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Simple and Sensitive Electrokinetic Supercharging in Capillary Electrophoresis for Online Preconcentration and Separation of Secbumeton in Water Samples

(Superpengesan Elektrokinetik Ringkas dan Sensitif dalam Elektroforesis Rerambut Prapemerkatan secara Terus dan Permisahan Sekbumeton dalam Sampel Air)

RASMEY SOEUNG, NADHIRATUL-FARIHIN SEMAIL, WAN ADNAN WAN OMAR, NUR NADHIRAH MOHAMAD ZAIN, MAZIDATULAKMAM MISKAM, YONG FOO WONG, AEMI SYAZWANI ABDUL KEYON, MUGGUNDHA RAOOV RAMACHANDRAN, SAZLINDA KAMARUZAMAN & NOORFATIMAH YAHAYA*

ABSTRACT

This study describes an electrokinetic supercharging for online preconcentration capillary electrophoresis (CE) technique of secbumeton in water samples. Important CE separation and preconcentration conditions, such as concentration and pH of the background electrolyte, applied voltage and ultraviolet wavelength, type and injection time of the terminating electrolyte, and injection time of the leading electrolyte and sample were investigated and optimized. The optimum conditions involved hydrodynamic injection of leading electrolyte (100 mM sodium chloride, 30 s, 50 mbar), electrokinetic injection of the sample as high as 250 s (at +7 kV voltage), and hydrodynamic injection of terminating electrolyte (100 mM Tris buffer, 40 s, 50 mbar). This strategy enhanced secbumeton detection sensitivity up to 3847-fold and 2267-fold when compared with hydrodynamic and electrokinetic injection, respectively, providing a limit of detection as low as 0.03 μg L^{-1} with good repeatability (relative standard deviation < 4%, n = 5). Wide linear range (0.1–500 μg L^{-1}) with good linearity (R^2 = 0.9997) was obtained. The limit of detection was adequate for the analysis of secbumeton in water samples with concentrations lower than its maximum residual limit (0.1 μg L^{-1}). The developed method was applied to environmental water samples, and recoveries were between 85.7 and 105.6%.

Keywords: Capillary electrophoresis; electrokinetic supercharging; environmental water samples; online preconcentration; secbumeton

ABSTRAK

Kajian ini menerangkan suatu superpengecasan elektrokinetik bagi prapemekatan secara terus teknik elektroforesis rerambut (CE) bagi sekbumeton di dalam sampel air. Pemisahan CE dan keadaan prapemekatan yang penting seperti kepekatan dan pH bagi latar belakang elektrolit, voltan gunaan dan panjang gelombang ultraviolet, jenis dan masa suntikan bagi elektrolit penamat dan masa suntikan bagi elektrolit pemula dan sampel telah dikaji dan dioptimumkan. Keadaan optimum termasuklah suntikan hidrodinamik bagi elektrolit pemula (100 mM natrium klorida, 30 s, 50 mbar), suntikan elektrokinetik bagi sampel setinggi 250 s (pada +7 kV voltan) dan suntikan hidrodinamik bagi elektrolit penamat (100 mM larutan penimbal TRIS, 40 s, 50 mbar). Strategi ini meningkatkan kepekaan penentuan sekbumeton sehingga 3847-gandaan dan 2267-gandaan apabila dibandingkan dengan masing-masing, suntikan hidrodinamik dan elektrokinetik, dengan memberi had pengesanan serendah 0.03 μg L^{-1} dengan kebolehulangan yang baik (sisihan piawai relatif < 4 %, n = 5). Julat linear yang besar (0.1–500 μg L^{-1}) dengan kelinearan yang baik (R^2 = 0.9997) telah diperoleh. Had pengesanan ini adalah mencukupi bagi menganalisa sekbumeton di dalam sampel air dengan kepekatan yang lebih rendah daripada had surih maksimum (0.1 μg L^{-1}). Kaedah yang telah dibangunkan telah digunakan bagi sampel air sekitaran dan pengembalian adalah antara 85.7 dan 105.6%.

