NORMAL STACKING MODE (NSM) IN IMPROVING THE DETECTION SENSITIVITY OF DIOXIN RELATED COMPOUNDS USING MICELLAR ELECTROKINETIC CHROMATOGRAPHY

(Mod Penyusunan Biasa Dalam Meningkatkan Pengesanan Sebatian Dioksin Dengan Menggunakan Kromatografi Rerambut Elektrokinetik Misel)

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Abstract

Micellar electrokinetic chromatography (MEKC) with diode array detection (DAD) was used to separate two dioxin related compounds viz. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzo-*p*-furan (TCDF) using 20 mM sodium tetraborate-decahydrate with 5% v/v organic modifier (3:1 acetonitrile-methanol). To enhance sensitivity of detection, normal stacking mode (NSM-MEKC) which is the simplest stacking mode was used just by dissolving the analytes in water and injected into the capillary as a long plug. The hydrodynamic injection time was optimized by varying the injection time length in the ranges of 1s, 4s, 10s, 20s, 30s, 40s and 50s at constant hydrodynamic pressure of 50 mbar. From the optimum injection time obtained, calibration graphs of both TCDF and TCDD were constructed to calculate the detection limit obtained. A detection sensitivity enhancement factor (SEF_{LOD}) of 330 was achieved for 2,3,7,8-TCDD achieving a detection sensitivity of 0.05 ppm while for 2,3,7,8-TCDF the SEF_{LOD} was 65 with a detection sensitivity of 0.34 ppm. Stacking efficiencies were compared to that of normal mode (NM- MEKC) whereby no stacking is involved.

Keywords: MEKC, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo-p-furans and stacking

Abstrak

Kromatografi rerambut elektrokinetik misel (MEKC) dengan menggunakan pengesan tatasusunan diod (DAD) telah digunakan untuk memisahkan 2 jenis sebatian dioksin iaitu 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). Untuk meningkatkan had pengesanan, mod penyusunan biasa (NSM-MEKC) yang merupakan mod penyusunan yang termudah telah digunakan dengan melarutkan analit dalam air dan disuntik kedalam kapilari dengan jangka masa yang lama. Jangka masa suntikan hidrodinamik telah dioptimumkan dengan mempelbagaikan masa suntikan dari 1s, 4s, 10s, 20s, 30s, 40s dan 50s pada tekanan hidrodinamik malar pada 50 mbar. Berdasarkan masa suntikan yang optimum, graf kalibrasi untuk kedua-dua TCDF dan TCDD dapat dilakarkan untuk menghitungkan had pengesanan. Faktor penambahbaikan had pengesanan (SEF_{LOD}) sebanyak 330 telah tercapai untuk 2,3,7,8-TCDD dengan had pengesanan sebanyak 0.05 ppm manakala untuk 2,3,7,8-TCDF, SEF_{LOD} ialah 65 dengan had pengesanan 0.34 ppm. Keberkesanan mod penyusunan ini dibandingkan dengan mod biasa (NM-MEKC) yang mana penyusunan tidak dilakukan.

Kata kunci: MEKC, sebatian poliklorin dibenzo-p-dioksin, sebatian poliklorin dibenzo-p-furan dan penyusunan

Introduction

Dioxins are a name given to 75 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs). The most toxic PCDD congener is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) while another closely related compound is 2,3,7,8-tetrachlorodibenzo-*p*-furan (TCDF) [1]. The risk posed by their toxicity is noted in their toxic equivalent factors (TEFs) with TCDD being rated the most toxic (TEF=1.00) while TCDF has a TEF of 0.1 [2]. These compounds are the byproduct of the manufacturing of phenoxy herbicides such as 2,4,5-trichlorophenol and pentachlorophenol which is usually used as a wood preservative [3,4]. Another source of dioxins are fly ash

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from municipal solid waste (MSW) and hazardous waste (HSW) incinerators due to the wet scrubbers in the flue gas cleaning system [5]. Furthermore, dioxins are also found airborne thus there is a risk of contamination via the food chain in humans [6].

