

ELECTROKINETIC SUPERCHARGING IN CAPILLARY
ELECTROPHORESIS FOR THE ON-LINE PRECONCENTRATION OF
SECBUMETON IN WATER SAMPLES

BY
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CERTIFICATE

This is to certify that the dissertation entitled "*Electrokinetic Supercharging in Capillary Electrophoresis for the On-line Preconcentration of Sebumeton in Water Samples*" is the bonafide record of research work done by Ms. Rasmey Soeung during the period from October 2016 to July 2017 under my supervision.

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ABSTRAK

Dalam kajian ini, suatu kaedah prapemekatan disebut superpengecasan elektrokinetik elektroforesis zon rerambut (EKS-CZE) telah dibangunkan bagi penentuan sebumeton (SEC) di dalam sampel air sekitaran. Kesan bagi panjang gelombang julat diod, pH dan kepekatan larutan penimbal, jenis dan kepekatan bagi elektrolit pemula dan penamat, voltan gunaan dan masa suntikan telah dikaji dengan teliti. Latar belakang elektrolit yang optimum adalah 100 mM larutan penimbal format (pH 2.5), suntikan hidrodinamik bagi 100 mM sodium klorida (30 s, 50 mbar) sebagai elektrolit pemula, suntikan elektrokinetik bagi sampel (250 s, 7 kV), suntikan hidrodinamik bagi 100 mM Tris (40 s, 50 mbar) sebagai elektrolit penamat dan 25 kV voltan bagi pemisahan (+25 kV). Di bawah keadaan optimum, kepekaan bagi SEC telah meningkat masing-masing sebanyak 3847-gandaan dan 2267-gandaan apabila dibandingkan dengan suntikan hidrodinamik biasa (HDI) dan suntikan elektrokinetik (EKI). Julat linear yang besar (0.1–500 $\mu\text{g L}^{-1}$) dengan kelinearan yang baik, $R^2=0.9997$, kebolehulangan yang memuaskan (% RSD < 4%, $n=5$) dan pengembalian yang baik (85.8–105.6%) telah didapati bagi SEC. Had pengesanan bagi SEC dengan sistem EKS-CZE ini adalah 0.03 $\mu\text{g L}^{-1}$. Keputusan yang didapati menunjukkan bahawa kaedah yang dibangunkan mempunyai keupayaan bagi penentuan SEC di dalam matriks akueus yang rumit.

ABSTRACT

In this study, an on-line preconcentration method termed electrokinetic supercharging capillary zone electrophoresis (EKS-CZE) was developed for the determination of sebumeton (SEC) in environmental water samples. The effects of diode array wavelength, buffer pH and concentration, the type and concentration of leading and terminating electrolyte, applied voltage and injection time were investigated thoroughly. The optimum background electrolyte was 100 mM formate (pH 2.5), hydrodynamic injection of 100 mM sodium chloride (30 s, 50 mbar) as leading electrolyte, electrokinetic injection of the sample (250 s, 7 kV), hydrodynamic injection of 100 mM Tris (40 s, 50 mbar) as terminating electrolyte and then a voltage of 25 kV for separation (+25 kV). Under the optimum conditions, the sensitivity of SEC was enhanced 3847-fold and 2267-fold when compared to normal hydrodynamic injection (HDI) and electrokinetic injection (EKI), respectively. Wide linear range (0.1–500 $\mu\text{g L}^{-1}$) with good linearity, $R^2=0.9997$, satisfactory repeatability (% RSD < 4%, $n=5$) and good recovery (85.7–105.6%) were obtained for SEC. The detection limit for SEC with this EKS-CZE system was 0.03 $\mu\text{g L}^{-1}$. The results demonstrated that the developed method has great potential for the determination of SEC in complicated aqueous matrices.

