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Determination of bisphenol AF (BPAF) in tissues, serum, urine and feces of orally dosed rats by ultra-high-pressure liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

As a homologue of bisphenol A (BPA), there is concern about the potential reproductive and developmental toxicity of bisphenol AF (BPAF) based on *in vitro* tests. In this study, a simple and universal analytical method was developed for the determination of trace BPAF in various tissues and excreta of rats after they were orally dosed. The samples were hydrolyzed with glucuronidase/arylsulfatase followed by ultrasonic extraction with acetonitrile. The crude extract was purified with a mixed-mode anion exchange (Oasis MAX) solid-phase extraction (SPE) cartridge. Separation and quantification was then conducted by ultra-high-pressure liquid chromatography/electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) in negative ionization mode. The recoveries at three fortification levels in different biological samples were from 71.0% to 102.3% with relative standard deviations no more than 13.2% ($n = 6$). The quantification limits of the method were from 0.5 μg/kg to 3 μg/kg depending on the matrix. This method was successfully applied to the determination of BPAF in tissues, serum, urine and feces of orally dosed rats.

1. Introduction

Bisphenol AF (1,1,1,3,3,3-hexafluoro-2,2-bis (4-hydroxyphenyl) propane, BPAF) has a structure of two phenolic rings joined together through a carbon bridge. It is a homologue of bisphenol A (BPA) in which the methyl groups are perfluorinated (Fig. 1). BPAF has broad applications in areas such as food processing equipment, electronic devices and optical fibers and especially in fluoroelastomers as the vulcanizer due to its excellent stability and hot tear strength [1]. Although industrial production of BPAF is increasing considerably, no data are available on the annual production or the occurrence of BPAF in the environment [2].

As the fluorinated homologue of BPA, a proven endocrine disrupting compound, there is concern that BPAF is potentially more harmful to human health because its CF_3 moiety may be much more electronegative and reactive than the CH_3 of BPA. The acute oral toxicity of BPAF in laboratory animals is low [3], but recent research indicates that this chemical may pose high potentiality as an endocrine disruptor for humans and wildlife *via* binding with hormone receptors. *In vitro* assays indicate that BPAF binds to estrogen receptor-alpha approximately 20 times more effectively than BPA and to estrogen receptor-beta almost 50 times more effectively. BPAF appears to shift endocrine action toward greater toxicity [2]. Another study found that BPAF exhibited both high estrogenic and anti-androgenic activities [3]. These potential risks have prompted the US National Institute of Environmental Health Science to nominate BPAF for comprehensive toxicological characterization [4].

Currently, the limited study of BPAF is mainly concentrated on the mechanism of its endocrine disrupting effect *in vitro*, and there are no published reports of an analytical method for the determination of BPAF. In order to assess the exposure level of BPAF in organisms and conduct further BPAF toxicological studies, a reliable analytical method for BPAF in bio-matrices was needed. The objective of this paper was to develop a fast and universal method for the determination of BPAF in bio-matrices after orally dosed exposure.

2. Experimental

2.1. Chemicals and reagents

BPAF (98% purity) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). HPLC-grade acetonitrile and methanol were

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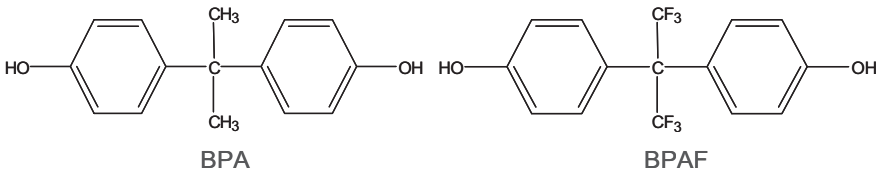

Fig. 1. Structure of BPA and BPAF.

supplied by Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was obtained by using an in-house Milli-Q Ultrapure water system (Millipore, Bedford, MA, USA). Formic acid (99%) was purchased from Acros Organics (New Jersey, NY, USA). Corn oil was supplied by Sigma–Aldrich. Oasis MAX SPE cartridges (60 mg, 3 mL) were purchased from Waters (Milford, MA, USA).

