APPLICATION OF AD-DR BIOASSAY ON ASH SAMPLES

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDD) and Polychlorinated dibenzo-furans (PCDF) referred to as dioxins are well-known environmental toxicants. Dioxins are soluble in fat and have high lipophilicity which when entered into a human body has the tendency to accumulate in fat. One of its major problems includes difficulty in metabolism due to bioaccumulation and biomagnification (Kojima et al., 2011). IARC confirmed 2,3,7,8-TCDD is a significant cause of liver cancer, lung cancer, stomach cancer and non-Hodgkin's lymphoma (IARC, 1997). A fast-screening test (the Ad-DR bioassay) for dioxins analysis was developed. The aryl-hydrocarbon-receptor (AhR) reporter system was utilized to transport dioxin-responsive-element (DRE) via an adenovirus vector into a rat hepatoma (H4IIE) before each experiment, and these DRE-H4IIE cells were utilized in the Ad-DR bioassay. The collected ash extracts were simultaneously analyzed by AD-DR bioassay and the high resolution gas chromatograph/ high resolution mass spectrometry (HRGC/HRMS) method. The preliminary result shows that Ad-DR bioassay following the column clean up pretreatment provided a reasonable result for ash samples.

Materials and methods

1. Extraction

- 2 g ash samples were extracted by 300 mL of three various organic solvents: toluene, n-hexane/ dichloromethane and n-hexane. Two extracted methods were used : soxhlet extraction and ultrasonic extraction. After extraction, direct concentration and column clean up were applied to pretreat these ash samples.

2. Soxhlet / Ultrasonic extraction

- Soxhlet extraction was conducted in 24 hours. Ultrasonic extraction was conducted with 100 mL organic solvent for 1 hour and repeated for three times to collect the supernatant.

3. Directly concentrated sample extract

- 300 mL extracted liquid was evaporated to dryness and dissolved in 200 μL dimethyl sulfoxide (DMSO).

4. Column purification

- Extracted samples were evaporated to near dryness and then transferred to the CAPE-coupled carbon-acid silica column for cleanup. The cleanup procedure using the CAPE- coupled carbon-acid silica column was previously described in detail (Chen et al. 2007, Lee et al. 2009).

5. Ad-DR Bioassay

- Using adenovirus infected cells to luminescence analysis, Ad-DRE-Luc/H4IIE cell lines was cultured in MEM at white 96-well dish (2 x 104 cells per well) overnight. Removed MEM and 90 μL virus/ well (Adeasy-TATA-4XDRE, MOI = 0.3) was added for 16 hours.
- Following incubation for 24 h, the cells were treated with the environmental extracts for 24 h. In parallel, a calibration curve of the Ad-DR bioassay system was created. After treatments, 50 μl of 1×lysis buffer (Promega, Madison, WI, USA) was added per well. To assure complete cell lysis, cells were freeze–thaw one time using liquid nitrogen and then were vortexed in 90 rpm at 37°C for 10 min. Luciferase activity was measured using Luciferase Assay System (Promega, Madison, WI, USA) according to the standard protocol provided. The luciferase activity was expressed as relative light units (RLU)/well. The sigmoid semi-logarithmic dose-response of TCDD calibration curve was fitted by the Hill equation(Chao et al. 2006).

Results and discussion

Ultrasonic extraction-Directly concentrated samples

A low correlation for the Ad-DR bioassay and chemical analysis was found for ultrasonic extraction-directly concentrated samples. If the sample extracts does not go through purification procedures, interference will affect the Ad-DR bioassay results. Different solvent extraction results are shown in Figure 1. From the figure, toluene has the lowest correlation (R^2 = -0.29), because the sample color is black and Ad-DR bioassay was disturbed. N-hexane extracted sample showed a better correlation (R^2 = 0.93) for ultrasonic extraction-directly concentrated samples.

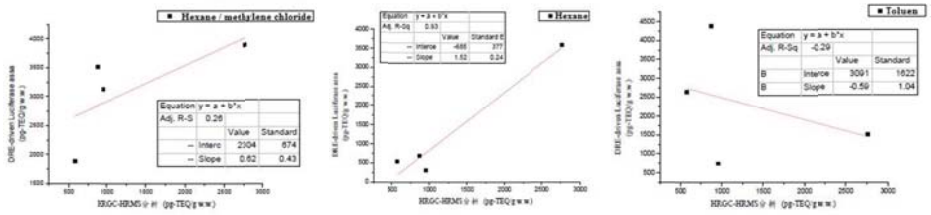

Figure 1. The relationship between Ad-DR bioassay and chemical analysis for ultrasonic extraction-directly concentrated samples

Ultrasonic extraction- column purification samples

The correlation coefficient is 0.873 for ultrasonic extraction-column purification samples. No matter what's the extraction solvent, a good correlation was found for the Ad-DR bioassay and chemical analysis. Toluene used as extraction solvent has the best result (R^2 = 0.98), followed by n-hexane / dichloromethane (R^2 = 0.93) and n-hexane (R^2 = 0.88) as shown in Figure 2.