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LIST OF SYMBOL, ABBREVIATIONS OR NOMENCLATURE

%	Percentage	LLE	Liquid -liquid extraction
$\mu\text{g L}^{-1}$	Microgram per litre	LOD	Limit of detection
μm	Micrometre	LOQ	Limit of quantification
AMDI	Advanced Medical and Dental Institute	LPME	Liquid phase microextraction
BGE	Background electrolyte	mbar	Millibars
CE	Capillary electrophoresis	MEKC	Micellar electrokinetic chromatography
cm	Centimeter	mg L^{-1}	Milligram per litre
CZE	Capillary zone electrophoresis	min	Minute
DAD	Diode array detection	mM	Millimolar
EF	Enhancement factor	MRLs	Maximum residue limits
EKI	Electrokinetic injection	MS	Mass spectrometry
EKS	Electrokinetic supercharging	NACE	Nonaqueous capillary electrophoresis
EOF	Electroosmotic flow	ng mL^{-1}	Nanogram per millilitre
EPA	Environmental protection agency	nm	Nanometre
FASI	Field-amplified sample injection	RSD	Relative standard deviation
FID	Flame ionization detector	s	Second
GC	Gas chromatography	SBSE	stir bar sportive extraction
HDI	Hydrodynamic injection	SEC	Secbumeton
HPLC	High performance liquid chromatography	SPE	Solid phase extraction
ITP	Isotachophoresis	SPME	Solid phase microextraction
kV	kilovolt	TE	Terminating electrolyte
LC	Liquid chromatography	tITP	transient isotachophoresis
LE	Leading electrolyte	USM	Universiti Sains Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The polluted substance in water, arising from contaminated or unwanted substances, are categorized into two forms, either the modification of the types and number of materials carried by water or by changing the physical properties of the water's body. The pollution possesses several characteristics and causes, may contribute to serious threats to the environment and kills 14000 lives of people on a daily basis due to the presence of toxins in water. The pollution cases are increased in the developing countries compared to the industrialized countries (Agrawal *et al.*, 2010).

Man-made activities contribute to the water pollution include the agricultural, industrial and domestic activities. The water ecosystem is polluted as a result of agriculture through the use of bulk amounts of toxic pesticides and fertilizer, industrial toxic substances as well as animal and people's wastes. The point and non-point is likely to produce pollution into the sources such as ground water, oceans, estuaries, seas, lakes and rivers. The point source of pollution is contributed by the effluents in a body of water, when the pollution is sent out directly from drain pipe. Contradictory, the discharged pollution from other spreading sources namely grazing lands, agricultural fields' construction sites, roads and streets, and pits and mines are examples of non-point sources (Slattery, 2012).

As stated by a published report from the Environment Protection Agency (EPA) in 1990; more than 50% of polluting water in miniature rivers as well as river derived from the removal process and combination of chemical substance from the operation of agriculture (Cook *et al.*, 2008; Rai and Pandey, 2017). Numerous cases of water pollution were reported due to the exposure to the toxic chemical substances, which are not naturally occurring in aquatic ecosystems. The pesticides, herbicides and industrial compounds are significant contributors to the toxic water pollution.

Besides that, the natural phenomena are able to make a significant change in quality of water and in status of ecology of water (Agrawal *et al.*, 2010).

In the process of development of agriculture industry, pesticides have become an important tool as a plant protection agent for boosting food production (Abhilash and Singh, 2009). However, pesticides are poisons and improper use or management of pesticides can be harmful to human. As pesticides constitute a potential risk to human health, their occurrence in the environment is a matter of public concern. Pesticides are classified by their target organism, chemical structure and physical state. Many pesticides can be grouped into chemical families such as organophosphorus, organochlorines, carbamates, triazines, pyrethroids and bipyridyl groups (Tadeo *et al.*, 2000).

Triazine herbicides are a class of pre- and post- emergent broadleaf herbicides with similar chemical structure that inhibit the growth of weeds through disruption of photosynthesis pathways (Graymore *et al.*, 2001). Main triazine herbicides are usually resulted from s-triazine which can persist in environmental samples from several months to many years as its stereochemical stability is high enough for it to do so (LeBaron *et al.*, 2008). Triazine and the degradation products produced by triazine are extremely toxic and permanent in environment and organism. Thus, they have become a potential concern on the environmental safety (Farland *et al.*, 2011). In addition, the maximum residue limits (MRLs) of triazines in environment have been established worldwide. For example, the maximum contaminant level of varies triazines which established by environmental protection agency (EPA) is approximately 1-4 $\mu\text{g L}^{-1}$ (EPA, 2012). Moreover, a maximum residue limits (MRLs) value of single pesticide approximately 0.1 $\mu\text{g L}^{-1}$ and total pesticides about 0.5 $\mu\text{g L}^{-1}$ in water have been established by the European Union Directive (The Council of the European Union, 1998). Due to the presence of triazines in lower concentration levels in the environment, a sensitive, simple, accurate and environmentally friendly method is required to monitor the concentrations of triazines in the environment.