Kata kunci: Elektroforesis rerambut; prapemekatan secara terus; sampel air sekitaran; sekbumeton; superpengecasan elektrokinetik

INTRODUCTION

Pesticides and herbicides are frequently used in humid regions and agricultural sites to provide high quality agricultural products. For instance, S-triazine pesticides have been applied all over the world to control weeds in forestry and industrial sites (Siripattanakul et al. 2009). Although soil bacteria have pesticide biodegradation properties, the amount of accidental exposure to pesticides experienced by ecosystems is increasing due to the intensive use making them more frequently detected in soil (Nousiainen et al. 2015). Moreover, heavy rainfall causes significant movement of herbicides into the drainage systems and run off, leading to flooding of chemical residues in surface water. Atrazine concentrations above the maximum residue limit (MRL) for drinking water have been frequently detected because of its high mobility and persistence in soil and its massive application to crops (Piutti et al. 2002; Sagarkar et al. 2013). The MRLs of various triazines established by the United States

Environmental Protection Agency are between 1 and 4 µg L^{-1} (Watershed Management Section 2016). Although atrazines were banned in October 2003 among different European countries, persistent pollutions of atrazine in surface-water and ground water systems have been frequently reported due to illegal applications of this pesticide in agriculture (Jablonowski et al. 2011).

Due to the long term and extensive use, the triazine pesticides have been found in many agricultural products (Li et al. 2017; Liang et al. 2018). Health issues, including dizziness, drowsiness, gastric, and intestinal issues, cracking or skin dryness, and corneal problems are caused by secbumeton (SEC) toxicity (Alvarsson 2012). This toxicant can spread to the environment and into aquatic organisms, including fish, algae, and bacteria, which could affect climate, and ecology (LeBaron et al. 2008). Monitoring of the influence of pesticide contamination on aquatic systems has been implemented in many parts of Europe, Japan, and America (Yamaguchi et al. 2003), in fact, in Canada, drinking water has the lowest acceptance level for triazine (i.e. 0.005 mg L^{-1}) among all types of agriculture-based food products of concerns (Safe Environments Programme 2006). Therefore, a rapid, sensitive, accurate, and environmentally friendly method is required to monitor the concentrations of triazines in the environment.

Numerous methods have been developed to separate and detect triazines from samples, including high performance liquid chromatography with an ultraviolet detector (HPLC-UV) (Gao et al. 2012; Liu et al. 2014; Wang et al. 2012; Yang et al. 2014), HPLC with a diode array detector (DAD) (Rodríguez-González et al. 2014; Zhao et al. 2011), gas chromatography-mass spectrometry (GC-MS) (Sanagi et al. 2012), liquid chromatography-mass spectrometry (LC-MS) (Ji et al. 2008), and micro-LC with UV-Vis detector (See et al. 2010). These methods are relatively less green and time consuming, as most require off-line extraction and a large volume of organic solvents as extractant (i.e. liquid-liquid extraction and solid-phase extraction) (Acedo-Valenzuela et al. 2004). As an alternative, utilizing capillary electrophoresis (CE) system due to its ability in online preconcentration prior to separation. One of the preconcentration techniques that has been developed can provide high sensitivity without a significant increase in sample-to sample time is termed electrokinetic supercharging (EKS) (Hirokawa et al. 2003).

EKS is the combination of field-amplified sample injection (FASI) and transient isotachophoresis (tITP) was introduced by Hirokawa in 2003. In comparison to the conventional hydrodynamic injection where all solution is introduced under pressure to the capillary, electrokinetic injection is preferable used only for charged species. Those selected species are introduced based on their electrophoretic mobility in to the capillary. When the injection proceeds under co-electroosmotic flow conditions, apparent mobilities should be taken into account (Khaledi 1998). This technique has been shown to have excellent preconcentration efficiency when used to detect non-steroidal inflammatory drugs, paraquat and sedative drugs in samples (Botello et al. 2013; Dawod et al. 2008; Qi et al. 2017). Enhancement factors up four orders of magnitude have been reported in the literature (Abdul Karim et al. 2016; Botello et al. 2013; Dawod et al. 2009; Lu et al. 2014; Wen et al. 2010). Thus, this technique overcomes the shortcoming of low sensitivity.

Thus, the aim of this study was to develop a simple and sensitive electrokinetic supercharging capillary zone electrophoresis (EKS-CZE) method for the online preconcentration and separation of SEC in selected environmental water samples. To the best of our knowledge, this is the first report of the use of EKS for SEC analysis in environmental waters samples.

Materials and Methods

The SEC standard (purity ≥ 97%) and Trizma base (Tris) used in this study were purchased from Sigma-Aldrich, Co., (St. Louis, MO, USA) (Figure 1). HPLC-grade methanol was obtained from Friendemann Schmidt Chemical (Parkwood, WA, Australia). Sodium chloride (NaCl) and sodium hydroxide (NaOH) were purchased from R&M Chemicals (Essex, UK). Formic acid was purchased from QRëC (ASIA) Sdn. Bhd. (Selangor, Malaysia). Milli-Q water used for stock and standard preparation was produced using a Sartorius Milli-Q water deionization system (Molsheim, France) with resistivity of 18.2 MΩ cm^{-1}. Standard solution of SEC (1000 mg L^{-1}) was prepared in methanol and stored in the dark at 20 °C. Standard mixtures of working solutions of SEC were prepared from appropriate dilutions of the stock made with deionized water. All other reagents were of analytical grade and used without any further purification.

