ANALYSIS OF DIOXIN AND FURAN RELATED COMPOUNDS AND ORGANOPHOSPHOROUS PESTICDES USING MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Dedicated to my beloved father, mother and brother and people who have supported me throughout this research.

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ABSTRACT

Micellar electrokinetic chromatography (MEKC) is increasingly popular in the analysis of organic pollutants in the environment due to its high separation efficiency, less solvent usage and shorter analysis time. In this study, MEKC was used for the separation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzo-*p*-furan (TCDF) with a separation buffer consisting of 20 mM sodium cholate, 20 mM sodium tetraborate decahydrate and 5% v/v organic modifier acetonitrile-methanol (3:1 v/v) at a final buffering pH of 9.16-9.22. Separation voltage was at 25 kV with anodic injection and optimum wavelength set at 225 nm. To improve the limit of detection (LOD), five on-line preconcentration techniques were evaluated. The techniques were normal stacking mode (NSM-MEKC), reversed electrode polarity stacking mode (REPSM-MEKC), high conductivity sample stacking mode (HCSSM-MEKC), sweeping and field enhanced sample injection (FESI-MEKC). High conductivity sample stacking method-MEKC (HCSSM-MEKC) that gave an LOD of 46 ppb for TCDF and 18.5 ppb for TCDD was chosen. Solid phase disc extraction (SPDE) was used to further improve the LOD during the extraction of TCDF and TCDD from water thus reducing the LODs by 1000 fold. LODs in the ppt range were achieved for both analytes. This is the first study conducted using MEKC for 2,3,7,8-TCDF and 2,3,7,8-TCDD. Selected hydrophilic organophosphorous pesticides were also included in this study. They were dicrotophos, monocrotophos and phosphamidon. Separations were carried out using acidic buffer (pH 2.3) with a buffering system consisting of 10 mM sodium dodecyl sulphate, 20 mM sodium dihydrogen phosphate and 10% v/v methanol. Optimum detection wavelength of 225 nm with separation voltage at -25 kV was used. MEKC separation gave LODs of 32.1 ppm for dicrotophos, 3.75 ppm for monocrotophos and 18.6 ppm for phosphamidon. Stacking with reverse migrating micelles (SRMM) gave better LODs of 0.15 ppm for dicrotophos, 0.39 ppm for monocrotophos and 0.22 ppm for phosphamidon. SPDE was able to reduce the LOD further by 250 fold. Sample matrix used was spiked pond water. MEKC was successfully used to separate the test analytes in pond water sample. These analytes consisted of 2,3,7,8-TCDF and 2,3,7,8-TCDD under basic conditions and three hydrophilic organophosphorous pesticides under acidic conditions with analysis time of less than 10 minutes.

ABSTRAK

Kromatografi rerambut elektrokinetik misel (MEKC) semakin kerap digunakan dalam analisis bahan pencemar alam sekitar kerana kecekapan pemisahan yang tinggi, penggunaan isipadu pelarut yang kecil dan masa analisis yang singkat. Dalam kajian ini, MEKC telah digunakan untuk memisahkan 2,3,7,8-tetraklorodibenzo-*p*-dioksin (TCDD) dan 2,3,7,8-tetraklorodibenzo-*p*-furan (TCDF) dengan menggunakan larutan penimbal 20 mM natrium kolat, 20 mM natrium tetraborat dekahidrat dan campuran pengubahsuai organik 5% asetonitrile-methanol (3:1 v/v) dengan pH 9.16-9.22. Voltan pemisahan adalah 25 kV dan panjang gelombang optimum pada 225 nm. Lima teknik pra-pemekatan talian terus iaitu mod penyusunan normal (NSM-MEKC), penyusunan kekutuban elektrod berbalik (REPSM-MEKC), mod penyusunan sampel kekonduksian tinggi (HCSSM-MEKC), sapuan (S-MEKC) dan suntikan sampel peningkatan medan (FESI-MEKC) telah diuji untuk membaiki had pengesanan analit. Mod penyusunan sampel kekonduksian tinggi (HCSSM-MEKC) telah dipilih dengan had pengesanan 46 ppb untuk TCDF dan 18.5 ppb untuk TCDD. Kaedah pengekstrakan cakera fasa pepejal (SPDE) telah digunakan untuk memperbaiki had pengesanan TCDF dan TCDD dalam sampel air dan berjaya menurunkan had pengesanan sebanyak 1000 kali ganda. Had pengesanan adalah dalam julat ganda bahagian per trillion (ppt). Ini adalah kajian pertama dilakukan dengan MEKC terhadap kedua-dua analit. Beberapa pestisid organofosforus hidrofilik turut dipilih dalam kajian ini iaitu dikrotofos, monokrotofos dan fosfamidon. Pemisahan dilakukan dalam penimbal berasid (pH 2.3) dengan larutan pemisahan yang mengandungi 10 mM natrium dodesil sulfat (SDS), 20 mM natrium dihidrogen fosfat dan 10% methanol. Panjang gelombang optimum 225 nm dan voltan pemisahan –25 kV. Pemisahan MEKC memberi had pengesanan sebanyak 32.1 ppm untuk dickrotofos, 3.75 ppm bagi monokrotofos dan 18.6 ppm dan fosfamidon. Mod penyusunan dengan misel penghijrahan berbalik (SRMM) memberikan had pengesanan yang lebih baik iaitu sebanyak 0.15 ppm untuk dikrotofos, 0.39 ppm bagi monokrotofos dan 0.22 ppm bagi fosfamidon. SPDE telah berjaya menurunkan lagi had pengesanan sebanyak 250 kali ganda. Sampel matriks yang digunakan ialah air tasik terpaku. MEKC telah berjaya digunakan untuk memisahkan analit-analit dalam sampel air tasik. Analit-analit ini terdiri daripada 2,3,7,8-TCDF dan 2,3,7,8-TCDD dalam keadaan berbes dan tiga jenis pestisid organofosforus hidrofilik dalam keadaan berasid dengan masa analisis kurang daripada 10 minit.