Several methods of analysis of triazine herbicides in environment and food samples have been developed, including gas chromatography (GC) and high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) system (Sanagi *et al.*, 2012; Fang *et al.*, 2014; Yang *et al.*, 2014). CE commonly requires low organic solvent and sample consumption, produces fast analysis of compounds and has become significant and a popular alternative to the HPLC, GC and LC-MS methods (Wang *et al.*, 2012). The detection of trace amount of triazine normally required preconcentration step such as solid phase extraction (SPE), liquid-liquid extraction (LLE), solid phase microextraction (SPME), liquid phase microextraction (LPME) and stir bar sorptive extraction (SBSE) methods too. However, these off-line preconcentration methods often take up longer time and contributing to the total cost of analysis (Osborn *et al.*, 2000). Therefore, various on-line preconcentration methods coupled to electrophoretic approaches have been developed to improve the sensitivity of CE system, including field-amplified sample injection (FASI), isotachopheresis (ITP), dynamic pH injection and sweeping. The combination of the on-line preconcentration and the CE system have contributed to high enhancement factors of analytes, cost-effective, shortened the analysis time, simple and environmentally friendly (Almeda *et al.*, 2010). In this study, a new on-line preconcentration method termed as electrokinetic supercharging (EKS) coupled to CE was developed for the determination of atrazine compound, secbumeton (SEC) in environmental water samples.

1.2 Problem Statement

In agriculture sector around the world, pesticides as well as herbicide are explored in the purpose of enhance the production yields. Selective pre- and post-emergence herbicides for control of broadleaf and grassy weeds which commonly used are triazines. Although, triazines have ability to destroy the unwanted planted, they are considered as the harmful reagents. Generally, the environment and crops

are polluted with extreme toxicity of triazines. Furthermore, human health impact including cancers, birth defects and interruption of hormone also can be harmed by this type of herbicide (Wu *et al.*, 2009). Thus, the maximum residue limits (MRLs) of triazines in environment as well as foods have been widely established.

Recently, liquid chromatography-mass spectrometry, high performance liquid chromatography, gas chromatography and gas chromatography-mass spectrometry are known as the common methods for triazine separation (Elbashir and Aboul-Enein, 2015). These methods are mostly sensitive, thus, with the purpose of avoiding contamination of chromatographic column, clean-up processes are frequently needed. The disadvantages of these methods are costly and time consuming due to the procedures need off-line extraction and much volume of solvent (Acedo-Valenzuela *et al.*, 2004). To avoid these problems, some researchers have considered on investigation of the CE system since it provides superior advantages including less analysis time, extreme efficiency of separation as well as minimal cost of operation. Nevertheless, the normal CE system results in poor sensitivity because of the limited UV detection (Chen *et al.*, 2012).

To overcome this shortcoming, different on-line preconcentration methods have been developed, including field-amplified sample injection (FASI), transient isotachopheresis (tITP), dynamic pH injection and sweeping. However, these methods are not adequate to determine SEC at trace MRLs under some conditions, due to the concentration and major interferences exist in the complex environmental water samples. EKS, the combination of FASI and tITP, was firstly introduced by Hirokawa at the year of 2003. It is a very powerful on-line preconcentration method, and enhancement factors up to three to four orders of magnitude have been reported in the literature (Dawod *et al.*, 2009; Botello *et al.*, 2013; Lu and Breadmore, 2010; Ning *et al.*, 2012; Lu *et al.*, 2015; Abdul Karim *et al.*, 2016). EKS is an appropriate method for environmental analysis since it is believed as a powerful on-line preconcentration strategy which concerns on tITP and FASI combination (Okamoto

and Hirokawa, 2003). To the best of our knowledge, there are no reports on the use of EKS for SEC analysis in environmental waters samples.

1.3 Objective of the Study

The main objective of this study is to develop a simple and sensitive electrokinetic supercharging capillary zone electrophoresis (EKS-CZE) in CE for the determination of sebumeton (SEC) in selected environmental water samples. In order to achieve the main objective, there are three specific objectives of this study include:

- i. To optimize capillary zone electrophoresis (CZE) conditions for the separation selectivity of SEC.
- ii. To enhance the separation and achieve lowest detection limit of SEC by optimizing the online EKS-CZE parameters.
- iii. To explore the application of the developed method for the determination of SEC in the real environmental water sample.

