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Simultaneous determination of phenylurea herbicides in *yam* by capillary electrophoresis with electrochemiluminescence detection

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ABSTRACT

A method of capillary electrophoresis (CE) coupled with electrochemiluminescence (ECL) detection has been applied to detect three major phenylurea herbicides (monuron, monolinuron and diuron) simultaneously. The effects of *yam* sample preparation, injection voltage and time, detection potential, detection buffer concentration and pH, $Ru(bpy)_3^{2+}$ concentration, separation buffer type, separation buffer pH and concentration, separation voltage were investigated in detail. Under optimum conditions, a good baseline separation and highly sensitive detection for monuron, monolinuron and diuron were achieved. The ECL intensity (I) was in proportion to three analytes concentration (ρ) in the range of 0.1–10,000 μg/L for monuron ($r \geq 0.9993$), 0.1–18,000 μg/L for monolinuron ($r \geq 0.9995$) and 0.1–20,000 μg/L for diuron ($r \geq 0.9997$). The detection limits for monuron, monolinuron and diuron were 0.05, 0.04 and 0.01 μg/L ($S/N = 3$), respectively. The developed method was successfully applied for the analysis of monuron, monolinuron and diuron residues in *yam* simultaneously. The average recoveries are in the ranges of 90.0–99.2% with relative standard deviations less than 3.2%. The limits of detection of the proposed method were 0.010 μg/kg for monuron, 0.008 μg/kg for monolinuron and diuron in *yam*.

1. Introduction

Phenylurea herbicides, a kind of pesticides which includes monuron, monolinuron, diuron and metobromuron, etc. has been widely used to suppress broadleaf and grass weeds in China and other countries [1]. It was reported that phenylurea herbicides have chronic toxic, and its residues and degradation products showed certain activities in crops, soil and water, which brought potential risks to the health of human beings by environmental accumulation and food chain. For example, it has been reported that people ate the foodstuff with excessive monuron and diuron would cause carcinogenic [2], which has already caused people's high attention. Therefore, some countries have set the maximum residue limit (MRL) of phenylurea herbicides in foodstuff.

Yam is one of the Medicine-Food agricultural products with high nutritional value, with people's awareness of health more and more strengthen, the demand for *Yam* are getting higher and higher. Therefore, in order to guarantee people's health, and to provide technical support for the effective monitoring of pesticide residues in agricultural products, it is highly desirable to develop a simple and effective method to monitor residues of phenylurea herbicides in the agricultural samples.

Analytical methods for the quantification of phenylurea herbicide residues are mainly based on the chromatographic analysis, such as high performance liquid chromatography (HPLC) [3,4], high performance liquid chromatography mass spectrometry-mass spectrometry (HPLC-MS/MS) [5], liquid chromatography time-of-flight mass spectrometry (LC/TOF-MS) [6], gas chromatography mass spectrometry (GC-MS) [7], thin layer chromatography (TLC) [8–10], immunoassay technique [11,12], photo-induced fluorescence (PIF) [13], graphene reinforced hollow fiber liquid phase microextraction (G-HF-LPME) [14], etc. However, these regular methods generally are high operation cost and long analytical time. Besides, the sample pretreatment procedures are rather cumbersome and expensive, which greatly diminished them application.

Capillary electrophoresis (CE) has been attracted many researcher's attention because of its advantage of high separation efficiency, short separation time, low reagent consumption, and ease of installation [15,16]. The features of the detector have great effect on the overall analytical efficiency for CE due to the small size of the separation capillaries and seriously low sample quantities injected into the capillaries. Actually, the detector should provide detection limits as low as possible without affecting the quality of separation. ECL is the procedure based on species generated at electrodes undergo high-energy electron transfer reactions

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to form excited states that emit light [17]. Nowadays, it has been widely used as a highly sensitive detection technique with its high sensitivity, wide linear range, and simple instrumentation. So, CE combining with ECL (CE-ECL) has been developed for the determination of various analytes containing tertiary amines group and their derivatives for a long time [18,19]. At present, it has become one of the high technology detection of pesticide residues, because of its advantages of high separation efficiency, good repeatability, short analysis time, high sensitivity and low solvent consumption, etc. For example, Chiu et al. [20] using 20 mM sodium phosphate (pH 9.0) and a separation voltage of 21 kV, working electrode at 1.6 V in a 300 mM sodium phosphate buffer (pH 8.0) containing 3.5 mM $Ru(bpy)_3^{2+}$, have been separated GLY and AMPA by CE-ECL detection, the limits of detection (LODs) for GLY and AMPA in water were 0.06 and 4.04 $\mu g\,mL^{-1}$, respectively. Liu et al. [21] have used the CE-ECL to analyze mefenacet, the detection limit of 4.0×10^{-9} M, and was also applied to analyze residues of mefenacet in seedling and soil. The optimized conditions were as follows: potential applied at 1.2 V, injection time and injection voltage was 10 s and 11 kV, running buffer pH value was 7.38. Cai et al. [22] have determined pretilachlor in soil and rice using MSPD extraction by CE with field amplified sample injection and ECL detection, the limits of detection of the proposed method were 0.01 mg/kg in rice matrix and 0.008 mg/kg in soil matrix. The optimized conditions were as follows: potential applied at 1.17 V, injection time and voltage was 9 s and 8 kV, respectively, running buffer concentration and pH was 50 mmol L^{-1} and 7.38, respectively, buffer pH in detection cell was 8.5, separation voltage was 10 kV. However, this method seldom apply to analysis three kinds of pesticide residues simultaneously, and cannot give full play to the advantages and application potential of this high technology coupling technique. On the other hand, the influencing factors of the above application were investigated not detailed enough, and so far, the CE-ECL method has not been reported for determination of monuron, monolinuron and diuron simultaneously, and no application to *yam* sample was demonstrated.

