

Capillary Electrophoretic Method for the Determination of Paraquat in Formulations and Biological Fluids

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Abstract : The use of capillary electrophoresis (CE) as an analytical method for the determination of the herbicide paraquat was evaluated. CE operating systems such as the kind of electrolyte used, its pH and other additives were investigated. The most satisfactory electrolyte tested was based on glycine-HCl buffer as it offers the best reproducibility in terms of peak areas. The final parameter adopted was : glycine-HCl buffer in the presence of 40 mM NaCl and 5% methanol at pH 3.0. In all cases, direct UV detection at 254 nm was done. Under such conditions and when operated at 10.0 kV, 20.0 kV and 30.0 kV using the electrokinetic injection mode at 25 °C, paraquat was eluted at about 10.0, 6.5 and 2.2 min, respectively. Key analytical characteristics of the CE method were : detection limit (signal-to-noise ratio, S/N of 3) 0.05 ppm; linearity of calibration curve 0.3 – 10.0 ppm; % RSD for the determination of 5 ppm paraquat that was spiked to artificial serum for run-to-run and day-to-day determinations were 0.93 and 0.64% respectively. The method was applied towards the assay of paraquat in formulations, urine, serum, vomitus and stomach-washout samples. In general, there was good agreement between results obtained from the CE and the high-performance liquid chromatographic (HPLC) method.

Abstrak : Kaedah elektroforesis rerambut (CE) telah dikaji kesesuaiannya sebagai satu kaedah analisis dalam penentuan racun tumbuhan parakuat. Sistem pengendalian CE seperti jenis elektrolit yang diguna, nilai pH-nya dan bahan tambahan lain telah diselidiki. Elektrolit yang telah dikaji yang memberi keputusan paling memuaskan adalah sistem penimbal glisin-HCl dengan kebolehdulungan luas di bawah puncak yang paling baik. Parameter yang telah dipilih akhirnya dan digunakan adalah : penimbal glisin-HCl dengan kehadiran 40 mM NaCl dan 5% metanol pada pH 3.0. Dalam semua kajian, pengesanan UV langsung pada 254 nm telah dilakukan. Di dalam keadaan ini, apabila CE dioperasikan pada 10.0 kV, 20.0 kV dan 30.0 kV dengan mod suntikan elektrokinetik pada 25 °C, parakuat telah dielusikan pada masing-masing lebih kurang 10.0, 6.5 dan 2.2 min. Ciri-ciri utama analisis dengan kaedah CE adalah : had pengesanan (nisbah isyarat terhadap bisingan, S/N 3), 0.05 ppm; kelinearan keluk tentukan antara 0.3 – 10.0 ppm; sisihan piawai relatif (% RSD) bagi penentuan dalam sehari dan antara hari untuk pakuan 5 ppm parakuat ke dalam serum tiruan adalah masing-masing 0.93 dan 0.64%. Kaedah ini telah digunakan untuk pencerakanan parakuat dalam formulasi, sampel air kencing, serum, muntah dan cucian perut. Secara umumnya, keputusan analisis yang diperolehi melalui kaedah CE adalah sepadan dengan yang diperolehi menggunakan kaedah kromatografi cecair prestasi tinggi (HPLC).

Received : 8.8.00, accepted 21.9.01

Introduction

Paraquat is a broad-spectrum contact herbicide that is used widely in the agricultural sector and sometimes as aquatic herbicide in more than 130 countries throughout the world [1,2]. Although the detrimental effect of paraquat exposure, such as its ability to cause pulmonary fibrosis, is well documented, there are still medical issues that remain unresolved. The effects of paraquat on the reproductive outcome when exposed during early pregnancy (in Malaysia 80 – 90% of workers who use pesticides are woman) is still not clear [2]. Paraquat has also been implicated as an environment toxin which may induce the syndrome of Parkinson's disease after exposure to this agent, eventhough the

exact biochemical mechanism by which paraquat causes cell death and neurodegeneration is not fully understood [3,4].

In Malaysia, 300 – 450 cases of poisoning has been contributed by paraquat annually, and 73% of the poisoning is due to suicide while the remainder is due to accidental and occupational exposure [5]. In the treatment and management of such cases, rapid analytical methods for the determination of paraquat in body fluids is of immense importance. The conventional method involves the formation of a blue complex of a pretreated alkaline solution upon the addition of sodium dithionate [6]. Measurement of the absorbance of the complex at 600 nm formed the basis of the analytical determination. However, this

method is plagued by several shortcomings, the major one is due to the instability nature of the radical formed which posed handling and reproducibility problems. To overcome this problem, flow injection analysis methodology has been proposed [7]. Our research group have reported on paraquat sensors that possessed attractive features such as simplicity, low cost and speed of analysis [8-10]. However, determination of paraquat remains problematic, mainly due to interference from endogenous component(s) [10]. HPLC methods have also been reported [11,12].

