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Determination of mefenacet by capillary electrophoresis with electrochemiluminescence detection

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Abstract

Electrochemiluminescence (ECL) detection with capillary electrophoresis (CE) separation system was used to the rapid analysis of mefenacet within 7 min. The linear response range of mefenacet was from 1.07×10^{-8} to 5.0×10^{-7} M with a detection limit of 4.0×10^{-9} M. This technique was also applied to analyze residues of mefenacet in seedling and soil.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Herbicide; Mefenacet

1. Introduction

Mefenacet, a herbicide containing tertiary amine substituent, has been widely employed to suppress broadleaf and grass weeds in Korea, Japan and China since it was commercialized in the middle of 1980s. However, compared with other herbicides such as propanil, diuron and simazine, mefenacet exhibits higher toxicity not only to weeds but also to crops. Negative effects of mefenacet have been found on growth of phytoplankton including *Scenedesmus acutus*, *Scenedesmus subspicatus*, *Chlorella vulgaris* and *Chlorella saccharophila* [1]. Many methods have been adopted especially for the detection of its residues. For example, gas chromatography-mass spectrometry (GC-MS) was used to detect herbicide residues in surface waters [2]. Other approaches, such as capillary gas chromatography (capillary GC) [3] and high performance liquid chromatography (HPLC) [4], have also been employed to determine mefenacet in food and in river water, respectively. However, these methods require more sophisticated instrumentation or are more time-consuming. Thus, it is very important to develop a simple and rapid method to detect mefenacet.

Capillary electrophoresis (CE) is a useful separation technique because of its high resolution, short analysis time, small sample volume and low operational cost. It has been widely used in analysis of DNA [5], RNA [6], proteins [7], enzymes [8], amino acids [9], anticancer drugs [10] and herbicides [11,12]. The most commonly used detection modes available for CE are fluorescence detection, laser-induced fluorescence detection [13,14], UV-visible spectrophotometric detection [15], mass spectrometry [16], chemiluminescence (CL) [17] and electrochemiluminescence (ECL) detection [18]. ECL detection, in comparison with other modes, offers lower background noise, higher detection sensitivity and requires simple and inexpensive instrumentation. The luminescence compounds undergo an electron-transfer reaction at the electrode surface to form excited states that can emit light. Tris(2,2′-bipydidyl)ruthenium(II), $Ru(bpy)_3^{2+}$, has been applied for the detection of hydrazine, amino acids [19], antibiotics [20], oxalate [21] and some clinical medicines [22,23].

In this paper, CE-ECL technique was employed to determine mefenacet. Influencing factors, such as applied potential, injection voltage, injection time, and pH of running buffer were investigated in detail. Residues of mefenacet in seedling and soil were also determined using the proposed technique.

2. Experimental

2.1. Reagents and solutions

Mefenacet (99.8%) was purchased from Haili Chemical Company (Hunan, China). Simazine (97%) and propanil (98%)

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were bought from Rainbow Chemical Co., Ltd. (Shandong, China). Urea (99%) was obtained from Guangzhou Chemical Company. Tris(2,2′-bipyridyl)ruthemnium(II) chloride hexahydrate (98%) was from J & K Chemical Company. Ethanol (≥99.7%) was purchased from Zhenxing Chemical Company (Shanghai, China). Phosphate buffer solutions with different pH values were prepared with Na_2HPO_4 and KH_2PO_4. 2.0×10^{-3} M mefenacet was prepared in ethanol as its solubility in water is very small (~4 mg/l). The stock solution was diluted to a series of concentrations before use. All chemicals used in this work were of analytical grade and double-distilled water was used throughout.

2.2. Apparatus

MPI-A CE-ECL was bought from Xi'an Remex Electronic Science-Tech Co. (China). The end-column detection cell of CE-ECL was composed of a three-electrode system (300 μm diameter Pt disc as working electrode, Ag/AgCl electrode as reference electrode, and Pt wire as counter electrode). The uncoated capillary (i.d. 25 μm, o.d. 375 μm) was bought from Yongnian Optical Conductive Fiber Plant (Hebei, China) and cut to 50 cm in length. The capillary was placed between two buffer reservoirs and the buffer was driven with the high potential apparatus.