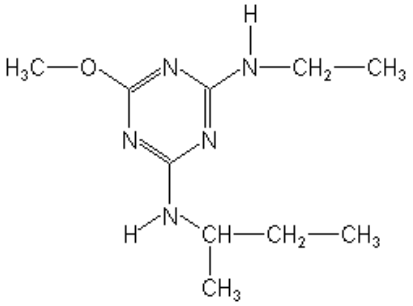

FIGURE 1. Chemical structure of SEC

INSTRUMENTATION AND CE CONDITIONS

Electrophoretic separation was carried out on an Agilent CE 7100 device (Agilent Technologies, Santa Clara, CA, USA) coupled to a UV-DAD system. A bare fused-silica capillary of 50 µm I.D. with total and effective lengths of 65 and 56 cm, respectively, was used (Agilent Technologies). Electrophoretic data were processed using ChemStation software (Agilent Technologies). New capillaries were preconditioned by flushing with 1 M NaOH for 20 min, 0.1 M NaOH for 15 min, and deionized water for 10 min. After each separation, the capillary was rinsed with 0.1 M NaOH for 5 min, deionized water for 5 min, and background electrolyte (BGE) for 15 min to maintain the repeatability of the analysis. The BGE was adopted from previously reported work (Arribas et al. 2011) with slight modifications. It consisted of 100 mM formic acid solution adjusted to pH of 2.5 with 1 M NaOH. The BGE was freshly prepared daily prior to analysis to avoid any possible degradation of formic acid. A typical hydrodynamic injection (HDI) procedure was performed at 50 mbar for 10 s. For FASI, a voltage of +5 kV and an injection time of 10 s were applied after filling the capillary with BGE.

ELECTROKINETIC SUPERCHARGING CAPILLARY ZONE ELECTROPHORESIS PROCEDURE

EKS was carried out by first filling the capillary with BGE followed by a short plug of 100 mM NaCl, which functioned as the leading electrolyte (LE). The LE was hydrodynamically injected at 50 mbar for 30 s. Next, the sample solution containing the analyte was injected at +7 kV for 250 s. Lastly, 100 mM Tris as the terminating electrolyte (TE) was injected hydrodynamically at 50 mbar for 40 s. A voltage of +25 kV was applied to re-stack the diffuse band of injected analyte between LE and TE, followed by a CZE separation.

PREPARATION OF WATER SAMPLES

Pond water was collected from a pond near the Advanced Medical and Dental Institute, Universiti Sains Malaysia, Pulau Pinang, Malaysia. River water was obtained from Kepala Batas, Pulau Pinang, Malaysia. All samples were stored in Schott bottles and transferred to the laboratory. Prior to analysis, each water sample was filtered through a 0.45 µm nylon filter membrane. The samples were kept in the refrigerator at 4 °C to avoid for sample preservation.

RESULTS AND DISCUSSION

OPTIMIZATION OF ELECTROKINETIC SUPERCHARGING CAPILLARY ZONE ELECTROPHORESIS PARAMETERS

The hydrodynamic injection capillary zone electrophoresis (HDI-CZE) system was firstly optimized prior to the development of EKS-CZE. Several important parameters affecting the sensitivity of the system to Secbumeton (SEC) as the model analyte for atrazine herbicides were investigated, including the optimum UV wavelength, selection of BGE, pH and concentration of BGE, separation voltage and injection time. All optimization experiments were carried out in triplicate using deionized water spiked with 20 mg L^{-1} of SEC.

EFFECTS OF UV WAVELENGTH, TYPE OF BACKGROUND ELECTROLYTE, AND BACKGROUND ELECTROLYTE PH ON THE CAPILLARY ZONE ELECTROPHORESIS SEPARATION OF SECBUMETON

The optimum UV wavelength within the range of 200 to 260 nm was obtained at 214 nm by a scanning UV/VIS spectrophotometer. This wavelength provided the highest sensitivity and the best peak area and peak height of SEC with an acceptable percentage relative standard deviation (% RSD < 3%). The buffer used for CE separation can be prepared from several types of electrolytes. Formate, acetate, and phosphate are frequently used as low pH buffers (Arribas et al. 2011; Yan et al. 2003). SEC is a weak base with pK_a ~4.4, thus it will be positively charged when present in an acidic medium. Therefore, a buffer with pH < 4.4 was needed as the BGE for this study; sodium phosphate at pH 2, sodium acetate at pH 4, and sodium formate at pH 2.5 were tested. Sodium formate at pH 2.5 was found to be the most suitable BGE, with acceptable repeatability (RSD < 4%; n = 5) and low background noise. This was in accordance with work by Arribas et al. (2011) that found formate buffer provided optimal resolution and efficiency and a moderate analysis time for the same group of pesticide (i.e. atrazine).