1.4 Scope of the Study

The presented study focuses into the analysis of SEC in environmental water samples using CE system. EKS, which combines FASI and tITP methods, is used as the preconcentration method in this study and the detection is carried out by CE with diode array detection (CE-DAD). Several important CE parameters affecting the analyte sensitivity were optimized comprehensively including the detection wavelength, pH and concentration of background electrolyte (BGE), separation voltage and injection time. For detection enhancement, EKS-CZE parameters were optimized including injection time of leading electrolyte (LE), terminating electrolyte (TE) and injection time of sample. The real environmental water samples that were analysed in this study include river water and pond water.

1.5 Significance of the Study

The on-line preconcentration method, namely electrokinetic supercharging capillary zone electrophoresis (EKS-CZE) in CE was developed for the determination of SEC in environmental water samples. The method is inexpensive, fast, simple, environmentally friendly and sensitive and it was shown to be suitable for the detection of SEC in water samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides

Pesticides are chemical substances designed to mitigate, repel, prevent and destroy pests namely insect, weeds, rodents and plenty of undesired organisms (Friis, 2012). Therefore, the pesticides played an important role amongst other chemicals that used in the environment aiming at injuring and killing some form of life. The pesticides of most common class, depends on the target species they work on. There are four significant classes of pesticides include insecticides, rodenticides, fungicides and herbicides. Beside this, pediculicides, larvicides, miticides, molluscides and acaricides are also known as pesticides. Furthermore, some of these including plant growth regulators, attractants (pheromones) and repellents also placed under this class of chemicals for purposes of regulation (Morrison and Murphy, 2010).

The pesticides are of a significant tool to protect plants and deemed as an agent for increasing food production as well as for maintaining numerous of dreadful diseases, this development is observed to be made in agricultural industry. In addition, the production yield was improved by the application of pesticides since they protect the cultivation (Morrison and Murphy, 2010). However, the foresaid target pests are only species of animals that divide plenty of similar type and characteristic of other animals. The susceptibility towards concrete toxins is considered one among the above characteristics (Koureas *et al.*, 2012). For instance, the function of sex hormones and reproductive performance mostly received negative impact from these does use of chemicals. The endocrine disruptors are resulted from the acting of pesticides as xenohormones or the disruption of processes of endocrine (Handford *et al.*, 2015). The utilization of pesticides has to consider the benefit with the possibility of risks to people health or the destruction of the quality of environment. The analysis of residues of pesticide is importantly used to monitor the contamination of food chain. The

pesticides give positive effect the crop yields, yet its residues in soils and water provide negative effect to the health of human (Koureas *et al.*, 2012). Therefore, the European Commission strictly manage the pesticide residues level produced by most popular agents such as organophosphorus, organochlorine, triazine, and carbamate groups in many different food commodities as well as environmental water (Hamilton *et al.*, 2003).

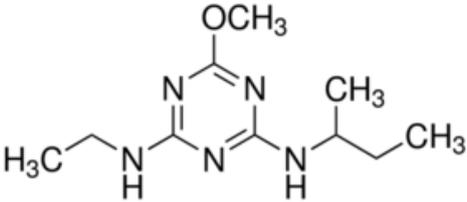
2.2 Triazine

The triazine herbicides are classified as a pre-post emergent broadleaved herbicide along with same chemical structure which prevents the weeds' growth via the disruption of pathways photosynthesis (Breckenridge *et al.*, 2010). They are also one of herbicides families that prevent the normal growth and improvement of plants (Casida, 2009). Approximately 1/3 of the herbicides utilized in agriculture are triazines. These widespread uses are operating in lots of fields namely industry, agriculture and urban location to soil for the management of grassy and broadleaved weeds in crops as follows: sorghum, sugarcane and corn (Farland *et al.*, 2011). Current reports atrazine and relevant triazine herbicides namely cyanazine and simazine, have been supposed to be endocrine disrupters. Furthermore, they are able to cause multiple varieties of cancers and birth defect as well as inhibit tumors' reproductive and function of hormone; as a result, these deemed hazardous compounds for human health and environment (Hayes *et al.*, 2002). In addition, their toxicity is also known as potential dangers. Because of the hazardous effects of triazine on human health and environment, for instance, activity of carcinogenic and endocrine disruption; they are deemed as compounds of critical concern. Moreover, there is a concern over the increase of triazine herbicide compounds in various sources of water and groundwater, in which this leads to the limits on their utilization in numerous countries. The extensive concern was seen occurred from the existence of triazines and their degradation products, this because of much toxic and persistent in soil, water, and

organism, which become an emergent concern upon the safety of environment (Armon and Hänninen, 2015).