CHI 660A electrochemical analyzer was purchased from Shanghai Chenhua Apparatus Company (China). Pt wire (1 mm in diameter) encapsulated in epoxy resin was used as working electrode, with an Ag/AgCl electrode and a platinum electrode as reference electrode and counter electrode, respectively. All potentials measured in the experiment were with respect to Ag/AgCl electrode.

2.3. Sample pretreatment

After burgeoning out, the paddy was planted in the soil, and then cultured in incubator under natural illumination for 5–7 days. Then 10 mg of mefenacet was sprinkled into the soil. Six days later, the seedling and the soil were collected and their contents of mefenacet were analyzed as follows.

Firstly, the soil was rinsed with distilled water (1 ml water per 1 g soil) for five times, and then the resulted slurry was filtrated out. Successively, the filtrate was concentrated at 72 °C under vacuum evaporation. Finally, the concentrate was filtrated twice using 0.22 μm cellulose acetate filters and collected. The extraction procedure for mefenacet from seedling was similar to that from soil. Then these extracts were analyzed by CE-ECL.

In addition, the draff of soil and seedling, which had been rinsed with water for five times, was further rinsed with water, respectively, then mefenacet content was analyzed again.

2.4. Electrophoresis procedure

Before each run, the capillary was flushed with running buffer for 10–15 min. The detection reservoir was filled with 250 μl of 5 mM $Ru(bpy)_3^{2+}$ solution (pH 7.38) before analysis, and replaced every 2 h to eliminate depletion effect. Injections were performed by electromigration at a constant voltage for several minutes. During the experiment, a 15 kV separation voltage was applied across the capillary and the potential of the photomultiplier tube (PMT) was set at 800 V.

3. Results and discussion

3.1. Cyclic voltammetry of reaction process

As shown in Fig. 1, curve a is the cyclic voltammogram of phosphate buffer solution. Curve b shows the cyclic voltammogram of 2 mM $Ru(bpy)_3^{2+}$ in phosphate buffer solution. As the potential increased positively, an oxidation peak was observed at 1.13 V (versus Ag/AgCl), which is attributed to the oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$. Curve c shows the cyclic voltammogram of 2 mM $Ru(bpy)_3^{2+}$ in the presence of 1.17 mM mefenacet. Compared with curve b, it can be seen that the reduction peak becomes weaker while the oxidation peak is greatly enhanced. In this reaction system, $Ru(bpy)_3^{2+}$ is first oxidized to $Ru(bpy)_3^{3+}$, then $Ru(bpy)_3^{3+}$ reacts with mefenacet and produces $Ru(bpy)_3^{2+*}$ after a series of reaction steps. $Ru(bpy)_3^{2+*}$, which is not stable, releases the energy and causes ECL emission [24]. Therefore, in the presence of mefenacet, $Ru(bpy)_3^{3+}$ is immediately consumed and an obvious catalytic oxidation peak can be observed. The proposed reaction mechanism between $Ru(bpy)_3^{2+}$ and mefenacet may be expressed as follows:

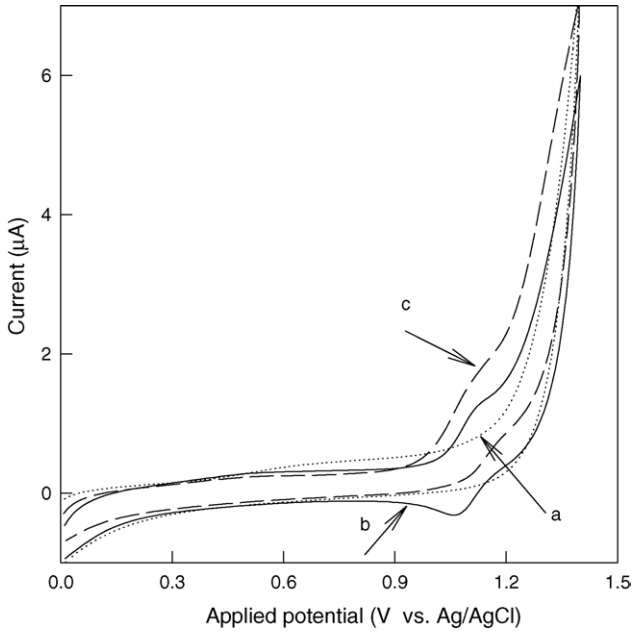
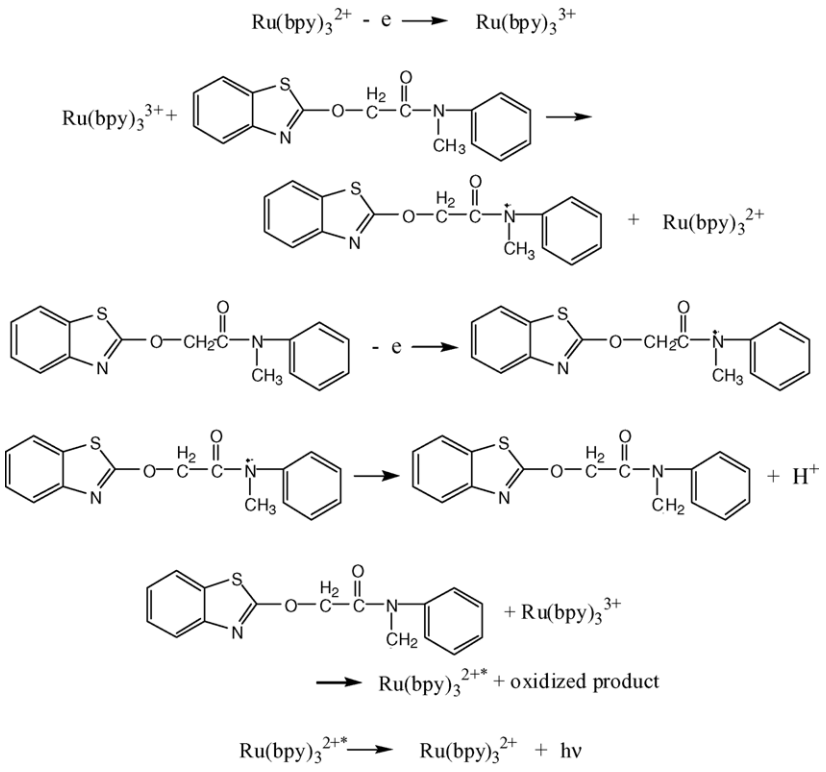


Fig. 1. Cyclic voltamograms of $Ru(bpy)_3^{2+}$ and mefenacet: (a) phosphate buffer solution (pH 7.38); (b) 2 mM $Ru(bpy)_3^{2+}$; and (c) 2 mM $Ru(bpy)_3^{2+}$ plus 1.17 mM mefenacet. Scanning rate: 100 mV/s.



3.2. Optimization of separation and detection conditions

Several factors that influence on the ECL intensity were investigated, including potential applied at the working electrode, injection time, injection voltage, and pH value of the running buffer.

3.2.1. Effect of the applied potential

The intensity of the emitted light is dependent on the rate of the light-emitting chemical reaction, and this reaction rate is relied on the potential applied to the electrode [25]. The relationship between ECL intensity and the applied potential was investigated in this work. It was found that with the increase of the applied potential, the ECL intensity increased and reached a maximum value at 1.2 V, then decreased slightly. Therefore, in the following experiment, the applied potential was set at 1.2 V.

3.2.2. Effect of injection time and injection voltage

As a key factor in CE, the effects of injection time and injection voltage were studied in detail. With the injection voltage being fixed at 11 kV, the effect of injection time of 2, 4, 6, 8, 10, 12 and 15 s on the ECL intensity and the number of theoretical plates is shown in Fig. 2. At the same time, fixing the injection time at 10 s, the influence of injection voltage of 2, 4, 6, 8, 10, 11, 13, and 15 kV on the ECL intensity and the number of theoretical plates is shown in Fig. 3. The theoretical plate numbers were calculated according to the following equation:

$$N = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

where N is the number of theoretical plates, t_m the migration time, and $W_{1/2}$ is the width at half height of the electrophoretic peak. It can be seen from Figs. 2 and 3, with the increase in injection time and injection voltage, ECL intensity increases while N decreases. The probable reason is that the longer the injection time and the higher the injection voltage, the more the analyte into the reservoir and hence the higher the ECL intensity. However, the analyte cannot reach the electrode surface immediately and diffuse into the solution, so the peak is retarded and broadened, and N decreased. Therefore, to compromise between the ECL intensity and the separation effect, 10 s and 11 kV were selected as the optimal injection time and injection voltage in the following experiments, respectively.