Due to their toxicity, various analytical methods have been developed to detect these compounds in the environment with different degree of sensitivity. Most analysis are carried out using gas chromatography (GC) coupled to mass spectrometry, MS achieving limits of detection as low as part per trillion [7,8]. Yet GC requires the usage of lots of organic solvents therefore capillary electrophoresis, CE comes in handy. CE only requires a few nanolitres of sample per injection and aqueous media is usually used. Due to the low UV sensitivity, stacking is utilized in CE analysis to improve on the detection limit. Normal stacking mode, NSM involves dissolving the neutral analytes in a solution that has a lower conductivity (water) than the buffer solution and injected as a long plug into the capillary [9]. The enhancement is due to the difference in electrical field between the sample zone and the running buffer zone as the analytes in the sample zone are stacked at the boundary between the sample zone and the running buffer zone. It is the simplest form of stacking and was used in detection of pesticides in food achieving a detection limit of 60-70ppb [10].

Experimental

Reagents and Apparatus

Sodium cholate from Wako (Japan) and di-sodium tetraborate decahydrate from Fisher Chemicals (UK are both analytical grade. HPLC Grade acetonitrile and methanol were from J.T. Baker (California, USA) while HPLC Grade 1,4-dioxane was from BDH Laboratory Reagents (UK). Sodium chloride was obtained from Merck (Germany). 2,3,7,8-TCDF and 2,3,7,8-TCDD as test analytes at 2 mg were from AccuStandard (New Haven, Connecticut, USA). Deionised water was obtained from Milipore UltraPure Water System purified up to 18 M Ω .

All experiments were conducted on a 220V Agilent Capillary Electrophoresis System (Hanover, Germany) equipped with a DAD detector working at wavelength 225 nm. 3D-CE ChemStation Software was used for system control. Standard bare fused silica capillaries (Polymicro Technologies, Phoenix, Arizona, USA) with 48.5 cm total length, 40 cm effective length and 50 µm i.d. were used. All samples and buffer solutions were degassed before use and filtered through a 0.45 µm Nylon filter disc (Whatman).

MEKC separation conditions and Conditioning of the capillary

Capillary was flushed for 10 minutes with 1N NaOH followed by 20 minutes of deionized water and lastly 5 minutes of running buffer. Before each sample injection, the capillary was rinsed for 5 minutes with 0.1N NaOH, followed by 5 minutes of the running buffer. In between runs, high pressure water was passed through the capillary for 10 minutes followed by 3 minutes of 1N NaOH and lastly 3 minutes of the running buffer. At the end of the day, the capillary was flushed with 20 minutes of water followed by 20 minutes of air. Both ends of the capillary were dipped in deionized water before shutting down for the day.

Stacking

Firstly, the buffering system consisted of 20 mM di-sodium tetraborate decahydrate , 20 mM sodium cholate and 5% v/v MeCN-MeOH (3:1) mixed modifier at a final pH range of 9.16-9.22.

To obtain the optimized injection time, 1 ppm of TCDD and 2 ppm of TCDF were prepared as mixture in water. The injection times used in the study were 1 s, 4 s, 10 s, 20 s, 30 s, 40 s and 50 s at constant hydrodynamic injection pressure of 50 mbar. From the optimized injection time obtained, calibration graphs of both 2,3,7,8-TCDF and 2,3,7,8-TCDD were constructed using external standard method and the LOD was calculated using the $3S_y/m$ formula where S_y refers to the relative standard deviation obtained from the calibration curve through least square fit (scatter of measured values around the regression line) while m refers to the slope of the calibration curve. The LODs obtained from NSM-MEKC was then compared with normal mode (NM-MEKC).

Optimization of Injection time

The injection time is optimized over a range of 1s, 4 s, 10s, 20s, 30s, 40s and 50s. The limit of detection is

Results and Discussion

proportional to the injected sample zone. The optimized injection time should also allow high separation efficiency because in real sample analysis numerous unknown matrix effects would lead to the appearance of unidentified peaks when on-line concentration techniques are used [11].

An increase in injection time from normal sample injection at 50 mbar for 1s resulted in an increase in peak area and peak height for both TCDF (2 ppm) and (1 ppm). The same observation was also reported by Süsse and Müller [12]. At normal injection time of 1 s, 2 ppm of TCDF is not detected (Figure 1). There is evidence of peak broadening for both analytes with increasing injection time till it reached 50 s whereby the peak height for TCDD and TCDF broadened.



Figure 1: Electropherograms showing the effect of different injection times on peak shapes of 2,3,7,8-TCDD and 2,3,7,8-TCDF. Separation buffer: 20 mM sodium cholate, 20 mM sodium tetraborate-decahydrate and 5% v/v MeCN-MeOH (3:1) at a final buffer pH 9.16-9.22. Separation wavelength, 225 nm; separation voltage, 25 kV; hydrodynamic injection of samples varied from 1s, 4s, 10s, 20s, 30s, 40s and 50s. Capillary total length, 48.5 cm; effective length, 40 cm.



