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Simultaneous identification and quantification of 4-cumylphenol, 2,4-bis-(dimethylbenzyl)phenol and bisphenol A in prawn *Macrobrachium rosenbergii*

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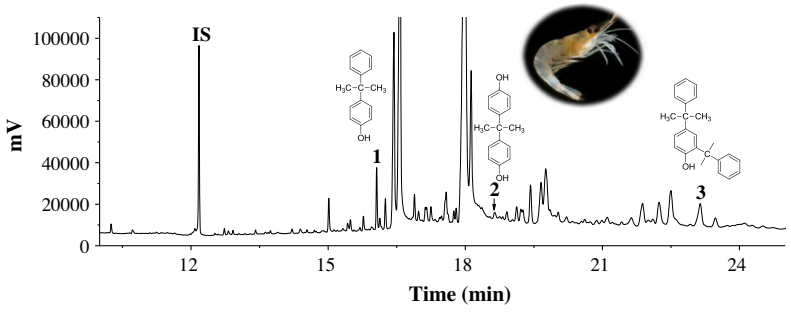
HIGHLIGHTS

- GC/FID/MS method was developed for determination of BPA and its analogs.
- The method is proven to be highly selective, accurate and sensitive.
- Estrogenic 4-CP, 2,4-DCP and BPA were found at ng g^{-1} concentration level in prawn.
- BPA, 4-CP and 2,4-DCP may negatively affect crustaceans at levels detected.

GRAPHICAL ABSTRACT



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ABSTRACT

Bisphenol A (BPA), 4-cumylphenol (4-CP) and 2,4-bis-(dimethylbenzyl)phenol (2,4-DCP) are all high production volume chemicals and widely used in plastic and other consumer products. During the past two decades, BPA has attracted a great deal of scientific and public attention due to its presence in the environment and estrogenic property. Although 4-CP and 2,4-DCP are much more estrogenic and toxic than BPA, little information is available about their occurrence and fate in the environment. In this study, a rapid, selective, accurate and reliable analytical method was developed for the simultaneous determination of 4-CP, 2,4-DCP and BPA in prawn *Macrobrachium rosenbergii*. The method comprises an ultrasound-accelerated extraction followed by capillary gas chromatographic (GC) separation. The detection limits range from 1.50 to 36.4 ng kg^{-1} for the three alkylphenols. The calibration curves are linear over the concentration range tested with the coefficients of determination, R^2, greater than 0.994. The developed method was successfully applied to the simultaneous determination of 4-CP, 2,4-DCP and BPA in prawn samples. The peak identification was confirmed using GC–MS. Bisphenol A, 2,4-bis-(dimethylbenzyl)phenol and 4-cumylphenol were found in prawn samples in the concentration ranges of 0.67–5.51, 0.36–1.61, and 0.00–1.96 ng g^{-1} (wet weight), respectively. All relative standard deviations are less than 4.8%. At these environmentally relevant concentration levels, 4-CP, 2,4-DCP and BPA may affect the reproduction and development of aquatic organisms, including negative influence on crustaceans' larval survival, molting, metamorphosis and shell hardening. This is the first study reported on the occurrence of 4-CP, 2,4-DCP and BPA in prawn *M. rosenbergii*.

1. Introduction

Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane (BPA), and two of its analogs, 4-cumylphenol (4-CP) and 2,4-bis-

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(dimethylbenzyl)phenol (2,4-DCP) are high production volume chemicals, with a total worldwide production rate over 8 billion pounds per year. BPA has been used as a monomer in the manufacture of polycarbonate plastics and epoxy resins, which are extensively used in the production of consumer goods, such as baby and water bottles, medical devices, dental fillings, sealants, sports equipment, coatings on metal lids, protective linings for canned foods and beverages, and household electronics. Due to its large quantity production and widespread application, BPA has been frequently detected in various environmental matrices, such as air, sewage, river, lake and coastal sea waters, sediments, soil, dust, foodstuffs, drinks, and biological samples, including human urine and blood samples (Zhu and Zuo, 2013). Detectable levels of BPA have been found in urine collected from over 92% of the general population in the United States (Calafat et al., 2008; Vandenberg et al., 2010).