3.2.3. Effect of pH

Knight and Greenway studied the effect of pH on the ECL signals and found that the ECL intensity was smaller at lower pH because of the protonation of amine [26]. Noffsinger and Danielson investigated the relationship between the ECL intensity and the analyte pK_a [27]. Since the pH of buffer solution has an influence on the ECL intensity, the relationship between the ECL intensity and pH value from 5.19 to 9.18 was studied. With the increase of solution pH, the ECL intensity firstly increased and reached a maximum value at pH 7.38, then decreased gradually. The ECL intensity at pH 7.38 is more than three times higher than that at pH 5.19. Hence, pH 7.38 was employed as the optimal pH value.

3.3. Standard concentration curves of mefenacet

The relationship between ECL intensity and the concentration of mefenacet is shown in Fig. 4. The actual mefenacet

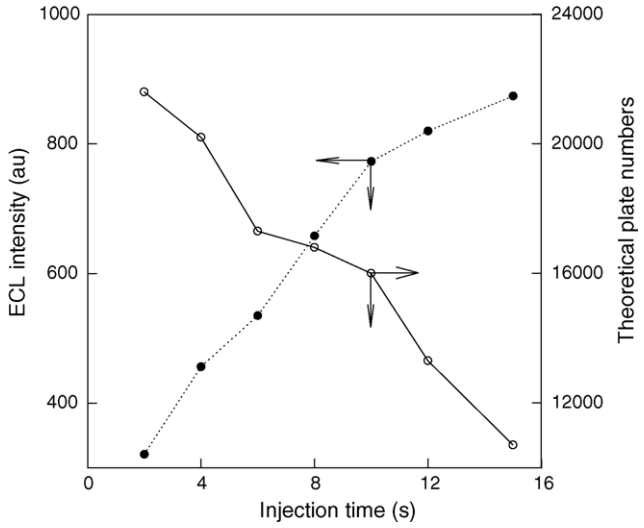


Fig. 2. Effect of injection time on the ECL intensity (●) of $Ru(bpy)_3^{2+}$ and the number of theoretical plate (○). Conditions: $Ru(bpy)_3^{2+}$, 5 mM; mefenacet, 2.0×10^{-5} M; injection voltage, 11 kV; phosphate buffer, pH 7.38.

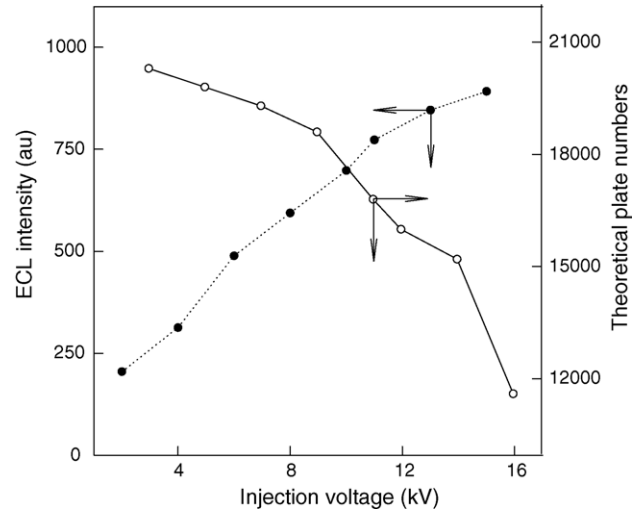


Fig. 3. Effect of injection voltage (●) on the ECL intensity of $Ru(bpy)_3^{2+}$ and the number of theoretical plate (○). Conditions: $Ru(bpy)_3^{2+}$, 5 mM; mefenacet, 2.0×10^{-5} M; injection time, 10 s; phosphate buffer, pH 7.38.

concentrations were 1.07×10^{-8}, 5.0×10^{-8}, 7.28×10^{-8}, 1.0×10^{-7}, 1.94×10^{-7}, 3.37×10^{-7}, 5.0×10^{-7}, 2.0×10^{-6}, 5.0×10^{-6}, 2.0×10^{-5}, 5.0×10^{-5}, and 2.0×10^{-4} M. With the increase in mefenacet concentration, the ECL intensity increases dramatically, then the intensity increase becomes smaller and smaller. It indicates that the amount of mefenacet