Sebumeton (N-ethyl-6-methoxy-N'-(1-methylpropyl)-s-triazine) is a critical type of triazine herbicide handled to manage of weed owing to the application of both selective and non-selective. This is of usefulness for the control of industrial weed for a miniature time at a certain place (See *et al.*, 2010). However, it is able to contaminate the environment especially water resources (Sanagi *et al.*, 2015). Moreover, it is commonly known that the health issue including dizziness, drowsiness, gastric, and intestinal issues, cracking or skin dryness and corneal are caused by the sebumeton toxicities (Alvarsson, 2012). In addition, the toxic is also be able to spreads to the environment and aquatic organisms including fish algae or bacterial which leading to trouble to climate changes and ecology (LeBaron *et al.*, 2008).

Table 2.1: Chemical structures and *pKa* value of studied anayte.

Sebumeton	pKa
	4.4

Thus, it is necessary to develop methods with high sensitive, accurate and reliable for the triazine separation and determination in different types of samples including environmental samples, food samples as well as biological samples.

2.3 Analytical Methods for Triazine Analysis

Recently, numerous methods have been developed for the determination and separation of triazine compounds. The developed methods included high performance liquid chromatography with UV detector (HPLC-UV) (Wang *et al.*, 2012; Liu *et al.*, 2014; Yang *et al.*, 2014; Zhou and Gao, 2014), HPLC with DAD detector (Zhao *et al.*,

2011; Rodríguez-González *et al.*, 2014), gas chromatography-mass spectrometry (GC-MS) (Sanagi *et al.*, 2012), liquid chromatography-mass spectrometry (LC-MS) (Ji *et al.*, 2008) and micro-liquid chromatography (See *et al.*, 2010).

These methods are known as the highly sensitive method but sample preparations which increase the cost operation and time consumption are usually required. A major disadvantage of HPLC was high organic solvent consumption which leads to high operating cost as well as waste generated (Yan, 2004). Micro LC was likely to develop, however, exist of hardship in designing high-pressure, low-volume solvent delivery systems and in controlling the decline of pressure in packed column chromatography discouraged the commercial improvement (Dziubakiewicz and Buszewski, 2013).

Beside these above method, capillary electrophoresis is commonly used in analytical field in order to obtain the high resolution, quantitative precision and the low costs of waste disposal as well as operation. CE is known as a separation method which provides a trouble-free, quick and cost-effective substitute to GC-MS or LC-MS for separation of toxic substances in water sample. The capillary has ability to eliminate efficiently the Joule heat made from the applied voltage which leads the electrophoretic separations to be able operating at up to 30 kV at ambient temperature (Landers, 2007). However, low sensitivity due to the characteristic of small dimensions in flowing cell and the capacity of sample volume is the significant limit of CE. Therefore, extraction procedures are also required. Nevertheless, off-line extraction can be avoided because CE has ability to do on-line preconcentration by stacking or sweeping (Zhao *et al.*, 2011; Rodríguez-González *et al.*, 2014). The analytical methods for SEC analysis are summarised in Table 2.2.

Table 2.2: Analytical methods for triazine analysis.

Triazine Analytes	Samples	Instrument	Preconcentration method	LOD ($\mu\text{g L}^{-1}$)	Recovery (%)	Reference
Simazine, atrazine, simetryn, propazine, ametryn, terbuthylazine, prometryn, terbutryn	Cereal and vegetable	MEKC	On-line sweeping	0.02-0.04	82.8-96.8	Fang <i>et al.</i> (2014)
Simazine, atrazine, cyanazine, simetryn	Milk	HPLC	Cloud point extraction	6.79-11.19	70.5-96.9	Liu <i>et al.</i> (2014)
Ametryn, atrazine, cyanazine, prometryn, propazine, simazine, simetryn, terbuthylazine terbutryn	Seaweed	HPLC-DAD	Matrix solid phase dispersion and solid phase extraction clean-up	1.4-3.8	75-100	Rodríguez-González <i>et al.</i> (2014)
Atrazine, simazine, ametryn cyanazine,	Fruit and vegetable	GC-FID	Ionic liquid-calixarene solid-phase microextraction	3.3-13	71.5–96.9	Tian <i>et al.</i> (2014)
Cyanazine, desmetryn, secbumeton, terbutryn, dimethametryn, dipropetryn	Vegetable and oil samples	LC-UV	Magnetic ionic liquid-based dispersive liquid–liquid microextraction	1.31–1.49	81.8–114.2	Wang <i>et al.</i> (2014)
Atrazine, simetryn, ametryne, propazine	Tap and river water	HPLC	Double water compatible molecularly imprinted polymers (DWC-MIPs-SPE)	3.2-8.6	69.2–95.4	Xu <i>et al.</i> (2014)
Atraton, desmetryn, atrazine, terbumeton and terbuthylazine	Milk	HPLC-UV	Aqueous two-phase extraction	2.1-2.8	86.3-120.6	Yang <i>et al.</i> (2014)