In CE separation, the most important parameter is the pH of BGE because it controls the direction and magnitude of electroosmotic flow (EOF), influences the charge of the compound, and affects the current and production of heat (Weinberger 2000). In this study, the effect of BGE pH on the separation of SEC was investigated under different pH values of sodium formate ranging from 2.5 to 4 (the pK_a of formic acid was 3.75) (Reutemann et al. 2011). The highest peak area was obtained at pH 4 (5.3 mAU) and the lowest at pH 2.5 (3.2 mAU). The results indicated that higher pH value (pH 4) produced highest peak area due to broadening effect. However, pH 2.5 resulted in the fastest analysis time (2.5 min), and reproducibility at pH 2.5 was also the lowest (< 6%). SEC was not further resolved at pH > 2.5, but instead only migrated with the EOF with a broad peak. In contrast, more acidic pH increased SEC mobility as it became positively charged. Based on these results, the most suitable separation electrolyte with optimal resolution, highest peak height, and shortest migration time was 100 mM sodium formate at pH 2.5 (25 kV separation voltage).

This result indicated that higher pH value may provide highest peak area as well as peak height. However, pH 2.5 gave the lowest migration time compared to other pHs, and RSD of pH 2.5 was also the lowest and satisfactory (RSD < 6). Moreover, the EOF appeared at minimum time at pH 3 up to 4 which may result in

overlapping peaks due to peak broadening of SEC and EOF in further optimization. Thus, the electroosmotic mobility was strongly raised at higher pH value of the buffer and extremely low at the acidic ranges.

EFFECTS OF BACKGROUND ELECTROLYTE CONCENTRATION, APPLIED VOLTAGE, AND INJECTION TIME ON THE CAPILLARY ZONE ELECTROPHORESIS SEPARATION OF SECBUMETON

Another parameter that significantly influences CE separation and detection is the BGE concentration. Therefore, the effect of BGE concentration ranging from 80 to 120 mM (fixed at pH 2.5) were tested on CZE of SEC. As a result, the migration time and peak area increased gradually from the lowest to the high concentration (80-100 mM), however, no significant enhancement was observed beyond 100 mM (Figure 2(a)). By considering the highest peak area with acceptable migration time (< 6 min) of SEC and %RSD < 3%, 100 Mm formate buffer was chosen as the optimum BGE concentration for subsequent experiments. Additionally, several previous studies reported that buffer concentrations higher than 100 mM were associated with the current and contributed to the Joule heating phenomenon (Arribas et al. 2011). The capacity of the capillary thermostatted system at higher separation voltage may be overwhelmed by the higher buffer concentration. Moreover, the stability of the analyte may be affected by this extreme Joule heating (Grossman 1992). Therefore, higher BGE concentration (> 100 mM) not used in this study.