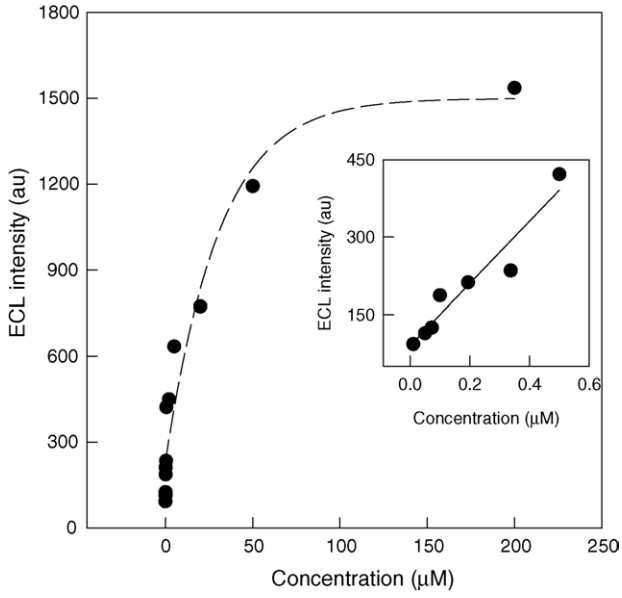


Fig. 4. The influence of mefenacet concentration on ECL intensity. Inset: the linear ECL intensity response to mefenacet concentration from 1.07×10^{-8} to 5.0×10^{-7} M. Conditions: $Ru(bpy)_3^{2+}$, 5 mM; injection voltage, 11 kV; injection time 10 s; phosphate buffer, pH 7.38.

reacting with $Ru(bpy)_3^{3+}$ was close to the maximum. The inset of Fig. 4 reveals a linear relationship between ECL intensity and mefenacet concentration over a range from 1.07×10^{-8} to 5.0×10^{-7} M as follows:

$$Y = 89.3 + 603.5X \quad (r = 0.961, \quad n = 7) \tag{2}$$

where Y is the ECL intensity (a.u.), X the concentration of mefenacet (μM). The recovery and relative standard deviation (R.S.D.) of the ECL intensity for 2.0×10^{-7} M mefenacet are 94.6 and 4.8%, respectively ($n = 5$). The limit of detection (LOD) for mefenacet is 4.0×10^{-9} M (obtained at S/N = 3), which is lower than that with other techniques. Comparison of the proposed method with other methods is listed in Table 1. Hence, CE-ECL is a sensitive method for determination of mefenacet.

3.4. Detection of mefenacet in seedling and soil

The proposed ECL technique was employed to detect mefenacet in seedling and soil. A typical example of the electropherograms of mefenacet extracted from soil is given in Fig. 5. The migration time was about 373 s, and the ECL intensity of mefenacet was 640, therefore, the concentration of mefenacet was about 5.0×10^{-6} M. The repeatability was studied by three

Table 1
Comparison of various methods for determination of mefenacet

Method	Application	Detection limit (M)	R.S.D. (%)	Recovery (%)	Reference
HPLC	Paddy field	2.1×10^{-6}	–	–	[4]
GC/MS	River water	8.7×10^{-8}	–	–	[27]
Capillary GC	Brown rice	3.4×10^{-8}	–	82.6 ± 7.3	[3]
CE-ECL	Mefenacet	4.0×10^{-9}	4.8[a]	94.6	This paper

[a] Stands for the R.S.D. of the migration time for 2.0×10^{-7} M mefenacet ($n = 5$).

