DETERMINATION OF DIOXINS IN FLUE GAS AND FLY ASH FROM INCINERATORS OF CHINA BY ELYSA

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Abstract

A good correlation between ELISA and HRGC/HRMS results of flue gas and fly ash samples from China was obtained, the cost per sample of ELISA analysis was low, and it was capable of multiple analyses of many samples. Therefore, the ELISA method was suitable for the daily dioxins monitoring in the flue gas, and for prescreening polluted sites. Furthermore, the ELISA method using 2,4,5-TCP-$(Gly)_2$ not only improved the safety of all experimental processes, but also attained excellent stability by enabling storage of every reagent in an ordinary refrigerator at 2-8℃. It also enabled transportation of all reagents without special handling, the ELISA method of rapid, low cost, multiple analyses of samples can be expected to provide an alternative.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated dibenzofurans (PCDFs) have been concerned by the public and government for a few decades, more attention are paid for which than other persistent organic pollutants (POPs) due to their high toxicity, the toxic equivalent factors (TEFs) are given by both USEPA and WHO. Effective dioxins monitoring requires large quantity of samples[1], however, the traditional methodwith high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) requires the complicated sample cleanup procedure, costly equipment and experienced analyst, so this expensive and time consuming method often severely limits the scope and thoroughness of a sample effort[2] . It's even more obvious in developing country, such as China, China has built lots of medical waste incineration plants (MWI), dioxins in flue gas, fly ash, and soil near the plants must be monitored once a year or even half a year according to China's regulation, if all the samples are tested by HRGC-HRMS, it will be a heavy stress to government and dioxin labs.

Several experts have agreed that the bioassays in vitro as an effective prescreening method for TEQs determination in environmental samples. Several bioanalytical detection methods (BDMs) have been applied for measuring dioxin-like compounds activity, including 7-ethoxyresorufin-o-deetthylase (EROD)-bioassay, aryl hydrocarbon hydroxylase (AHH) bioassay, enzyme immunoassay (EIA), reporter gene assay [e.g. chemical-activated luciferase gene expression (CALUX) or P450HRGS], gel retardation of AhR DNA binding (GRAB) assay, recAhr DELFIA assay kit, Ah receptor (AhR) (or filtration) assay with radio labeled dioxins, and Ah-Immunoassay (AhIA). These methods are based on the ability of key biological molecules (e.g. antibodies, receptors, enzymes) to recognize a unique structural property of dioxins-like compounds, or on a specific response between cells (or organisms) and dioxins-like compounds. Many research reports have been published about biomarkers or bioassays of dioxins and dioxins-like compounds[3]. EPA Method 4425 (Reporter gene assay) and EPA Method 40255 (Immunoassay) are official methods in US, there are ten BDMs have been approved by Japan EPA, and EU also has set the same standards.

In China, many dioxins labs have been set up in recent years but can't satisfy the need of dioxins analysis yet, government is thinking about introducing BDMs, and setting relevant standards for dioxin analysis as well. Some research to improve the performance of BDMs in Chinese samples is necessary. The main aim of this study is validating feasibility of ELISA used as a prescreening method for TEQs determination in flue gas and fly ash samples from municipal solid incinerator of China. An ELISA method developed by Japan Tsuruga Institute of Biotechnology, TOYOBO Co. LTD is applied to analyzing 24 flue gas samples and 20 fly ash samples collected in China. The statistical data from ELISA derived 2,4,5-TCP-$(Gly)_2$ equivalents (ELISA-EQ) are compared with the TEQs values of HRGC-HRMS. The performance of ELISA method in China is evaluated for the first time.

Materials and Methods

Sampling

Dioxins in flue gas were collected by a glass or quartz fiber filter cylinder and XAD-2 resin with a vacuum pump and a volumetric meter located downstream under an isokinetic sampling mode (gas flow of approximately $3M^3$/4hr), fly ashes in the bag filter from municipal solid incinerators were collected as well.

Clean up Procedure for ELISA and HRGC/HRMS

Solid samples (filter, XAD-2 resin, fly ash) were Soxhlet extracted with toluene for at least 19 hours. The conventional cleanup method consisted of sulfuric acid treatment, multilayer silica purification (from top to bottom, sodium sulfate/ 10% silver nitrate-silica/ silica/ 22% sulfuric acid-silica/ 44% sulfuric acid-silica/ silica/ 2% potassium hydroxide-silica/ sodium sulfate) and fractionation by alumina or active carbon. Half of extract were completely dried and redissolved with Dimethyl sulfoxide (DMSO), for ELISA analysis, the other half was dried and redissolved with decane, for HRGC/HRMS analysis**.**

ELISA Experiment

Each 100µl of extract redissolved in DMSO was thoroughly mixed with equal volume of PBS, then 200µl of anti-dioxin monoclonal antibody solution was added. 100µl of this mixture was dispensed in the wells of micro plate coated with TCP-BSA. After each mixture was placed in wells, the whole micro plate was incubated in a cold room (2-8℃) for an hour, and all wells were washed 3 times in a plate washer. 100µl of goat antibody labeled with horseradish peroxidase (HRP) solution was dispensed into each washed well. The plate was incubated for an hour at room temperature (around 25℃), and all wells were washed in the washer. Next, 100µl of Tetramethylbenzidine (TMB) solution was added to each well and developed a color at 25℃ for 30 mins. Finally 100µl of 0.5N H_2SO_4 was added into each well to stop reaction, and absorbency of each well were measured at a wavelength of 450nm. Experiments were performed in triplicate (n=3) for every sample, the calibration curve was obtained using 2,4,5-TCP-$(Gly)_2$ as the standard substance, although other ELISA systems generally used highly toxic 2,3,7,8-TeCDD[4].