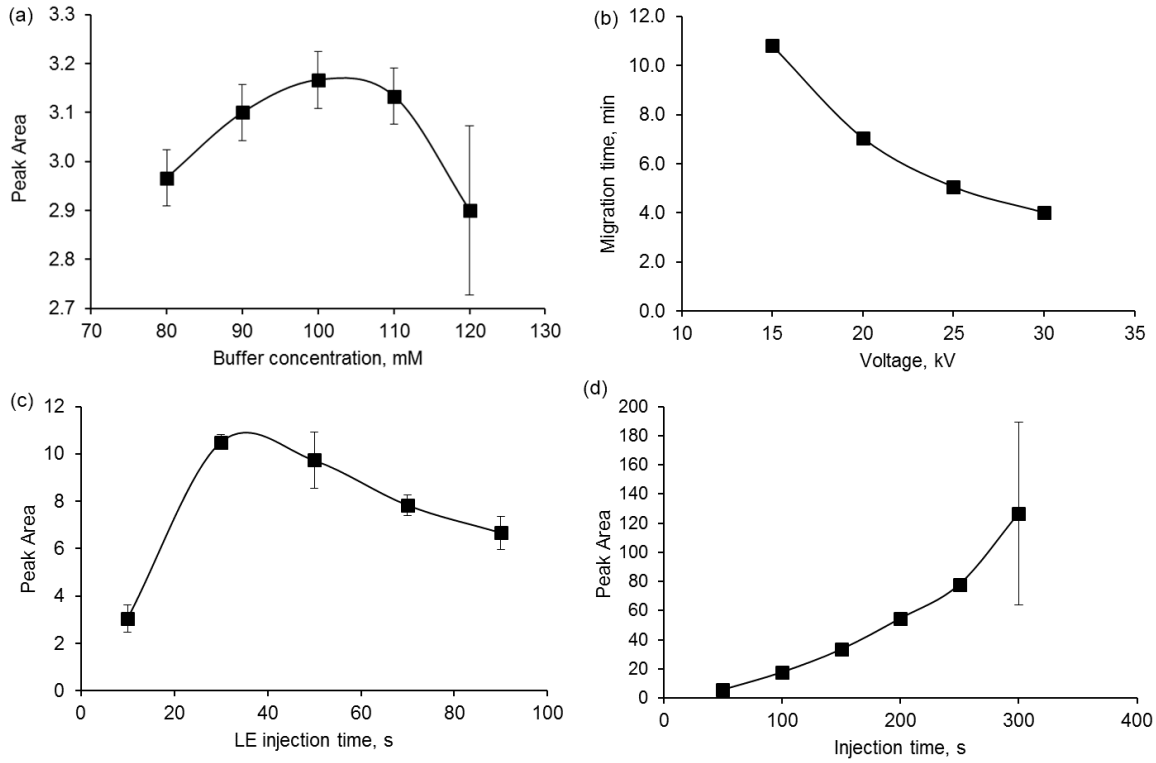

FIGURE 1. Effects of (a) BGE concentration, (b) applied voltage, (c) LE injection time and (d) EKS injection time on EKS-CZE separation of SEC. CZE conditions: capillary 65 cm × 50 µm i.d.; BGE 100 mM formate at pH 2.5, separation at 25 °C and 25 kV; analytes mixture was injected at 7 kV for 250 s. UV detection at 214 nm

Applied voltage is another parameter that is critical in CE optimization. Applied voltages ranging from +15 to +30 kV were investigated, and the shortest migration time of the analyte obtained when the highest voltage (+30 kV) was applied, followed by +25 kV (Figure 2(b)). Solvent viscosity decreased with the rise in temperature, and because electrophoretic mobilities were inversely proportional to viscosity, this caused a decrease in viscosity of the BGE that led to higher mobilities as the voltage increased to 30 kV. However, in term of peak area, the lowest voltage used (+15 kV) produces the highest peak area of SEC. Taking into consideration both migration time and peak area, 25 kV was identified as the most suitable and optimum voltage for use in this study. Increasing applied voltage usually resulted in decreasing migration time of the analytes and increasing sharpness

of the peak as well as enhancement of the resolution. However, increasing the applied voltage could also increase the speed of EOF, which could affect the peak of the analytes (Cazes 2010). Several investigations have shown that a low separation voltage resulted in prolonged migration time, and the peak of the analyte could not be resolved at voltages ≤ 10 kV (Landers 1998).

The last effective parameter of HDI-CZE is the sample injection time. In this study, the hydrodynamic injection times were varied from 1 to 10 s. As the injection time increased, the peak area was also amplified. However, a slightly broad SEC peak was observed in the electropherogram at injection times > 5 s. Thus, 10 s was chosen as the maximum injection time for HDI-CZE. The LOD of SEC was calculated based on the optimum HDI-CZE method. Based on the results obtained, the LOD (115.4 μg L^{-1}) in HDI-CZE mode was not adequate to reach the MRLs (0.1 μg L^{-1}) of SEC in the environmental water samples. To address this problem, another alternative injection mode (EKI) was evaluated.

OPTIMIZATION OF ELECTROKINETIC INJECTION CAPILLARY ZONE ELECTROPHORESIS

Due to the lower sensitivity of the optimized HDI-CZE method, an EKI-CZE was further explored for the separation and preconcentration of SEC. The difference between HDI and EKI was that different injection mode was used to introduce the sample. HDI used pressure to introduce the sample into the capillary, whereas EKI used voltage. With the EKI injection mode, the sample could be injected from 1 to 90 s with the low applied voltage (1–10 kV) (Landers 1998).