Because of its estrogenic properties and widespread occurrence in consumer products and the environment, BPA has attracted a great deal of attention from scientists, general public and regulatory agencies all over the world. As early as 1936, before its use as a chemical that makes plastics, BPA was discovered as a synthetic estrogen (Dodds and Lawson, 1936). The estrogenic activities of BPA have been consistently shown by *in vitro* assays (Staples et al., 1998; Vandenberg et al., 2010; WHO, 2011). Bisphenol A has some structural similarity to estradiol (E2) (Zuo et al., 2006) and can bind human estrogen receptors ERα and ERβ. Until recently, BPA was considered a weak environmental estrogen with a binding affinity and transcriptional activity for these ERs more than 1000-fold lower than that of E2 (Gould et al., 1998; Kuiper et al., 1998). Nevertheless, a number of studies have shown that exposure to BPA at concentrations of real life exposure, (<50 μg BPA kg^{-1} body weight d^{-1}, the tolerable daily intake set by the EU Commission and the reference dose established by US-EPA), resulted in decreased sperm production, increased prostate gland volume, altered development of the mammary gland, altered vaginal morphology and estrous cycles, disruption of sexual differentiation and earlier puberty (Vandenberg et al., 2010; WHO, 2011). At present, the effects of BPA *in vivo* at low doses are still a hot scientific debate (Melnick et al., 2002; Ryan et al., 2010). However, most of previous research efforts have been focused on BPA itself. Fewer studies have been made on its analogs, metabolites, and other related degradation products from the plastics and resins. Some of these analogs and degradation products could have a higher estrogenic activity and toxicity than BPA. Okuda et al. (2010) and Yoshihara et al. (2004) have revealed that one of such BPA metabolites, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), has an estrogenic activity approximately 1000 times higher than BPA. It is recently assumed that not BPA but one its metabolite MBP is more problematic as an endocrine disrupting compound. Terasaki et al. (2005) have also reported that 4-cumylphenol (4-CP), a BPA analog in plastics, resins and the degradation products, has an estrogenic activity over 12-folds higher than BPA. Therefore, it is important to identify BPA analogs, metabolites and related degradation products of polycarbonate plastics and epoxy resins, and examine their concentrations, estrogenicity and toxicity in various environment matrices.

So far, analysis of BPA and other endocrine disrupting chemicals in water and biological samples has mainly been accomplished by different chromatographic methods such as GC, GC–MS, HPLC and LC-MS (Berkner et al., 2004; Stuart et al., 2005; Fukata et al., 2006; Zuo et al., 2006, 2011, 2013; Zuo and Lin, 2007; Calafat et al., 2008; Shi, 2012; Wang et al., 2013; Zuo, 2014). Although enzyme linked immunosorbent assays (ELISA) and other bioassay techniques have also commonly used to measure BPA concentration in water and biological samples, they are less specific than chromatographic methods, may not distinguish BPA from other endocrine disrupting compounds, particularly other bisphenols and analogs (Fukata et al., 2006). Due to the requirement of high selectivity and sensitivity for the analysis of environmental and biological samples, GC–MS has been extensively applied to determine these chemicals without derivatization or after derivatization to improve the evaporative ability of the analytes. In this study, an accurate and rapid GC–MS method has been developed for the simultaneous determination of bisphenol A and two its analogs, 4-cumylphenol and 2,4-bis-(dimethylbenzyl)phenol, in environmental and biological samples. For the first time, 4-CP and 2,4-DCP have been found in prawn tissue samples. The chemical structures of these alkylphenols studied and biphenyl, the internal standard, are presented in Fig. 1.

2. Experimental

2.1. Chemicals and prawn samples

Bisphenol A, 4-cumylphenol, and 2,4-bis-(dimethylbenzyl)phenol standards were all purchased from Sigma–Aldrich (St. Louis, MO). Biphenyl (internal standard) was purchased from Acros Organics (Morris Plains, NJ). Acetonitrile, acetone and hexane, all of HPLC grade, were obtained from Pharmco products (Brookfield, CT). Except where noted, all reagents were of analytical grade and all aqueous solutions were prepared by using doubly-distilled and deionized water. Prawn samples were purchased from local supermarkets in New Bedford and North Dartmouth, MA and stored at 4 °C until used in this study.

2.2. Preparation of standard solutions and samples

2.2.1. Standard solutions

The standard stock solution of BPA (1.000 mg mL^{-1}), 2,4-bis-(dimethylbenzyl)phenol (1.080 mg mL^{-1}) and 4-cumylphenol (1.000 mg mL^{-1}) were prepared by dissolving the standard chemicals in 10 mL of methanol. Internal standard stock solution (1.030 mg mL^{-1}) was prepared by dissolving 10.30 mg of biphenyl in 10.00 mL methanol. All these solutions were stored in the dark at 4 °C. The lower concentrations of working solutions were freshly prepared by dilution of these stock solutions.

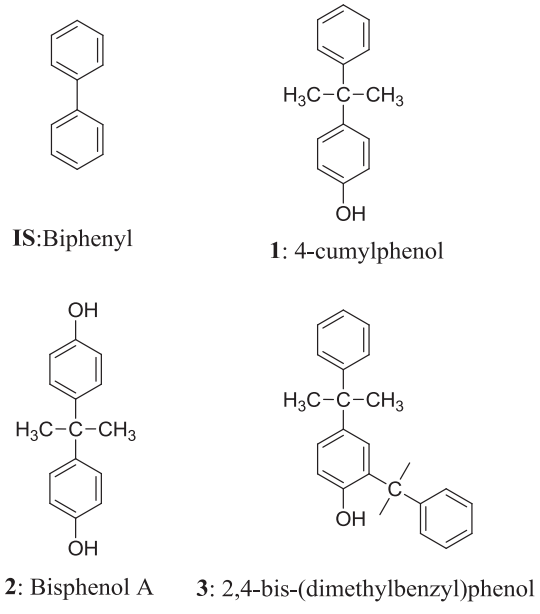

Fig. 1. Chemical structure of biphenyl, bisphenol A and 2,4-bis-(dimethylbenzyl)phenol.

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