CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 43, Issue 7, July 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2015, 43(7), 1027–1032.

RESEARCH PAPER

Indirect Competitive Chemiluminescent Enzyme Immunoassay Method for Determination of Dimethyl Phthalate in Soy Sauce and Liquor

ZHU Bin, ZHU Fan, XU Zhen-Lin, YANG Jin-Yi, LIU Chun-Hong, SUN Yuan-Ming, WANG Hong, LEI Hong-Tao, SHEN Yu-Dong*

College of Food Science, South China Agricultural University, Guangdong Provincial Key Laboratory of Food Quality and Safety, Guangzhou 510642, China

Abstract: 4-Amino dimethyl phthalate (ADMP) as hapten was coupled to carrier protein and subsequently used to immunize New Zealand rabbits. Polyclonal antibody which showed specific binding to dimethyl phthalate (DMP) was obtained, and on the basis of this, an indirect competitive chemiluminescent enzyme immunoassay (icCLEIA) was developed. The experimental parameters for icCLEIA were optimized as follows: the concentration of coating antigen was 50 μg L^{-1}, the primary antibody concentration was 92.5 μg L^{-1}, the secondary antibody concentration was 1 μg mL^{-1}, ultrapure water (pH 6.0) was used as dilution solution and the time for competitive reaction was 40 min. Under the optimal conditions, the icCLEIA exhibited a linear working range from 0.74 μg L^{-1} to 30.32 μg L^{-1}, with the limit of detection of 0.29 μg L^{-1}. The cross-reactivity with thirteen structural analogues was less than 5%. The recoveries of DMP from spiked liquor and soy sauce samples were from 80.2% to 116% and the average RSD was less than 3.6%. The detection results of spiked liquor and soy sauce by icCLEIA were consistent with those by standard GC-MS method. The developed icCLEIA method exhibited a practical potential for detecting DMP residue in food samples.

Key Words: Dimethyl phthalate; Indirect competitive chemiluminescent enzyme immunoassay; Liquor; Soy sauce

1 Introduction

Phthalate acid esters (PAEs), as one kind of environmental estrogen-like substances, are widely used as plasticizer in a variety of plastic materials such as engineering materials, medical materials, food packaging materials and agricultural adjuvant[1]. It has been found that PAEs exhibit various harmful effects on human body including central nervous suppression, endocrine disruption, carcinogenicity, etc., which has attracted worldwide attention[2–5]. Dimethyl phthalate (DMP), one of the most commonly used PAEs, can easily migrate into the food chain to contaminate food and agricultural products due to its relatively better solubility in water than other PAEs[5–7]. It has been listed as one of the primary pollutants and strictly banned from using in food industry by the United States and China[8–10]. Therefore, it is of important significance to develop accurate, rapid, sensitive and low-cost detection methods for DMP contamination monitoring in food.

Currently, the detection of DMP is mainly performed by using chromatography and chromatography-mass spectrometry technologies including gas chromatography (GC)[11,12], high performance liquid chromatography (HPLC)[13–15], high performance liquid chromatography coupled with mass spectrometry (HPLC-MS)[16,17], gas chromatography coupled with mass spectrometry

Received 20 February 2015; accepted 16 March 2015
* Corresponding author. Email: syd_tyx@163.com
This work was supported by the Guangdong Province Science and Technology Plan Project of China (Nos. 2013B040402006, 2014A020219008), the Guangzhou Science and Technology Plan Project of China (Nos.2011Y2-00007, 2013J4100053), the National Natural Science Foundation of China (No. 31371769), and the Guangdong Province Outstanding Doctoral Dissertation Grant of China (XU Zhen-Lin).