For EKI-CZE mode optimization, injection time ranging from 1 to 40 s were tested at a constant injection voltage of +5 kV. All other conditions were similar as the HDI-CZE conditions. The highest peak area was obtained from the highest applied injection time (40 s), and the lowest was obtained from the lowest applied injection time (1 s). However, a high % RSD (33%) was also obtained from the 40 s injection time, which indicated that this injection time was not stable and produced inconsistent peak area of SEC. Partial deterioration of peak shape was observed for 40 s long injections and 10 s was chosen as optimum as it produced an efficient separation of SEC. A further increment in sensitivity could be obtained by using longer EKI injection time, however, for such conditions, different instrumental set-up (e.g. longer effective length of separation capillary) would be necessary to achieve sufficient separation efficiency of the large electrokinetically injected sample plugs (Pantůčková et al. 2015). Therefore, injection time of 10 s and voltage of 5 kV were identified as the best conditions for the EKI-CZE.

In preliminary investigations, the higher EKI injection time produced a rather broad SEC peak, likely due to the higher concentration being injected. Therefore, a lower concentration of SEC (500 μg L^{-1}) was injected in triplicate under the optimized conditions of EKI-CZE and HDI-CZE. The analyte was successfully detected in < 7 min for both the HDI and EKI modes. EKI resulted in better sensitivity (LOD = 68 μg L^{-1}) as compared to the HDI-CZE (LOD = 115.4 μg L^{-1}). Therefore, the calculated enhancement factor of EKI-CZE was ~ 1.7-fold greater than that of HDI-CZE. At the end of the capillary, EKI regularly amplified the electric field and improved on the quantification of trace level amount by varying the injection time, and concentration of sample with different capillary internal diameter (Altria 1996). Thus, the analyte could enter the capillary per second due to mobilize and ions of the analytes were more improved.

Under optimized conditions, the highest enhancement factor obtained for SEC was 1.7 for the EKI-CZE system. This was not sufficient to detect concentrations of SEC lower than the reported MRL (0.1 μg L^{-1}). Thus, an online pre-concentration method, viz. EKS-CZE was developed to further enhance the sensitivity and to obtain the lowest LOD that was sufficient to analyze SEC near the MRL value in water samples.

OPTIMIZATION OF ELECTROKINETIC SUPERCHARGING CAPILLARY ZONE ELECTROPHORESIS

EKS involves electrokinetically injecting the sample between leading and terminating ions. In EKS-CZE, the BGE was first added into the capillary, followed by hydrodynamically injecting a small amount of LE. Then, the target analyte was introduced into the capillary by EKI for a certain amount of injection time. Lastly, the capillary was filled with a small volume of TE and BGE by HDI (Hirokawa et al. 2003). This process caused the diffused band of SEC to be restacked according to isotachophoresis (ITP) principle. After the ITP step was disrupted, the analyte was separated by CZE (Lu et al. 2014).

The LE, TE, and EKI time were the most important factors that affected the sensitivity of the EKS-CZE (Hirokawa et al. 2003). A co-ion with electrophoretic mobility higher than that of the analyte was chosen as the LE, whereas one with lower mobility was chosen as the TE. According to Wang and Chen (2009), the mobility of Na^+ ion (51.9 × 10-9 $m^2v^{-1}s^{-1}$) was higher than that of many organic anions and SEC. Thus, 100 mM NaCl was chosen as the LE in this study. According to formula on mobility;

$$\mu A = \mu E + \mu EOF = (lL/\ tV)\ \text{(Altria 1996)}$$

The electrophoretic mobility of Tris (0.15 × 10-9 $m2v^{-1}s^{-1}$) was lower than that of the effective mobility of the analyte (18.79 × 10-9 $m^2v^{-1}s^{-1}$), thus 100 mM Tris was chosen as the TE. Two main part in stacking process involved were LE and TE with different mobility injected using pressure first and electrokinetic injection assists in stacking (tITP) and the analyte forming band

or concentrating and stack between LE and TE ions when separation voltage applied (Abdul Karim et al. 2016).

The influence of the LE injection time was investigated over the range of 10 to 90 s for injection of 100 mM NaCl. LE was hydrodynamically injected into the capillary at 50 mbar. Increasing LE injection time from 10 to 30 s resulted in a significant increase of SEC peak area, but it decreased beyond 30 s (Figure 2(c)). Thus, 30 s was chosen as the best LE injection time. Other recent studies of the separation of pollutants by EKS also demonstrated that 30 s was the optimum injection time for LE (Abdul Karim et al. 2016; Lu et al. 2014). In the TE optimization step, 100 mM Tris was injected hydrodynamically at 50 mbar at injection times of 20 s (21 nL), 40 s (41 nL), and 60 s (60 nL). Injection time of 40 s was optimal for the system, as it produced the highest peak area among the times tested. Previous studies (Abdul Karim et al. 2016; Dawod et al. 2008) reported that among TE injection times ranging from 10 s to 100 s, the optimum TE injection time was 40 s (approximately 2.7% of capillary volume). Thus, 40 s was used as TE injection time for subsequent experiments.