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LIST OF SYMBOLS

D, d - Diameter (µm)

I_p - Sample plug length

L - Length (cm)

N - Efficiency

P - Pressure (mbar)

r^2 - Correlation coefficient

R_s - Peak resolution

T - Temperature (˚C)

t_m - Migration time

V_p - Volume of sample loaded

α - Selectivity

η - Viscosity

LIST OF ABBREVIATIONS

ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
ASE	-	Accelerated solvent extraction
BGE	-	Background electrolyte
BGS	-	Background solution
CD	-	Cyclodextrin
CD-MEKC	-	Cyclodextrin assisted MEKC
CHES	-	2-(N-Cyclohexylamino)ethane sulfonic acid
CMC	-	Critical micelle concentration
CZE	-	Capillary zone electrophoresis
DAD	-	Diode-array detection
DMSO	-	Dimethyl sulphoxide
EPA	-	Environmental Protection Agency
EOF	-	Electroosmotic flow
FESI	-	Field enhanced sample injection
HCSSM	-	High conductivity sample stacking mode
HRGC	-	High resolution gas chromatography
HRMS	-	High resolution mass spectrometry
HSW	-	Hazardous solid waste
ID	-	Isotope dilution
i.d.	-	Internal diameter
LD	-	Detection limit
LLE	-	Liquid-liquid extraction
LMT	-	N-lauroyl-N-methyltaurate
LOD	-	Limit of detection
MASE	-	Microwave-assisted solvent extraction

MeCN - Acetonitrile
MEKC - Micellar electrokinetic chromatography
MeOH - Methanol
MSPD - Matrix solid phase dispersion
MSW - Municipal solid waste incinerator
NACE - Non-aqueous capillary electrophoresis
NaCl - Sodium chloride
NM - Normal mode
NSM - Normal stacking mode
OPPs - Organophosphorous Pesticides
PBDE - Polybrominated diphenyl ether
PCB - Polychlorinated biphenyls
PCDD - Polychlorinated dibenzodioxins
PCDF - Polychlorinated dibenzofurans
PLE - Pressurized liquid extraction
poly-SUS - Polysodium undecyl sulfate
REPSM - Reversed electrode polarity stacking mode
RSD - Residual standard deviation
SC - Sodium cholate
SDS - Sodium dodecyl sulphate
SEF - Sensitivity enhancement factor
SPE - Solid phase extraction
SPDE - Solid phase disc extraction
SPME - Solid phase microextraction
SDME - Single drop microextraction
SPMD - Semi permeable membrane devices
SRM - Standard Reference Material
SRMM - Stacking with reverse migrating micelles
SRW - Stacking with reverse migrating micelles and a water plug
TEF - Toxic Equivalent Factors
TEQ - Toxic Equivalent Concentrations
TCDD - Tetrachlorodibenzodioxins
- 2,3,7,8-tetracholordibenzo-p-dioxin

TCDF	-	Tetrachlorodibenzofurans
	-	2,3,7,8-tetrachlorodibenzo-p-furan
UV	-	Ultraviolet

CHAPTER I

SUMMARY OF THESIS

1.1 Background

Since its introduction in the late 1980s, micellar electrokinetic chromatography (MEKC) has been widely used in the pharmaceutical industry and in environmental analysis. MEKC is a mode of capillary electrophoresis able to separate both ionic and neutral analytes with the usage of charged micelles in a single run. Separation by electrophoresis is obtained via differential migration of solutes of charged species in an electric field performed in narrow-bore capillaries with inner diameter (i.d) of 25-75 µm filled only with buffer. Its advantages lie in its flexibility in manipulating various parameters on-column in order to obtain the best separation and to improve sensitivity. Furthermore, separation time is faster compared to conventional methods such as gas chromatography and high performance liquid chromatography with very little sample and solvent requirement.