CE has emerged as a powerful analytical tool that has been applied successfully to the separation and analysis of numerous simple and complex molecules ranging from ions, vitamins, drugs, amino acids, and nucleotides, to peptides, and glycoproteins [13]. Inherent characteristics of the technique, such as small sample size capability, high efficiency, high speed analysis, low reagent consumption, naturally lends itself to the analysis of clinical samples. Carneiro *et al.* [11] and Wigfield *et al.* [14] have reported on the CE method for the determination of paraquat, diquat and difenzoquat residues in crop waters, and paraquat and diquat residues in potatoes, respectively. The use of CE for the determination of paraquat and diquat in fortified sera has been reported by Tomita *et al.* [15]. In view of the widespread interest in CE, here we wish to report on our evaluation of the technique for the separation and quantitation of paraquat in several types of samples with different matrices, namely weedicide formulations, artificial serum, urine, stomach-washouts and vomitus. A solid phase extraction (SPE) was used as a clean-up procedure for the pretreatment of biological samples prior to the analytical determinations.

Experimental

Reagents and Chemicals

All solutions, electrolytes and standards were prepared using 18.2 M Ω -cm Milli-Q water generated by Milli-Q Plus Water System (Millipore, Bedford, MA, USA). Paraquat dichloride was obtained from Aldrich (Milwaukee, WI, USA), glycine and potassium hydroxide from Merck (Darmstadt, Germany) while the control serum was supplied by Sigma (St. Louis, MO, USA). Chemicals required for the preparation of buffer solutions for the working electrolyte were purchased from Ajax Chemicals (Sydney, Australia). Fresh working electrolytes and working standards were prepared daily, vacuum-filtered and degassed prior to use.

Test Samples

All the six formulation herbicides were purchased from various agricultural outlets in Penang. Human serum, vomitus and stomach-washout were obtained from the Chemistry Department, Penang, while urine samples were obtained from student volunteers. Paraquat content in the formulations was determined after dilution of

1:10000 (formulation:water). Run-to-run and day-to-day determinations were carried out on control serum that contained 5 ppm spiked standard paraquat.

Solid Phase Extraction

Urine and serum samples were subjected to the solid phase extraction (SPE) procedure as described by Gill *et al.* [16]. C18 cartridges were used for the clean-up of 1 mL sample that was made alkaline by adding concentrated ammonia. The final solution from the elution was evaporated to dryness and the residue was dissolved in 1 mL water before injecting into the HPLC or CE unit.

Instrumentation

HPLC

Reversed phase ion-pair HPLC separation was carried out on a Hitachi L-4250 unit using a Phenomenex Spherisorb 10 ODS (250 x 4.60 mm) column at room temperature, in conjunction with a Hitachi L-6200 intelligent pump. The detection wavelength was at 257 nm and the volume of sample injected was 20 μ L. An aqueous solution containing 10 mM sodium heptanesulphonic acid, 22 mM sodium dihydrogen orthophosphate, 35 mM disodium hydrogen dodecahydrate and 100 mM triethylamine that were mixed with acetonitrile at a ratio of 78:22 (v/v) was used as mobile phase. The solutions were passed through a 0.45 micron filter before degassed for 15 min in an ultrasonic bath.

CE

Analytical separation was carried out on a Waters Capillary Ion Analyzer (Milford, MA, USA) which was interfaced to a Waters PC 800 Workstation. The capillaries (75 μ m internal diameter x 60 cm) used were constructed of fused silica and was supplied by Waters. Direct UV detection was performed at 254 nm with a mercury lamp and a 254-nm optical filter. The samples were introduced into the capillary using 10-V electrokinetic injections. Paraquat determinations were performed by using a positive power supply with the applied voltage set, unless stated, at 10.0 kV and thermostated at 25 $^{\circ}$ C. Each day before starting any analysis, the capillary was conditioned by sequential purging with 100 mM potassium hydroxide solution and then followed by Milli-Q water for 5 min. Between each run, the capillary was rinsed with electrolyte for 2 min. Before powering down, the capillary was flushed and cleaned with Milli-Q water for 5 min. These steps were crucial for the required reproducibility and effectiveness of the capillary electrophoretic separation.

Results and Discussion

The type of electrolyte used as running buffer that were reported in the literature were evaluated. This was done by using the respective electrolyte and the repeated injection of 10 ppm paraquat standard. The peak area reproducibility, as indicated by the % RSD of 10 successive injections is shown in Table 1.

Table 1. Comparison of the Effect of Electrolyte Systems for the Repeated Injections of 10 ppm Paraquat Standards

Type of Electrolyte	Electrophoretic Mobility, $\text{cm}^2\text{V}^{-1}\text{min}^{-1}$	Peak Area Reproducibility, % RSD
Acetate buffer, pH 4.0 in the presence of 10.0 mM NaCl	0.309	7.94
Phosphate buffer, pH 3.5 in the presence of 200 mM NaCl	0.325	13.90
Glycine-HCl buffer, pH 3.0 in the presence of 40.0 mM NaCl and 20% methanol	0.315	0.896

* Mean migration times of 10 min, 6.5 min and 2.2 min for the applied voltage of 30.0 kV, 20.0 kV and 10.0 kV, respectively.

