloromethyl)pyridine-2-ylthio) propanoate **5** (420 mg, 1.3 mmol) in acetonitrile (2.5 mL) at 10–15 °C (ice-water bath). The mixture was stirred for 3 h at room temperature. Then the solvent was removed and the residue was treated with 5% $NaHCO_3$ solution (20 mL). The product was extracted with ethyl acetate and dried with Na_2SO_4. The solvent was removed and purified using column chromatography on silica (ethyl acetate/methanol (1:1, v/v) containing 1% (v/v) triethylamine) to give **6** (274.8 mg) in 67% yield. **6:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, 1H, *J* = 1.83 Hz, H-6 Py), 7.48 (dd, 1H, *J* = 1.83, 7.93 Hz, H-4 Py), 7.14 (dd, 1H, *J* = 0.61, 7.93 Hz, H-3 Py), 4.18-4.22 (m, 2H, -OC*H*$_2$CH$_2$Si-), 3.70 (s, 2H, -PyC*H*$_2$N-), 3.42 (t, 2H, *J* = 7.32 Hz, -SC*H*$_2$CH$_2$CO-), 2.75 (t, 2H, *J* = 7.32 Hz, -SCH$_2$C*H*$_2$CO-), 2.44 (s, 3H, -NC*H*$_3$), 0.97-1.01 (m, 2H, -OCH$_2$C*H*$_2$Si-), 0.04 (s, 9H, -Si(C*H*$_3$)$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 156.5, 149.0, 136.0, 130.9, 121.8,

62.6, 52.5, 35.6, 34.6, 24.8, 17.0, -1.7 ppm. HRMS (DART) *m/z*: Calcd for $C_{15}H_{27}N_2O_2SSi$ ($M+1^+$) 327.1563, Found 327.1560. IR (neat) 3329, 2952, 2897, 1731, 1592, 1464, 1250, 1110 cm^{-1}.

Synthesis of ethyl N-cyanoethanimidate (7)
A mixture of ethyl acetimidate hydrochloride (0.99 g, 8.0 mmol) and cyanamide (1.68 g, 40 mmol) in EtOH (12 mL) was stirred for 4 h at 40 °C. After the mixture was cooled to room temperature, the mixture was filtered off and condensed, followed by addition of water. The product was extracted with ethyl acetate. The organic phase was separated and dried with Na_2SO_4. The solvent was removed to give almost pure product **7** (0.89 g) in 99% yield. Product **7** was used for next step with no further purification. **7:** ^{1}H NMR (500 MHz, $CDCl_3$) δ4.29 (q, 2H, *J* = 6.88 Hz, -OC*H*$_2$CH$_3$), 2.40 (s, 3H, -C*H*$_3$), 1.35 (t, 3H, *J* = 6.88 Hz, -OCH$_2$C*H*$_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.0, 114.1, 65.8, 20.8, 13.5 ppm. HRMS (DART) *m/z*: Calcd for $C_5H_9N_2O$ ($M+1^+$) 113.0715, Found 113.0713. IR (neat) 2204, 1606, 1325 cm^{-1}.

Synthesis of acetamiprid hapten, 3-((5-((N'-cyano-N-methylacetimidamido) methyl)pyridine-2-yl)thio)propanoic acid (9)
A mixture of 2-(trimethylsilyl)ethyl 3-(5-((methylamino)methyl)pyridin-2-ylthio) propanoate **6** (778.6 mg, 2.38 mmol) and ethyl *N*-cyanoethanimideate **7** (280.3 mg, 2.50 mmol) in EtOH (4 mL) for 19.5 h at room temperature. Then the solvent was removed under reduced pressure. The residue was dissolved in *N*,*N*-dimethylformamide (DMF) (14 mL) followed by addition of tetra-*n*-butylammonium fluoride (TBAF) (5.71 mL, 5.71 mmol, 1 mol L^{-1} THF solution). The resulting mixture was stirred for 3 h at room temperature. The mixture was acidified with 1.2 mol L^{-1} HCl solution until pH = ca. 4. Then the solvent was removed and the residue was purified using column chromatography on silica gel eluted with *n*-hexane/ethyl acetate/methanol (5:5:1, v/v/v) to give the desired hapten **9** (312.3 mg) in 45% isolated yield from **6**. **9:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, 1H, *J* = 2.14 Hz, H-6 Py), 7.51 (dd, 1H, *J* = 2.14, 8.24 Hz, H-4 Py), 7.18 (d, 1H, *J* = 8.24 Hz, H-3 Py), 4.67 (s, 2H, PyC*H*$_2$N-), 3.42 (t, 2H, *J* = 7.02 Hz, -SC*H*$_2$CH$_2$CO-), 3.08 (s, 3H, -NC*H*$_3$), 2.85 (t, 2H, *J* = 7.02 Hz, -SCH$_2$C*H*$_2$CO-), 2.45 (s, 3H, -CC*H*$_3$), 12.32 (bs, 1H, -COOH, in DMSO-d^6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4, 172.0, 158.4, 148.9, 136.5, 126.9, 122.6, 117.5, 50.8, 37.2, 34.5, 24.7, 19.4 ppm. HRMS (DART) *m/z*: Calcd for $C_{13}H_{17}N_4O_2S$ ($M+1^+$) 293.1072, Found 293.1070. IR (neat)3412, 2938, 2179, 1725, 1575, 1464 cm^{-1}.