The final EKS-CZE parameter optimized was sample injection time, with times ranging from 50 to 300 s. Increasing the injection time resulted in increasing peak area (Figure 2(d)). The highest peak area was obtained at 300 s, but the repeatability was not poor (RSD of 50%). EKS-CZE electropherogram showed that the peak area of the analyte became slightly broad when injection time increased to 300 s. Thus, sample injection time of 250 s at +7 kV was chosen as the best injection time for further SEC analysis. In summary, the optimum conditions for EKS-CZE were as follows: HDI of LE (100 mM NaCl) and TE (100 mM Tris) at 50 mbar for 30 s and 40 s, respectively, together with sample EKI at +7 kV for 250 s. These conditions were expected to achieve LOD values lower than the MRL and high peak area with acceptable migration time. Other optimized conditions included BGE of 100 mM sodium formate at pH 2.5, wavelength 214 nm, and applied voltage of 25 kV. The analyte was successfully preconcentrated and separated in < 10 min by applying these conditions. Electropherograms of SEC were compared among HDI-CZE, EKI-CZE, and EKS-CZE, and the SEC peak was significantly improved under the EKS-CZE system as compared to the others (Figure 3(a)).

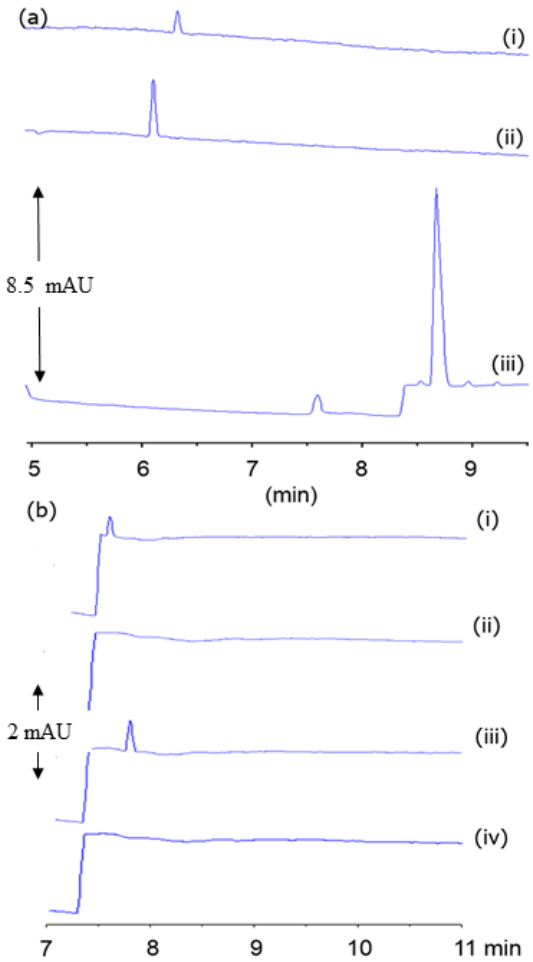

FIGURE 1. (a) Electropherograms of 500 µg L^{-1} SEC which were analyzed by HDI-CZE (i), EKI-CZE (ii), and EKS-CZE (iii). CE conditions as in Figure 2, (b) Electropherograms of the unspiked pond water (i), spiked pond water (100 µg L^{-1} SEC) (ii), unspiked river water (iii), spiked river water (100 µg L^{-1} SEC) (iv) samples. CE condition as in Figure 2

METHOD VALIDATION

Under optimized EKS-CZE conditions, the method was validated in terms of linearity, LOD, LOQ, repeatability, and recovery. A matrix-matched calibration curve was obtained from the corresponding peak areas over eight-points of concentrations of spiked pond water samples. The proposed method provided good linearity in the range of 0.1 to 500 µg L^{-1} with a good coefficient of determination (R^2 = 0.9997) (Table 1). The enhancement factor was calculated based on the LOD values obtained for EKS-CZE, HDI-CZE, and EKI-CZE. The LOD and LOQ were calculated based on a signal to noise ratio of three and ten, respectively. The obtained LOD for SEC was 0.03 µg L^{-1}, which is below its MRL. The enhancement factor obtained by the EKS-CZE method was extremely high compared to that of the HDI-CZE (3847-fold) and EKI-CZE (2267-fold) methods (Table 1). Intra-day and inter-day repeatability (or precision) of the method was studied using two concentrations (low and high) from the calibration curve (10 and 100 µg L^{-1}). For the intra-day analysis, these two concentrations were analyzed over five independent series on the same day. For the inter-day analysis, the two concentrations were analyzed over five different days. All samples were injected in triplicate. The results were expressed as the percentage of RSD of the peak area and migration time (Table 2(a)). Good repeatability was obtained for SEC with RSD < 4% for both migration time and peak area.