Of the 3 electrolytes evaluated, the use of glycine-HCl buffer was found to be the most satisfactory in terms of peak area reproducibility. The electropherograms obtained were all of good quality, and paraquat peaks were observed at around 10 min, regardless of the type of buffer used when operated at 10.0 kV. Predictably, the migration time was reduced to around 2 min when the system was operated at 30.0 kV. Based on this consideration, the glycine-HCl buffer was used for further studies.

The effect of pH of the glycine-HCl buffer used was studied by preparing the buffer over the pH range of 2.0 – 5.0. This range was chosen as it is well recognized that paraquat is only stable in neutral and acidic conditions, but are destroyed in alkali medium [1]. The optimal pH was 3.0 as higher or lower pH values caused serious reduction in the signal sensitivity. The effect of methanol was also studied. It was found that the used of 5% and 20% methanol gave % RSD values of 0.276 and 0.896% respectively. The final electrolyte composition adopted for the studies was similar to that reported by Tomita *et al.* [15], except that the percentage of methanol used was reduced from 20% to 5% : 10 mM glycine-HCl buffer at pH 3.0 containing 40 mM NaCl and 5% methanol. Under these operating conditions, the detection limit of the technique (S/N 3) was found to be 0.05 ppm. Calibration curve was found to be linear over the concentration range of 0.3 – 10.0 ppm, and the correlation coefficient was at least 0.9700. The precision of the method was determined on spiked 5 ppm paraquat standard to artificial serum solution. Average recoveries and % RSD of 97.8 and 0.93%, respectively for run-to-run, and 97.0 and 0.64% for day-to-day determinations were obtained, suggesting that the method possesses not only good accuracy but also excellent reproducibility.

Results for the determination of 6 diluted commercial herbicide formulation is shown in Table 2. There is good agreement between the CE and thereversed-phase HPLC method. The CE method was also applied for the determination of paraquat in serum and urine samples that were fortified with

Table 2. Comparison of Results for the Determination of Paraquat Dichloride in Weedicide Formulation Samples Using the CE and HPLC Methods.

Brand	Paraquat Dichloride Concentration (% w/w)	
	CE	HPLC
<i>Action</i>	30.30 ± 0.12	25.05 ± 0.32
<i>Contact</i>	22.00 ± 0.05	22.78 ± 0.21
<i>Gramoxone</i>	23.30 ± 0.09	25.16 ± 0.56
<i>PQ XTRA</i>	30.33 ± 0.12	26.32 ± 0.29
<i>Silverquat</i>	21.33 ± 0.07	23.35 ± 0.38
<i>Superzone</i>	28.33 ± 0.13	24.70 ± 0.69

* All samples were analysed without using the SPE procedure.

paraquat standard. Percent recoveries of paraquat that were obtained from the CE and HPLC are shown in Table 3. Table 4 shows a comparison of the values of paraquat that were determined in real samples such as vomitus and stomach-washouts. Good agreement between the CE and HPLC results were obtained in these fortified and real samples (Tables 3 and 4). Typical electropherogram obtained from a serum sample is shown in Figure 1.

Conclusions

A CE method for the determination of paraquat in herbicide formulations, fortified serum, urine, vomitus and stomach-washouts was carried out. The complementary features of the CE method such as high efficiency, rapid separation, low reagent consumption and requiring small quantities of samples made it a natural candidate for the routine determination of paraquat in real samples.

Table 3. Results for the Percent Recoveries of Paraquat that were Spiked to Various Samples.

Sample	Spiked Paraquat Concentration (ppm)	Percent Recovery (%)	
		CE	HPLC
Artificial Serum (Normal)	5.0	90.82 ± 0.35	–
Real Serum	5.0	78.60 ± 0.03	–
Artificial Serum (Normal)	20.0	92.79 ± 0.03	87.37 ± 0.71
Artificial Serum (Abnormal)	20.0	94.05 ± 0.03	92.43 ± 0.51
Urine	20.0	83.60 ± 0.03	88.07 ± 0.53
Urine	25.0	92.21 ± 0.01	90.41 ± 0.60
Urine	30.0	91.92 ± 0.02	90.16 ± 0.73

*n ≥ 3

Table 4. Comparison of Results for the Determination of Paraquat in Body Fluids using the CE and HPLC Methods.

Sample	Paraquat Concentration (ppm)	
	CE	HPLC
Vomitus	98.0 ± 0.1	98.8 ± 0.7
Stomach-Washout 1	1567.0 ± 0.1	1612.0 ± 0.5
Stomach-Washout 2	2067.3 ± 0.1	2031.8 ± 0.4

*n ≥ 3

Figure 1. Typical electropherogram obtained from the injection of a serum sample when a voltage of 30 kV was used. Please refer to the text for the details of the electrophoretic conditions.

Acknowledgment

We wish to thank En. Shaharudin Hasan of Chemistry Department, Penang, for the kind provision of the body fluid samples. Financial support from the Universiti Sains Malaysia for a short term research grant is also greatly appreciated.

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