

METHODS DEVELOPMENT FOR SEPARATION OF FUNGICIDES USING
MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT

In this study, micellar electrokinetic chromatography (MEKC) was used for the simultaneous separation of three different classes of fungicides. Separations were done at 210 nm with on-column UV detection. Two different types of separation buffer system (borate and formate) were used. The influence of separation buffer concentration and pH, surfactant concentration, separation voltage and addition of organic solvents and modifiers on migration time, efficiencies and resolutions were investigated. Four fungicides from three different classes viz. carbendazim and thiabendazole (benzimidazole), propiconazole (triazole) and vinclozolin (dicarboximide) were successfully separated in less than 15 min using both separation buffer systems with propiconazole giving two stereoisomer peaks. A buffer system consisting of 4 mM borate buffer at pH 10, with 60 mM sodium cholate (SC) gave the best separation result. On the other hand, good separation was also achieved with a running buffer composed of 20 mM formate buffer at pH 7, containing 60 mM SC and 5 mM β -cyclodextrin (β -CD). The limit of detections (LODs) of fungicides in borate buffer system were in the range of 9 – 23 ppm and 29 – 52 ppm for formate buffer system. In an effort to reduce the LODs, two on-line preconcentration techniques with formate buffer system were used because of its higher LODs. The two on-line preconcentration techniques, sweeping and stacking with hydrodynamic (HDI) and electrokinetic (EKI) injection were contrasted. The effect of sample matrix, injection time and injection voltage on fungicides separation was studied using the two online preconcentration techniques. Using on-line preconcentration techniques, height and area sensitivity enhancement factor (SEF_{height} and SEF_{area}) were found to be between 10 to 99. LODs in the sub-ppm (0.3 – 4 ppm) level were obtained. At least 10-times improvement in detector response was achieved with normal stacking mode MEKC using hydrodynamic injection, NSM-MEKC-HDI. The proposed method was applied to the analysis of spiked fungicides in lake water samples at 2 – 4 ppm. The recovery was between 46.22 % to 93.30 % with RSDs of 10 % to 27 %.

ABSTRAK

Dalam kajian ini, kromatografi rerambut elektrokinetik misel (MEKC) telah digunakan untuk pemisahan serentak tiga kelas fungisid. Pemisahan dilakukan pada 210 nm menggunakan pengesanan ultra lembayung pada turus. Dua jenis larutan penimbal (borat dan format) yang berbeza telah digunakan. Pengaruh kepekatan dan pH larutan penimbal, kepekatan surfaktan, voltan pemisahan dan penambahan pelarut dan pengubahsuai organik terhadap masa perpindahan, kecekapan dan resolusi telah dikaji. Empat fungisid daripada tiga kelas yang berbeza iaitu karbendazim dan tiabendazol (benzimidazol), propikonazol (triazol) dan vinklozlin (dikarboksimida) berjaya dipisahkan dalam masa kurang daripada 15 minit dengan kedua-dua sistem larutan penimbal dengan propikonazol memberikan dua puncak stereoisomer. Sistem larutan penimbal terdiri daripada 4 mM penimbal borat pada pH 10 dan 60 mM natrium kolat memberikan pemisahan terbaik. Pemisahan terbaik juga dicapai menggunakan larutan penimbal 20 mM format pada pH 7 yang mengandungi 60 mM natrium kolat dan 5 mM β -siklodekstrin (β -CD). Had pengesanan fungisid dengan sistem larutan penimbal borat ialah antara 9 – 23 ppm dan 29 – 52 ppm bagi larutan penimbal format. Teknik prapemekatan talian terus menggunakan larutan penimbal format telah dikaji dalam usaha untuk menurunkan had pengesanan fungisid. Dua teknik prapemekatan secara terus, iaitu sapuan dan himpunan menggunakan suntikan hidrodinamik dan elektrokinetik telah dibandingkan. Kesan matriks sampel, masa dan voltan suntikan terhadap pemisahan fungisid telah dikaji menggunakan dua teknik prapemekatan talian terus. Faktor peningkatan kepekaan tinggi dan luas (SEF_{tinggi} dan SEF_{luas}) antara 10 – 99 telah diperoleh. Had pengesanan sub-ppm antara 0.3 – 4 ppm telah diperoleh. Sekurang-kurangnya 10 kali peningkatan dalam gerak balas pengesanan telah diperoleh dengan nilai tertinggi untuk semua fungisid diperoleh menggunakan mod himpunan normal MEKC secara suntikan hidrodinamik, NSM-MEKC-HDI. Kaedah yang dibangunkan telah diaplikasikan untuk analisis fungisid pakuan di dalam sampel air tasik pada kepekatan 2 - 4 ppm. Peratus pengembalian fungisid ialah antara 46.22 % hingga 93.90 % dengan kebolehhilangan masing-masing antara 10 % hingga 27 %.