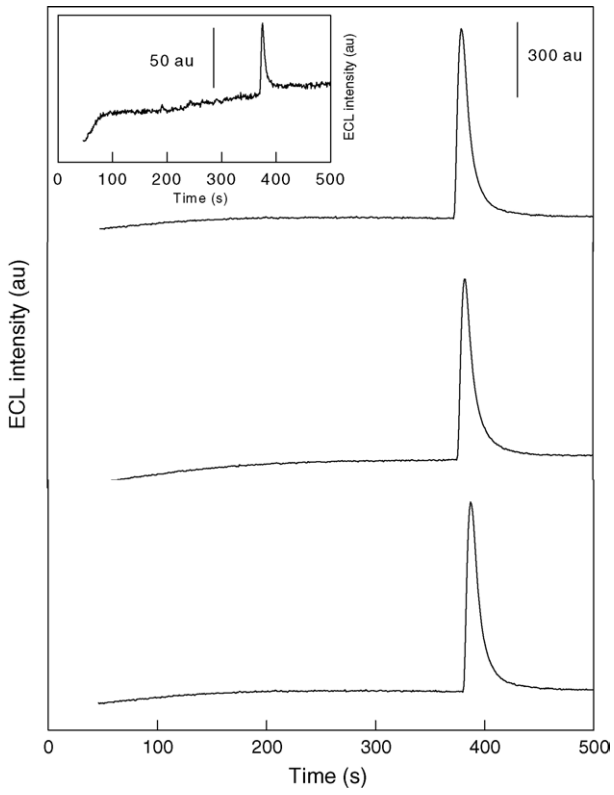


Fig. 5. CE-ECL electropherograms of extracts of mefenacet from soil. The inset stands for extract from seedling. Conditions: $Ru(bpy)_3^{2+}$, 5 mM; injection voltage, 11 kV; injection time 10 s; phosphate buffer, pH 7.38.

consecutive injections of mefenacet sample. The R.S.D. of the migration time and ECL intensity was 2.56 and 5.0%, respectively. The inset was the electropherograms of the extract from seedling. The ECL intensity was about 60, which was a little larger than the value of 53 (LOD, 4.0×10^{-9} M). Hence, the concentration of mefenacet in seedling was a little higher than 5.0×10^{-9} M. Compared with that in soil, the residue in seedling is much lower. According to the literatures [28,29], the reason may be that mefenacet absorbed readily onto soils at the surface layer of field when it was sprayed into the paddy field. The depth of soil where herbicides mainly remained was 0.5–2 cm, where most of weed seeds existed should be killed while no harm to the paddy.

Subsequently, the draff of soil and seedling, which had been rinsed with water for five times, was further rinsed with water, respectively, then the filtrate was analysed. No peak was found either in draff of seedling or soil. It indicates that the amount of mefenacet in the draff of seedling and soil is too little to be detected by this method.

3.5. Interference of other substances

A number of substances have been investigated to study the possible interference on mefenacet determination. These substances include nitrogen, phosphorus, and potassium salts, fertilizer, herbicide and pesticide. The analytical process is depicted as follows. Firstly, 2.0×10^{-5} M mefenacet was tested under the optimal conditions. A peak was found with the migration time at about 373 s. Then, a mixture of 2.0×10^{-5} M mefenacet with 2.0×10^{-5} M of the tested interferent was detected. The results are given in Table 2. The separation factor α is defined as,

$$\alpha = \frac{\Delta t}{t} \quad (3)$$

where Δt is the difference of migration time between mefenacet and the interferent, and t is the migration time of mefenacet. It can be seen that no peak was observed for the substances, such as diammonium hydrogen phosphate, ammonium ferric sulfate, ammonium chloride, potassium bicarbonate, potassium bisulfate, dipotassium hydrogen phosphate, propanil, and potassium nitrate. For calcium nitrate, sodium oxalate, cyhalothrin, and imidacloprid, the α values of them are 0.035, 0.051, 0.021 and 0.035, respectively. However, the ECL intensity of them is

Table 2
Study on interference of other substances on the determination of mefenacet

Interfering substance		Migration time (s)	Δt (s)	Intensity (a.u.)	α
Diammonium hydrogen phosphate	Hydrogen				
Ammonium ferric sulfate					
Ammonium chloride		No peak observed			
Potassium bicarbonate					
Potassium bisulfate			–	–	–
Dipotassium hydrogen phosphate	Hydrogen				
Propanil					
Potassium nitrate					
Calcium nitrate		360	13	20	0.035
Diphenylamine		310	63	500	0.168
Sodium oxalate		354	19	75	0.051
Sodium acetate		735	362	22	0.970
Aminoacetic acid		310	63	440	0.168
Nicotine		310	63	500	0.168
Urea		730	357	280	0.957
Simazine		620	247	60	0.662
Cyhalothrin		365	8	135	0.021
Imidacloprid		386	13	190	0.035

Each value represents the mean for five measurements.

smaller than that of mefenacet. Therefore, these substances do not cause any significant interference on mefenacet determination.

4. Conclusion

The CE-ECL method developed in this work is simple, rapid, specific, and sensitive. A linear response range of mefenacet from 1.07×10^{-8} to 5.0×10^{-7} M was obtained with a detection limit of 4.0×10^{-9} M. Residues of herbicide mefenacet in seedling and soil were investigated with this technique. The result obtained shows that this technique is suitable for analyzing the residues of herbicides in water and soil. It may offer a rapid detection method for the monitoring of environmental pollution problem or the residue content of herbicides in food.

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