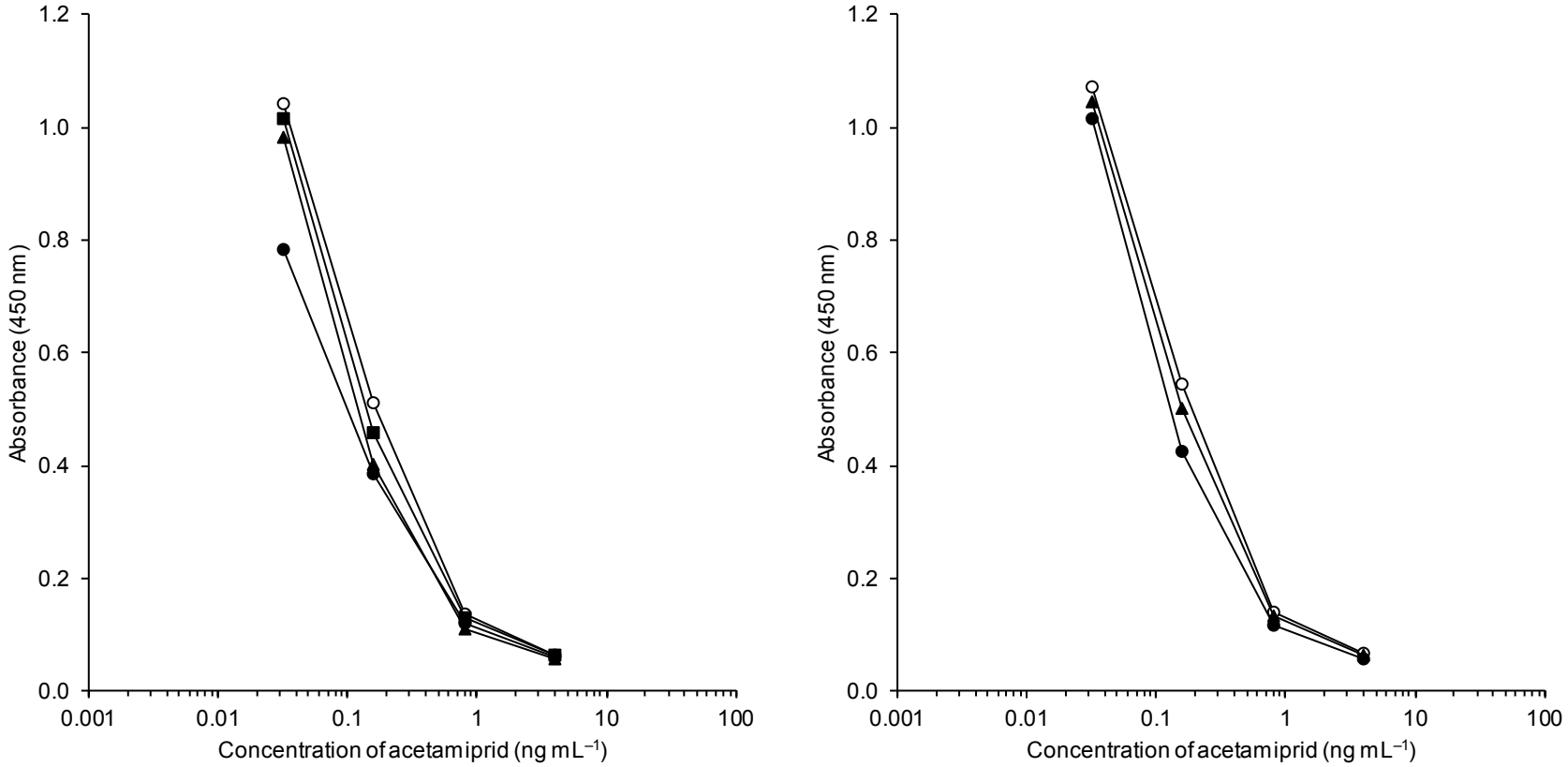

Figure 2S. Influence of the sample matrix on the ELISA standard curve. Extract of spinach (a) and Welsh onion (b). Each data point is the average of duplicate determinations: (*open circles*) control (standard solutions prepared in water), (*closed circles*) sample extract diluted with water at ten-fold, (*closed triangles*) 20-fold dilution, and (*closed squares*) 40-fold dilution.