TABLE 1. LOD, LOQ, enhancement factors (EFs), regression equation in EKS-CZE

Analyte	LOD[a] (µg L^{-1})	LOQ[b] (µg L^{-1})	EF[c]	EF[d]	Regression equation	Coefficient of determination (R^2)	Linear range (µg L^{-1})
SEC	0.03	0.10	3847	2267	Y= 0.0336X + 0.2412	0.9997	0.1–500

aBased on three times noise, bBased on ten times noise, cCompared to HDI, & dCompared to EKI

TABLE 2. Precision and recovery of the developed EKS-CZE method

(a) Intra-day and inter-day precision of the EKS-CZE method

Analyte	Concentration (µg L^{-1})	Intra-day (RSD %) (n=5)		Inter-day (RSD %) (n=5)	
		Migration time	Peak area	Migration time	Peak area
SEC	10	2	2.7	3.2	3.8
	100	1.1	3.2	2.5	3.6

(b) Results of water samples analysis and recovery

Analyte	Spiked (µg L^{-1})	Pond water		River water	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
SEC	10	105.7	3.5	91.9	2.6
	100	95.7	3.9	85.8	4.7

APPLICATION TO REAL SAMPLES

To test the applicability of the developed method, the technique was used to analyze SEC in river and pond water samples. Figure 3(b) shows the electropherograms of the unspiked and spiked (100 µg L^{-1} SEC) samples. No SEC was detected in all blank samples, indicating that the samples were free from SEC contamination. For the recovery study, SEC was measured in water samples that had been spiked with 10 or 100 µg L^{-1} of SEC. Good recoveries ranging from 86 to 106% with acceptable RSD (< 5%) were obtained for SEC (Table 2(b)).

CONCLUSION

A new method for the analysis of SEC in environmental water samples by EKS-CZE was developed. The sensitivity improved by 3847-fold, providing a detection limit of 0.03 µg L^{-1}, which was lower than the MRLs established by the European Union Directive (0.1 µg L^{-1}). The optimized EKS-CZE conditions were as follows: HDI of LE (100 mM NaCl) at 50 mbar for 30 s; EKI of sample at 7 kV for 250 s; HDI of TE (100 mM Tris) at 50 mbar for 40 s; 25 kV separation voltage; wavelength of 214 nm; and BGE of 100 mM formate at pH 2.5. The developed method provided good linearity, acceptable repeatability, and satisfactory recovery. This method is inexpensive, environmentally friendly, rapid, and simple, and it was shown to be suitable for the detection and measurement of SEC in water samples. Overall, the developed method had great potential for routine monitoring of SEC and other compounds in environmental water samples.

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Rasmey Soeung, Nadhiratul-Farihin Semail, Wan Adnan Wan Omar, Nur Nadhirah Mohamad Zain & Noorfatimah Yahaya*
Integrative Medicine Cluster
Advanced Medical and Dental Institute (AMDI)
Universiti Sains Malaysia
13200 Bertam Kepala Batas, Penang
Malaysia

Rasmey Soeung
Royal University of Agriculture
12400 Dangkor District
Phnom Penh
Cambodia

Mazidatulakmam Miskam & Yong Foo Wong
School of Chemical Sciences
Universiti Sains Malaysia
11800 Minden Penang
Malaysia

Aemi Syazwani Abdul Keyon
Department of Chemistry
Faculty of Science
Universiti Teknologi Malaysia
81310 UTM Johor Bahru, Johor Darul Takzim
Malaysia

Muggundha Raoov Ramachandran
Department of Chemistry, Faculty of Science
Universiti Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Sazlinda Kamaruzaman
Department of Chemistry, Faculty of Science
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

Aemi Syazwani Abdul Keyon
Centre for Sustainable Nanomaterials
Ibnu Sina Institute for Scientific and Industrial Research
Universiti Teknologi Malaysia
81310 Johor Bahru, Johor Darul Takzim
Malaysia

*Corresponding author; email: noorfatimah@usm.my

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