Figure 2: Influence of injection time on (A1) peak area, (A2) enlarged for TCDF peak area, (B) peak height and (C) efficiency on the separation of TCDF and TCDD. Hydrodynamic injection pressure constant at 50 mbar.

There was an increase in peak height for TCDF till it reached a maximum at 30 s while for TCDD it reached a maximum at 40 s before decreasing with a further increase in injection time, s (Figure 2B). This may be due to the sample plug being too long generating a strong laminar flow. This laminar flow is the result of a mismatch of the EOF velocity in the sample and buffer zones [13]. There is also an increase in peak height followed by a reduction in peak efficiency. Peak efficiency for TCDF was good (above hundred thousand) till a 40 s injection time is used. While for TCDD, the peak efficiency deteriorated from 10 s onwards. In order to maintain high separation

efficiency and reasonable peak height and area, the optimized injection time chosen is 10 s at 50 mbar.

Calibration Lines, Linearity (r^2) , LODs

The calibration curves for both TCDF and TCDD are constructed with concentrations 10 times lower than in NM-MEKC and using the optimized injection time of 10 s. Each run was done in triplicates. The calibration curves based on peak heights are shown in Figure 3 while the calibration equations, linearity and LODs at S/N = 3 are shown in Table 1. The calibration curves for TCDF and TCDD are linear in the range of 1-4 ppm for TCDF and 0.25-1.5 ppm for TCDD. Both curves show r^2 values of greater than 0.995 and the LOD of 0.34 ppm and 0.05 ppm for TCDF and TCDD respectively.



Figure 3: Calibration curves based on peak height for (A) TCDF and (B) TCDD using NSM-MEKC. Injection time at 50 mbar for 10 s. Separation conditions as in Figure 1.

Table 1: Equation of calibration curves, r^2 , and LODs (for S/N = 3) based on the calibration curves in Figure 3.

ANALYTES	EQUATION	r^2	LOD $(S/N = 3)$, ppm
2,3,7,8-TCDF	y = 0.1827x + 0.1572	0.9950	0.34
2,3,7,8-TCDD	y = 3.3013x + 1.6329	0.9993	0.05

Repeatability and Reproducibility

Table 2: Repeatability of migration time, peak height and peak area for TCDF and TCDD using NSM. Conducted at triplicates for each run.

ANALYTES		% RSD, n=3				
	Migration time	Peak Height	Peak Area			
2,3,7,8-TCDF	0.50	6.35	6.89			
2,3,7,8-TCDD	0.34	0.92	2.08			

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The repeatability of migration time, peak height and peak area for each analyte are given in Table 2. Migration times of both TCDF and TCDD peaks are uniquely reproduced with RSDs below 1 % while RSDs for peak heights and peak areas are in the acceptable range of 0.9-6%. Electropherogram of three intraday replicated runs are shown in Figure 4. In this experiment, buffers are freshly prepared after every four runs in order to maintain stability. To avoid errors, the buffers were prepared in large quantities for 4 days for 20 runs.



Figure 4: Electropherogram of three successive replicated runs for the separation of TCDF and TCDD in NSM-MEKC under same conditions as in Figure 1 but with an injection time of 10 s.

Stacking Efficiency

Table 3 shows the comparison of LOD obtained for TCDF and TCDD using NM-MEKC and NSM-MEKC. Stacking efficiencies in terms of peak height (SEF_{height}), peak area (SEF_{area}), and LOD (SEF_{LOD}) are calculated for each test analyte to evaluate quantitatively the degree of stacking to compare between NSM and NM. A stacking efficiencies in the form of sensitivity enhancement factors were calculated based on the ratio of the peak height, peak area and LOD obtained by NSM-MEKC to NM-MEKC multiplied by the dilution factor of 10. All three enhancement factors are shown in Table 3.

Table 3: Sensitivity Enhancements Factor (SEF) in NSM-MEKC over NM-MEKC in the separation of TCDF and TCDD.