In this study, a simple and rapid CE-ECL method was adopted to determine phenylurea herbicides residues (monuron, monolinuron and diuron) simultaneously, and the matrix solid-phase dispersion method (MSPD) was developed to handle sample before determining [23]. Influencing factors, such as *yam* sample preparation, injection voltage and time, detection potential, detection buffer concentration and pH, $Ru(bpy)_3^{2+}$ concentration, separation buffer type, separation buffer pH and concentration, separation voltage were investigated in detail. Residues of monuron, monolinuron and diuron in *yam* sample were also determined using the proposed technique. It was expected to be able to provide some theoretical basis for the quality evaluation and judgment of *yam*, and can provide some effective technical support for the agricultural product quality safety testing.

2. Experimental

2.1. Apparatus and reagents

An MPI-A CE-ECL system was produced by Remax Electronic Science-Tech Co. Ltd. (Xi'an, China); a 42 cm length of 25 μm i.d. uncoated fused-silica capillary was used (Yongnian Optical Fabric Factory, Hebei, China); ECL detection was employed using a three-electrode system: a 300 μm diameter Pt disk as the working electrode, a Pt wire as the counter electrode, and a Ag/AgCl electrode (in saturated KCl solution) as the reference electrode.

Tris(2,2-bipyridyl) ruthenium(II) chloride hexahydrate [$Ru(bpy)_3Cl_2$-$6H_2O$], and monuron, monolinuron and diuron standard compounds were obtained from Bailingwei Technology Company (Beijing, China). *Yam* was purchased from a local pharmacy. 0.22 μm membrane filters was purchased from Xinya Purification Material Factory (Shanghai, China). All water used throughout the study was double-distilled water, and reagents and chemicals used were analytical grade.

2.2. Yam sample preparation

MSPD was developed to handle sample [23]: *yam* was weighted accurately after drying at 55 °C for 4 h by oven, and grounding into powders for samples. 0.5 g sample (blank or spiked) was blended with 2.5 g dried Florisil material, and then ground together for 12 min to obtain a uniform mixture. Next, the mixture was transferred to a 10 mL syringe barrel covered in turn with a filter, anhydrous alumina at the bottom. Then, 3 cm height of anhydrous sodium sulfate and another filter were placed on the top of the mixture and then the sample mixture was compressed tightly. After successive elution with three aliquots of ethyl acetate (5 mL) for 4 min, the solutions were combined together and evaporated to dryness under a nitrogen stream. The residue was then dissolved in 100 mL 70% ethanol and soaked at room temperature for 24 h. Finally, 25 mM phosphate buffer solution (pH 8. 0) had been added and was volumed to 250 mL. Before analysis, all the *yam* sample were filtered through 0.22 μm membrane filters.

2.3. Experimental methods

Before the experiment, 350 μL volume of 5 mM $Ru(bpy)_3^{2+}$ and 45 mM phosphate buffer at pH 7.5 was injected into the detection reservoir, 25 mM phosphate buffer at pH 8.0 was used as separation buffer, 16 kV voltage was used as separation voltage, electrokinetic injection 10 s at 10 kV was used for sample introduction and detection potential at 1.13 V was applied at the working electrode. The biased potential of photomultiplier tube was set at 800 V. Before use, the working electrode was polished with alumina powder (0.3 and 0.05 μm in turn) until a mirror-smooth surface appeared. Prior to CE injection, all the solutions were filtered through membrane filters. Then the injector end of capillary was put into phosphate buffer and electrophoresis voltage was applied, and the ECL analyzer was opened at the same time. In order to obtain accurate result, electrokinetic injection, separation and detection were began after the baseline of intensity signal to be stable (about 10 s).

3. Results and discussion

3.1. ECL behaviors of monuron, monolinuron and diuron

The corresponding ECL curves of $Ru(bpy)_3^{2+}$, monuron, monolinuron and diuron were shown in Fig. 1, when a Pt electrode in 45 mM PBS (pH 7.5) with 5 mM $Ru(bpy)_3^{2+}$ in the potential range from 0 to 1.3 V, only relatively weak ECL signal could be observed (Fig. 1(a_0)). With the addition of the same concentration of monuron, monolinuron and diuron, respectively. As is clearly shown in Fig. 1, the ECL intensity of three analytes increased remarkably, and the ECL intensity of diuron (Fig. 1(c)) is a little stronger than monuron (Fig. 1(a)) and monolinuron (Fig. 1(b)) at the same concentration.

3.2. Optimization of the yam sample preparation

The *yam* samples were handled based on the MSPD method that have been reported [23]. Firstly, Florisil was chosen as the dispersant because it led to a cleaner chromatographic profile with lower baseline than C_8, C_{18} and silica. Various mass ratios of the dispersant to the sample matrix (0.5 g) from 7:1 to 3:1 were assayed with