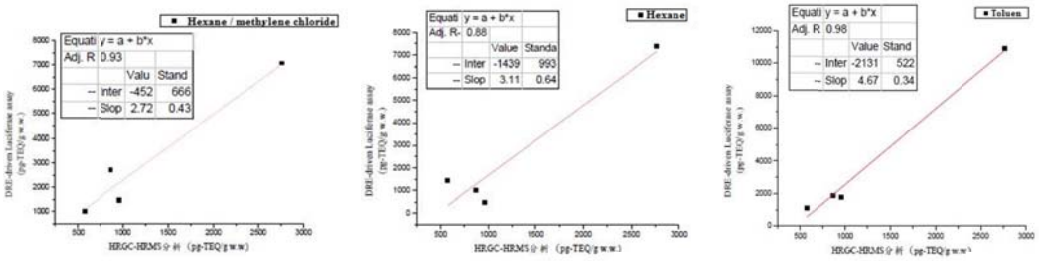

Figure 2. The relationship between Ad-DR bioassay and chemical analysis for Ultrasonic extraction- column purification samples

Soxhlet extraction - Directly concentrated of samples

A low correlation for the Ad-DR bioassay and chemical analysis was also found for soxhlet extraction-directly concentrated samples. N-hexane / dichloromethane (R^2 = -0.47) and n-hexane (R^2 = -0.47) have poor correlation for the Ad-DR bioassay and chemical analysis because the bioassay result was interfered by orange color. Toluene-based extraction showed a good correlation (R^2 = 0.93) for the Ad-DR bioassay and chemical analysis. The results were shown in Figure 3.

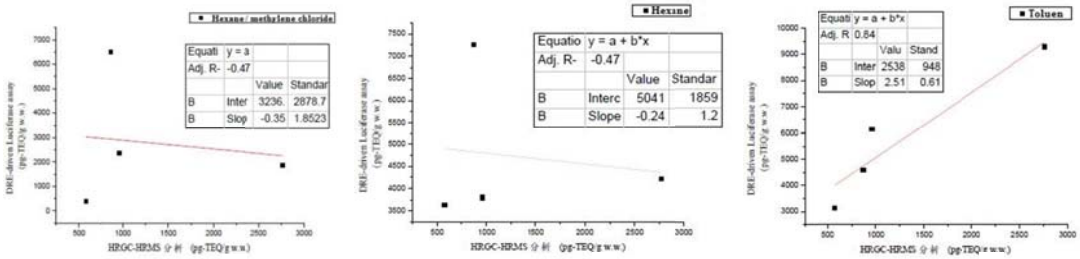

Figure 3. The relationship between Ad-DR bioassay and chemical analysis for Soxhlet extraction - Directly concentrated of samples

Soxhlet extraction- column purification

N-hexane/dichloromethane and the toluene solvent extracts showed good correlation for the Ad-DR bioassay and chemical analysis, R^2=0. 898 and R^2=0.887, respectively. The results were shown in Figure 4.

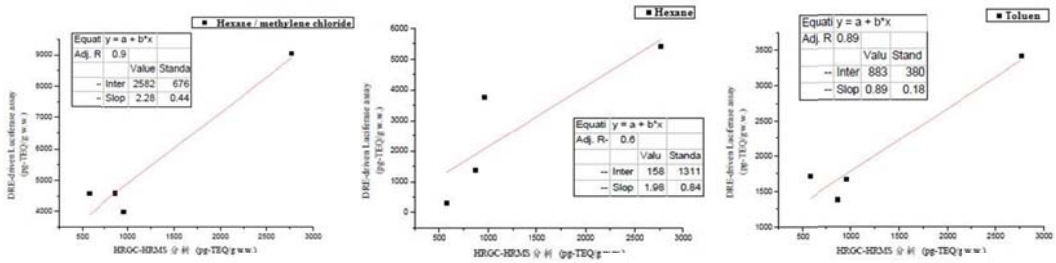

Figure 4. The relationship between Ad-DR bioassay and chemical analysis for Soxhlet extraction- column purification

1. A good correlation between Ad-DR bioassay and chemical analysis for ultrasonic extracted samples following column clean up procedure. The correlation coefficient is 0.873 for all three solvent samples.
2. For soxhlet extraction column cleanup process, n-hexane/ dichloromethane and the toluene solvent extracts also had good correlation, R^2=0.898 and R^2=0.887, respectively. The low correlation coefficient of Ad-DR bioassay and chemical analysis method for directly concentrated samples between ultrasonic extraction and soxhlet extraction was only 0.010 and -0.026.
3. The results suggest that the extensive cleanup procedure is critical for dioxin detection using the Ad-DR bioassay. In the present study, combining an effective cleanup system with an Ad-DR bioassay for PCDD/F TEQ levels analysis is an alternative.

Acknowledgements

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