Analytes	SEF _{height}	SEF _{area}	SEF _{LOD*}	LOD _{NM-MEKC} (ppm)	LOD _{NSM-MEKC} (ppm)
TCDF	7.4	53	65	2.20	0.34
TCDD	32	24	329	1.71	0.05

SEF_{height}, SEF_{area} and SEF_{LOD}: see text, *LODs are based on peak heights. Conditions as in Figure 4.

 SEF_{area} gave a much higher value for both analytes compared to SEF_{height} but this implies that with stacking, peaks broadened in NSM. Similarly, LODs were improved by almost 65 times lower for TCDF and 329 fold lower for TCDD. A sensitivity enhancement of 7.4-32 fold was obtained with the NSM-MEKC (based on peak height).

Conclusions

The LOD obtained via this study for TCDF (0.34 ppm) and TCDD (0.05 ppm) is significant as this is the first study conducted using the two specific analytes with NSM-MEKC. A sensitivity enhancement of 7.4-32 fold was obtained with the NSM-MEKC (based on peak height) as peak area linearity of the calibration graph was very poor. Further study is required in order to improve on the detection limit such as by using off line concentration methods.

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References

- 1. G. Choudhary, L. H. Keith and C. Rappe (1983). *Chlorinated Dioxins and Dibenzofurans in the Total Environment*. Butterworth Publishers.
- Danielsson, C., Wiberg, K., Korytár, P., Bergek, S., Brinkman, U. A. Th and Haglund, P. (2005). Trace analysis of polychlorinated dibenzo-p-dioxins, dibenzofurans and WHO polychlorinated biphenyls in food using comprehensive twodimensional gas chromatography with electron-capture detection. J. Chromatogr. A. 1086. 61-70.
- 3. Lacorte, S., Latorre, A., Barceló, D., Rigol, A., Malmqvist, A., Welander, T. (2003). Organic compounds in paper-mill process waters and effluents. *Trends Anal. Chem.* 22. 725-737.
- 4. Nakamata, K. and Ohi, H. (2003). Examination of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in process water of kraft pulp bleaching mill using chlorine dioxide from the aspect of environmental quality. *J. Wood Sci.* **49**. 525-530.
- Ferré-Huguet, N., Nadal, M., Schumacher, M. and Domingo, J. L. (2005). Environmental impact and human health risks of polychlorinated dibenzo-p-dioxins and dibenzofurans in the vicinity of a new hazardous waste incinerator: A case study. *Environ. Sci. Technol.* 40. 61-66.
- Gómara, B., Bordajandi, L. R., Fernández, M. A., Herrero, L., Abad, E., Abalos, M., Rivera, J. and González, M. J. (2005). Levels and Trends of Polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) and Dioxin-like polychlorinated biphenyls (PCBs) in Spanish commercial fish and shellfish products, 1995-2003. J. Agric. Food. Chem. 53. 8406-8413.
- 7. Focant, J., Pirard, C. and Pauw, E. D. (2004). Automated sample preparation-fractionation for the measurement of dioxins and related compounds in biological matrices: A review. *Talanta* **63**. 1101-1113.
- 8. Singh, S. A. and Kulshrestha, G. (1997). Gas chromatographic analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans. J. Chromatogr. A. 774. 97-109.

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- 9. Quirino, J. P and Terabe, S. (1997). On-line concentration of neutral analytes for micellar electrokinetic chromatography I. Normal Stacking Mode. J. Chromatogr. A. **781**, 119-128.
- 10. Hernández-Borges, J., Cifuentes, A., Garcia-Montelongo, F. J. and Rodriguez-Delgado, M. (2005). Combining solid-phase microextraction and on-line preconcentration-capillary electrophoresis for sensitive analysis of pesticides in foods. *Electrophoresis*. **26.** 980-989.
- 11. Huang, H. and Lin, C. (2005). Methanol plug assisted sweeping-micellar electrokinetic chromatography for the determination of dopamine in urine by violet light emitting diode-induced fluorescence detection. J. Chromatogr. B. 816. 113-119.
- 12. Süsse, H. and Müller, H. (1996). Pesticide analysis by micellar electrokinetic capillary chromatography. J. Chromatogr. A. 730. 337-343.
- Wang, S., Wu, Y., Ju, Y., Chen, X., Zheng, W. and Hu, Z. (2003). On-line concentration by field-enhanced sample injection with reverse migrating micelles in micellar electrokinetic capillary chromatography for the analysis of flavonoids in *Epimedium brevicornum* Maxim. J. Chromatogr. A. 1017. 27-34.