Results and Discussion

24 flue gas samples and 20 fly ash samples were measured using this ELISA method, the convert formula between ELISA and HRGC-HRMS were $y=0.0129\ (x/1000)^{1.4437}$ in flue gas and $y=0.003\ (x)^{0.8638}$ in fly ash. The results showed good correlation between two methods, with $R^2=0.944$ in flue gas and $R^2=0.9957$ in fly ash, respectively. The results indicated that ELISA is suitable for dioxin prescreening in flue gas and fly ash samples from China.

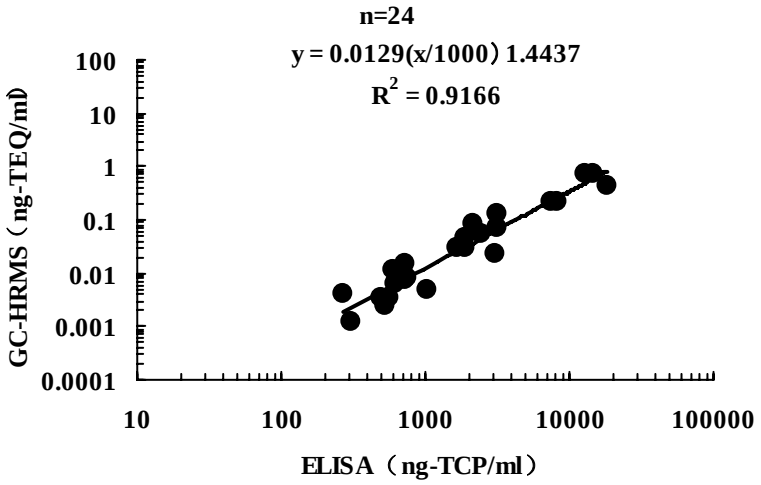

Figure1 Convert formula between HRGC-HRMS and ELISA values in flue gas samples

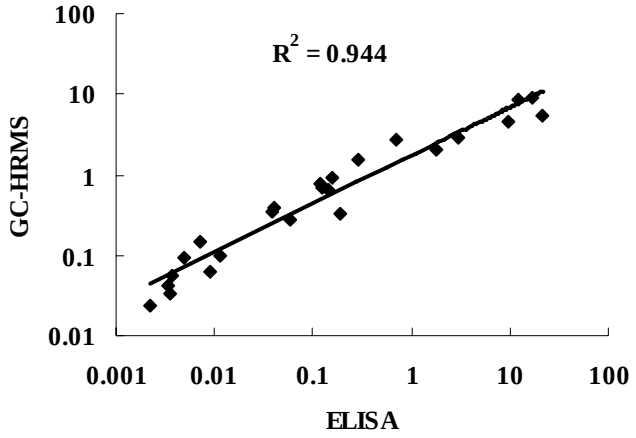

Figure2 Correlation between HRGC-HRMS and ELISA values in flue gas samples

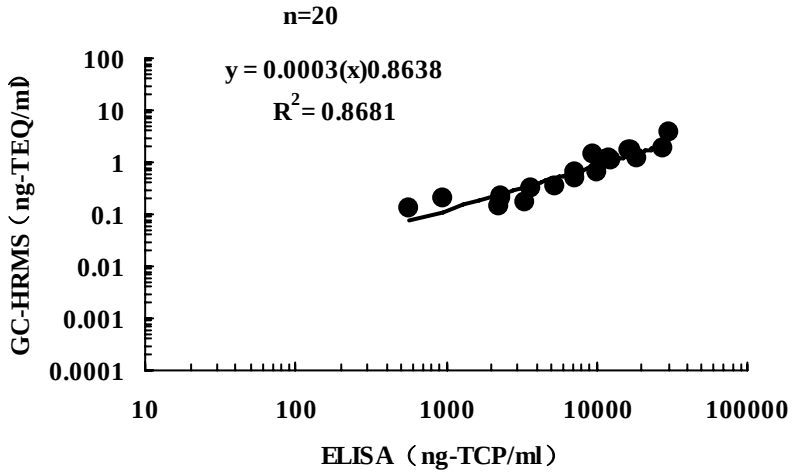

Figure3 Convert formula between HRGC-HRMS and ELISA values in fly ash samples

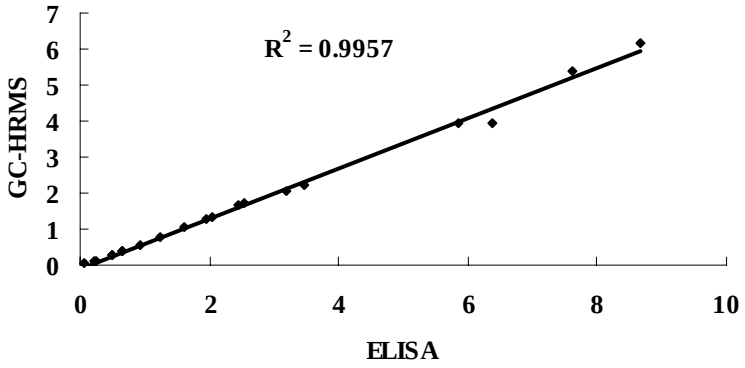

Figure4 Correlation between HRGC-HRMS and ELISA values in fly ash samples

Acknowledgements

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Reference

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