DOI: 10.1016/S1872-2040(15)60839-8

(GC-MS)[18–22], etc. With these methods, DMP can be detected in the mg L^{-1} to pg L^{-1} level and multi-analyte detection of a variety of PAEs including DMP can be achieved. However, these methods generally suffer from inherent disadvantages such as expensive equipment, time-consuming sample preparation procedure, high cost, sophisticated operation, which limit the extensive application and can't meet the practical requirement for monitoring DMP in China because of the characteristics of decentralized management of agricultural production. Immunoassay based on specific antigen-antibody interaction is recommended as an excellent determination technology to developing countries by the United Nations Food and Agriculture Organization (FAO), due to the features of high throughput, low cost, rapidity, good sensitivity and accuracy. Meanwhile, the immunoassay method, by combining with instrument-based methods, can effectively improve the detection efficiency of DMP and greatly reduce the cost, thus is suitable to Chinese situation. For example, the ELISA and the FIA methods for detecting DMP in environmental water developed by Zhang *et al*[23,24] showed a quantitative limit of 0.02 μg L^{-1}. The detection limit of the biotin-avidin-based ELISA developed by Sun *et al* was down to pg level in the determination of DMP in milk samples[25]. However this biotin-avidin system is still complicated with low storage stability and high cost, which limits its practical application.

In this work, the artificial antigen of DMP was prepared by diazotization reaction method and used to immunize New Zealand white rabbits to produce polyclonal antibody against DMP with high sensitivity. Subsequently, an indirect competitive chemiluminescent enzyme immunoassay (icCLEIA) method for detecting DMP in liquor and soy sauce samples was developed and the result obtained here was compared with that of national standard confirmation techniques. The result showed that the developed icCLEIA method had a good practicability for rapid screening of DMP in food samples.

2 Experimental

2.1 Instruments and reagents

SpectraMax L Luminescence Microplate Reader (MD, USA), 96-well luminescence microplate (Shenzhen Jincanhua, China), DEM-3 microplate washer (Beijing Tuopu, China), EYELA rotary evaporator (Shanghai EYELA, China) and QP2010 Plus Gas chromatography-mass spectrometer (Shimadzu, Japan) were used in the experiment.

4-Aminophthalic acid (APA) was purchased from Energy Chemical (China). $SOCl_2$ and other reagents were purchased from Kemiou Chemical Reagent (China). DMP and other PAEs standards were from Dikma (China). Silica gel (100–200 mesh) for column chromatography was purchased from Qingdao Puke (China). Horse radish peroxidase (HRP) labeled goat-anti-rabbit IgG was bought from Wuhan Boster (China). Luminol chemiluminescence substrate was obtained from Guangzhou Wanlian (China). Ultrapure water (18.2 MΩ·cm) was used throughout. Liquor and soy sauce samples were purchased from local supermarket.

2.2 Experiment methods

2.2.1 Synthesis of hapten and antigens

0.2 g of APA was dissolved in 10 mL of anhydrous methanol, and 5 mL of $SOCl_2$ was then added drop by drop. After refluxing for 12 h at 70 °C, the solvent was rotary evaporated and the residue was purified by silica gel column chromatography. The obtained white powder hapten ADMP was identified by NMR and MS. Subsequently, hapten was coupled to carrier proteins bovine serum albumin (BSA) and ovalbumin (OVA) to get immunogen ADMP-BSA and coating antigen ADMP-OVA respectively through diazotization method[24], which were then identified by UV-vis spectroscopy[26].

2.2.2 Preparation and purification of antibody

According to the immunization protocol in Reference [27], two female New Zealand white rabbits (2.0–2.5 kg) were immunized with immunogen ADMP-BSA to produce antiserum against DMP. Subsequently, the polyclonal antibody against DMP (anti-DMP pAb) was purified from antiserum by ammonium sulfate precipitation method[28] and stored at –20 °C. The titer and inhibitory ability of the antibody were identified.

2.2.3 icCLEIA method

The icCLEIA was carried out as described in Reference [29]. Firstly, the coating antigen stock solution was diluted to 50 μg L^{-1} with carbonate buffer and added to a 96-well luminescence microplate at 100 μL per well. After incubation overnight at 37 °C, the microplate was washed twice with PBST washing buffer, and then blocked with blocking agent (120 μL per well) at 37 °C for 3 h. The liquid was discarded and the plate was dried at 37 °C for 1 h. Seven concentration levels of DMP standard solutions were obtained by diluting the DMP stock solution of 8000 μg L^{-1} with distilled water (pH 6.0) by 8-fold dilution, and the anti-DMP pAb was diluted 60000 times with PBS dilution buffer. Then 50 μL of the DMP standard solution and 50 μL of diluted pAb solution were added to each well and mixed thoroughly. After incubation for 40 min at 37 °C, the plate was washed with PBST for five times, and then 5000-fold diluted HRP-labeled secondary antibody was added at 100 μL per well and