Table 2.2: Continued

Triazine Analytes	Samples	Instrument	Preconcentration method	LOD ($\mu\text{g L}^{-1}$)	Recovery (%)	Reference
Cyanazine, Simazine, Atrazine	Water	HPLC-UV	Ionic liquid dispersive liquid-phase microextraction	0.05-0.06	85.1-100	Zhou and Gao (2014)
Simazine, atrazine, secbumeton, cyanazine	Farm, tap and lake water	GC-MS	Dispersive liquid-liquid microextraction	0.037-0.008	95.7-116.9	Sanagi <i>et al.</i> (2012)
Ametryn, atrazine, desmetryn, propazine, simazine	Cereal	HPLC-UV	Dynamic microwave-assisted extraction (DMAE-SFO system)	1.1-1.5	80-102	Wang <i>et al.</i> (2012)
Atrazine, prometon, propazine, prometryn	lake, river, reservoir water	HPLC	graphene-based Fe ₃ O ₄ magnetic nanoparticles (G-Fe ₃ O ₄ MNPs)	0.025- 0.040	89-96.2	Zhao <i>et al.</i> (2011)
Simazine, cyanazine, atrazine, propazine	River water	Micro-LC	Membrane-protected carbon nanotubes solid phase membrane tip extraction	0.2-0.5	95-101	See <i>et al.</i> (2010)
Chloro-s-triazine, viz. atrazine, simazine, propazine, thiomethyl-s-triazine ones, viz. ametryn, prometryn, prometon	Fruits and vegetables	LC-MS	Dispersive solid-phase extraction	0.05-0.2	80-110	Ji <i>et al.</i> (2008)
Atrazine, simazine	Drain water	GC-MS	Solid-phase microextraction	0.25-0.5	-	Rocha <i>et al.</i> (2008)

Table 2.2: Continued

Triazine Analytes	Samples	Instrument	Preconcentration method	LOD ($\mu\text{g L}^{-1}$)	Recovery (%)	Reference
Simazine, prometryn, atrazine	Water	MEKC	On-line sweeping	0.5-10	83.7-94.3	Zhang <i>et al.</i> (2008)
Atrazine, desmetryn, simazine prometryn, propazine, ebuthylazine, secbumeton simetryn	River and tap water	GC-MS	Dispersive liquid-liquid microextraction	0.021- 0.12	24.2-115.6	Nagaraju and Huang (2007)
Atrazine, simazine	Water	MEKC	Solid-phase extraction	0.6-1	80-95	Acedo- Valenzuela <i>et al.</i> (2004)
Simazine, prometon, atrazine, simetryn, ametryn, propazine, prometryn, trietazine, terbutylazine, terbutryn	Ground water samples	MEKC	Solid-phase extraction	0.02-0.03	> 87	Frías <i>et al.</i> (2004)
Atrazine	Fruit juices	CE	Solid-phase extraction	30	40-60	Khrolenko <i>et al.</i> (2002)
Simetryn, ametryn, prometryn terbytryn	Deionised water	MEKC	Stacking	9-15	-	Lin <i>et al.</i> (2001)

2.4 Electrokinetic Supercharging (EKS) On-line Preconcentration

A major disadvantages of CE with UV detection is Low sensitivity due to the characteristic of small dimensions in flowing cell and the capacity of sample volume. The total capillary volume can be result in negative to resolution if the amount of the sample volume introduces into the capillary more than 1-5%. Hence, CE is considered as weak concentration detector even though it is a useful mass detector. According to this reason, the using of CE as a separation technique for sample consisting supposedly high concentration ($10 \mu\text{g L}^{-1}$ or greater) is limited (Grossman and Colburn, 2012). As the result, some approaches development for on-capillary sample concentration is necessary to obtain micro analytical technique of CE. There are some on-capillary sample concentration techniques which are commonly used include stacking of sample, focusing of sample, enrichment of isotachoporetic sample and on-line preconcentration (Osborn *et al.*, 2000).