1.2 Summary of Thesis

This study was conducted into two parts as two different separation conditions were used. The first part was the study on separating polychlorinated dibenzo-*p*-dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and polychlorinated dibenzo-*p*-furans (2,3,7,8-tetrachlorodibenzo-*p*-furan) which were conducted under basic conditions. The second part was the study of three hydrophilic organophosphorous pesticides (OPPs) under acidic buffer conditions. The three hydrophilic organophosphorous pesticides were monocrotophos, dicrotophos and phosphamidon. This chapter summarizes every chapter covered in this work.

Chapter 2 introduces the background behind this work. It explains in detail the physical and chemical properties of the test analytes which are the polychlorinated compounds (PCDDs and PCDFs) and the hydrophilic pesticides. The potential routes of PCDDs and PCDFs in humans and wildlife are also discussed. While for the OPPs, we touched on the toxicity of the pesticides. Various extraction and detection methods are also discussed for both class of analytes in this chapter. On-line preconcentration methods using MEKC are also discussed in detail. The objectives of this study and the problem statements are also covered.

Chapter 3 discusses the experimental methods used in this work. The instrumental aspect covers the capillary electrophoresis system used for our analysis and the extraction set-up used. Conditioning of the capillary was also discussed in detail for both polychlorinated compounds and pesticides analysis. For both polychlorinated compounds and the pesticides, the methodology used in optimizing parameters and online preconcentration techniques used are found in Chapter 3. Sample preparation and extraction methods for both analytes are covered in Chapter 3.

Chapter 4 covers the optimization of parameters used in the MEKC analysis of TCDF and TCDD. The parameters optimized are the fraction of organic modifier used in the running buffer in order to improve peak resolution. Single modifiers used were methanol and acetonitrile at various percentages in the running buffer. These were then compared with mixed modifier mode at different fractions of acetonitrile and methanol in the running buffer. The second parameter involved the type of sample matrix used. In aqueous mode, the analytes were diluted in water and in the same fraction as the running buffer. While for non-aqueous mode, analytes were diluted in the organic solvent which was ethanol. Different sample matrixes were investigated to study the effect of the sample matrix on separation efficiency.

Chapter 5 explores the various on-line preconcentration techniques used to reduce the detection limit for both TCDF and TCDD. Five types of on-line preconcentration techniques were used which were normal stacking mode (NSM), reversed electrode polarity stacking mode (REPSM), high conductivity sample stacking mode (HCSSM), sweeping and field-enhanced sample injection (FESI). Out of the five, the on-line preconcentration technique which gives the best limit of detection (LOD) would be chosen to be applied to the real sample. HCSSM was chosen as the optimized stacking method. This is then followed by off-line preconcentration technique which is solid phase disc extraction (SPDE) to further reduce the existing limit of detection (LOD) by offering sample enrichment. SPDE is also able to do sample clean-up by removing interference in the matrix that would interfere with the analysis. The combined HCSSM-SPDE was then applied to real sample analysis of spiked deionised water and effluent water obtained from a pulp mill at Temerloh, Pahang.

Chapter 6 involves the analysis of three hydrophilic OPPs which are dicrotophos, monocrotophos and phosphamidon. In this chapter, the analysis using MEKC was conducted under acidic conditions. This section discusses the wavelength optimization for analyzing the three analytes using diode-array detection (DAD). The wavelength that offers the best peak area and peak height was used in the stacking method. Stacking with reverse migrating micelles (SRMM) was used to reduce the detection limit (LODs) of the three analytes. This is followed by solid phase disc extraction (SPDE) in order to reduce the LODs further by offering sample enrichment and clean-up. The combined SRMM-SPDE was then applied to analyzing spiked pond water obtained from the UTM lake.

Chapter 7 presents the conclusions and suggestions for further studies. This chapter summarizes the results obtained throughout the study such as the optimized conditions and recovery studies from the extraction method. Suggestions are presented and discussed for further improvement of the study for future usage.