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LIST OF SYMBOLS

%	-	Percentage
μm	-	Micro meter
cm	-	Centi meter
g	-	Gram
k'	-	Capacity factor
K_{ow}	-	Octanol-water partitioning coefficient
kV	-	Kilo volt
mL	-	Mili liter
mM	-	Mili molar
mmol	-	Mili mol
MT	-	Metric ton
$M\Omega$	-	Mega ohm
N	-	Efficiency
ppb	-	Parts per billion
ppm	-	Parts per million
R_s	-	Resolution
t_0	-	Solvent migration time
t_m	-	Analytes migration time
v/v	-	Volume per volume

LIST OF ABBREVIATIONS

β -CD	-	β -cyclodextrin
γ -CD	-	γ -cyclodextrin
AcN	-	Acetonitrile
AN	-	Aggregation number
BGS	-	Background solution
CE	-	Capillary electrophoresis
CEC	-	Capillary electrochromatography
CGE	-	Capillary gel electrophoresis
CIEF	-	Capillary isoelectric focusing
CITP	-	Capillary isotachopheresis
CMC	-	Critical micellar concentration
CZE	-	Capillary zone electrophoresis
DAD	-	Diode array detector
DNA	-	Deoxyribonucleic acid
DTAB	-	Dodecyltrimethylammonium bromide
ECD	-	Electron capture detector
EI	-	Electron impact
EKI	-	Electrokinetic injection
EOF	-	Electroosmotic flow
FESI	-	Field enhanced sample injection
FPD	-	Flame photometric detector
GC	-	Gas chromatography
HDI	-	Hydrodynamic injection
HPLC	-	High performance liquid chromatography
HTAB	-	Hexadecyltrimethylammonium bromide

ID	-	Inner diameter
LC	-	Liquid chromatography
LLE	-	Liquid-liquid extraction
LOD	-	Limit of detection
MBC	-	Carbendazim
MEKC	-	Micellar electrokinetic chromatography
MeOH	-	Methanol
MRL	-	Maximum residue limits
MS	-	Mass spectrometry
MSPD	-	Matrix solid phase dispersion
NA	-	Not available
ND	-	Not detected
NM	-	Normal Mode
NPD	-	Nitrogen phosphorus detector
NSM	-	Normal stacking method
OD	-	Outer diameter
PCZ	-	Propiconazole
PS	-	Pseudostationary phase
RP	-	Reverse phase
S	-	Sample zone
SC	-	Sodium cholate
SDS	-	Sodium dodecyl sulfate
SFC	-	Supercritical fluid chromatography
SPE	-	Solid phase extraction
SRM	-	Standard reference material
SRMM	-	Stacking reverse migration micelles
SRW	-	Stacking reverse migration micelles and a water plug
TBZ	-	Thiabendazole
t-ITP	-	Transient isotachopheresis
TLC	-	Thin layer chromatography
TSD	-	Thermionic specific detector
TTAB	-	Tetradecylammonium bromide
UV	-	Ultra violet

VCZ - Vinclozolin

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CHAPTER 1

SUMMARY OF THESIS

1.1 Background

Micellar electrokinetic chromatography, MEKC is a mode of CE and has been used to separate neutral and ionic solutes. Surfactant is used in MEKC to form micelles that are used for the analytes separation. Separation of the analytes is dependent on the interaction between the analytes with the micellar phase and aqueous phase. The more hydrophobic the analytes, the more time is needed to separate the analytes.

Numerous works have been done for the separation of pesticides with MEKC. Different kind of buffers, surfactants and also modifiers were used to obtain the optimum results. From the literature survey carried out, to the best of our knowledge, the combination of separation and detection of triazole, dicarboximide and benzimidazole fungicides have not been attempted using MEKC. Herein, the study was undertaken to explore the potential of MEKC use in the separation. However, due to some limitation with UV detector, detection limits with MEKC was found higher than other separation technique. To overcome the problem, two on-line preconcentration techniques were implemented to lower the detection limit of analytes. Since the use of on-line preconcentration techniques are quite new, attempts were made to separate fungicides with the optimized conditions in order to achieve lower detection limits.

1.2 Summary

An introduction to pesticide and CE system in general with research objectives and scopes are discussed in Chapter 2.

All experimental work including instruments, reagents and chemicals used are described in Chapter 3.

Chapter 4 explores the separation of the selected four fungicides with borate buffer system. Optimization of the separation conditions such as borate concentration and pH, SC concentration, applied voltage, addition of organic solvents and modifiers were varied to obtain the optimum conditions for the separation of the four fungicides. Quantitative analysis was done using the developed optimum conditions to obtain the calibration curves, detection limits and reproducibility of separation. Apart from using conventional optimization technique, a factorial design technique for optimization was also carried out and compared. A full factorial design at two levels with five factors (2^5) were implemented in the fungicides separation.

Optimizations of separation conditions with formate buffer system were explored in Chapter 5. Formate concentration and pH, SC concentration, separation voltage, addition of organic solvents (methanol and acetonitrile) and organic modifiers (β -cyclodextrin and γ -cyclodextrin) were varied. The optimum conditions obtained were then used to test the analytical performance of the developed method. The analytical performances with different buffer systems (borate and formate) were also compared.

In Chapter 6, two on-line preconcentration techniques were used to enhance the sensitivity of the system. Normal stacking mode MEKC with hydrodynamic injection (NSM-MEKC-HDI) and electrokinetic injection (NSM-MEKC-EKI) and sweeping were used in an attempt to further reduce the limit of detection. For NSM-MEKC-HDI, sample matrix concentrations and injections were varied. Concentration of sample matrix, injection voltage and injection time were also varied to obtain

optimum results with NSM-MEKC-EKI. For both stacking technique, formate buffer was used as the sample matrix. In sweeping MEKC, only injection time of the analytes was varied.

Finally in Chapter 7, the conclusions of the research are presented and suggestions made for future study.

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