In current reports, stacking techniques such as; either field amplified sample injection (FASI) or transient ITP (tITP), are able to improve sensitivity of separation which lead the limit of detection down to ppb and ppt. Nevertheless, in order to obtain this sensitivity level, additional off-line extraction processes are still needed when apply on real sample (Zdena *et al.*, 2009; Almeda *et al.*, 2010). As mentioned earlier, CE also has capacity to do on-line preconcentration together with detection, and other additional instrumentations are regularly not required for this technique. Thus, online preconcentration is the best choice for on-capillary sample concentration in order to get the lowest sensitive of separation.

There are several on-line preconcentration techniques which recently used. Among those, EKS has been considered as the suitable technique which FASI together with tITP has been used in CE. The important substances in EKS are leading electrolyte (LE) and terminating electrolyte (TE) which has faster and slower mobility than the analytes, respectively. In order to assist in the stacking process during tITP, LE and TE are injected into the capillary by hydrodynamic injection mode before and

after samples, respectively while the samples are introduced by electrokinetic injection (Hirokawa *et al.*, 2003). EKS has been used firstly by Hirokawa's group who did separation on rare-earth ores samples (Okamoto and Hirokawa, 2003). Then, several currently studies (Table 2.3) have used this method to separate many different types of sample by adopted Hirokawa's method. According to Table 2.3, EKS together with CZE was commonly used rather than other CE mode. These investigations demonstrated that the separation and preconcentration by EKS method to detect the different toxic substances provided the low LOD ($0.01-53 \mu\text{g L}^{-1}$) and high enhancement factor (100-11800 fold) compared with hydrodynamic injection (Dawod *et al.*, 2008, 2009, Botello *et al.*, 2013; Lu and Breadmore, 2010; Ning *et al.*, 2012; Lu *et al.*, 2015; Abdul Karim *et al.*, 2016). Until now, there is lack of reports on utilized of EKS as preconcentration on the separation of triazine. However, there is a reports on the melamine compound analysis in milk powder and liquid powder using EKS-CZE which conducted by Lu and co-workers (Lu *et al.*, 2015). The result from that finding demonstrated that EKS-CZE was the sensitive and reliable method with the enhancement factor of 2285 fold compared to HDI-CZE whereby the LOD was 0.7 ng mL^{-1} . Therefore, due to the ability of EKS system on improving the separation sensitivity without off-line preconcentration processes requirement, this on-line preconcentration method together with capillary zone electrophoresis will be used for study in order to obtain the lowest LOD as well as high enhancement factor. The reported methods involved with EKS are summarised in Table 2.3.

Table 2.3: Application of electrokinetic supercharging (EKS) with different mode of CE for toxic substances separation.

Analytes	Samples	Analytical method	Enhancement factor (fold)	LOD ($\mu\text{g L}^{-1}$)	Recovery (%)	Reference
Disrupting chemical and phenolic compound	Environmental water	EKS-CZE	100-737	4.89-53	56-64	Abdul Karim <i>et al.</i> (2016)
Melamine	Milk	EKS-CZE	2285	0.7	29.8-42.1	Lu <i>et al.</i> (2015)
Three barbiturate drugs	Urine Sample	EKS-CZE	1050	1.5-2.1	97-102	Botello <i>et al.</i> (2013)
Acromatic Acids	River water	EKS-NACE	3868-6480	0.08-0.3	21-75.5	Ning <i>et al.</i> (2012)
Five non-steroidal anti-inflammatory drugs	water and human plasma	EKS-CZE	2000	0.9-2	56-73	Botello <i>et al.</i> (2010)
Phenolic acids	Water	EKS-NACE	1333-3440	0.22-0.51	42.25-75.24	Lu and Breadmore (2010)
Seven Non-steroidal anti-inflammatory drugs	Waste water	CF-EKS	11800	0.01-0.04	-	Dawod <i>et al.</i> (2009)
Seven Non-steroidal anti-inflammatory drugs	Waste water	EKS-CZE	2400	0.5-1.8	-	Dawod <i>et al.</i> (2008)