2.2. Collection of murine specimens

Male Sprague Dawley (SD) rats of 8–9 weeks old (250–300 g) were obtained from the experimental animal center at the Academy of Military Medical Sciences, China. All experiments were carried out in compliance with the guidelines of the experimental animal center of the Beijing Center for Disease Control and Prevention. The animals were housed in a temperature-controlled room (25 °C) under a 12 h/12 h light/dark cycle with free access to standard food and water. Four rats were caged individually in steel metabolism cages to collect the urine and feces that was used for blanks. After the collection of excreta, whole blood samples were obtained by femoral artery puncture, and the rats were euthanized by cervical dislocation. The following tissues were dissected and used as blanks: kidney, liver, testis, adipose and muscle. Serum was obtained by centrifugation (15 min, 3000 rpm, 4 °C) and stored at −20 °C. Tissues were immediately placed on ice and then stored at −80 °C until analyzed.

To investigate the applicability of this method in rats and the distribution of BPAF in different tissues, 4 SD male rats were allowed to acclimate 1 week before treatment. A 10 mg/kg dose of BPAF dissolved in corn oil (a non-toxic dose, approximately 350-fold lower than the published acute LD50 data in rats) was given to rats by oral administration for 2 consecutive weeks. Immediately after the administration, the rats were returned to the metabolism cage, and urine and feces samples were collected. At the end of the exposure, animals were euthanized for necropsy. The obtained serum was stored at −20 °C. Tissues (kidneys, liver, testis, adipose and muscle) were immediately placed on ice and then stored at −80 °C until analyzed. The concentration of BPAF in the biological matrices was determined by processing the samples as described, followed by detection using LC–MS/MS.

2.3. Analytical procedure

2.3.1. Instruments and conditions

BPAF identification and quantification were performed with an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA). LC separation was conducted with an Acquity BEH C18 column (2.1 mm × 50 mm; 1.7 μm; Waters). The mobile phases were A (ultrapure water) and B (methanol). The injection volume was 10 μL. A total flow rate of 0.3 mL/min was used with a gradient elution starting with 30% mobile phase B, followed by a 4 min linear gradient to 100% mobile phase B, which was continued for 2.0 min. The system was re-equilibrated for 3 min between runs. Samples were ionized in negative electrospray ionization mode (ESI). The capillary voltage was 2.5 kV. The source temperature and desolvation temperature were set at 150 °C and 400 °C. The nitrogen flow rate was 700 L/h, and ultra-pure argon was used as the collision gas at a flow rate of 0.13 mL/min.

2.3.2. Preparation of stock and standard solution

Approximately 10 mg of BPAF powder was accurately weighed and dissolved in methanol to yield a final concentration of 1.0 mg/mL and then stored at −20 °C in amber glass vessels. Working standards were prepared by diluting the stock solution in methanol/water (50:50, v/v) to a final concentration of between 0.1 μg/L and 500 μg/L.

2.3.3. Preparation of biological matrices sample

Aliquots of 100 mg of homogenized tissue, 50 mg of freeze-dried feces, or 200 μL of serum/urine samples were transferred into 5 mL polypropylene centrifuge tubes containing 500 μL of 0.2 mol/L acetate buffer (pH 5.2). After which, 20 μL of glucuronidase/arylsulfatase from *Helix Pomatia* (Roche Diagnostics GmbH, Mannhein, Germany) was added and mixed thoroughly with a vortex mixer. The mixture was incubated overnight at 37 °C. After the sample cooled to room temperature, 1.5 mL of acetonitrile was added, and the mixture was sonicated at room temperature for 15 min. This extract was centrifuged at 9000 × *g* for 10 min at 4 °C. The supernatant was transferred into another 5 mL tube, and the residue was re-extracted with 1.5 mL of acetonitrile. The combined supernatants were diluted with a 4-fold volume of water and purified with an Oasis MAX cartridge (60 mg, 3 mL) that was preconditioned with 3 mL of methanol and 3 mL of water. After the sample was loaded, the cartridge was sequentially washed with 1 mL of water containing 5% ammonia, 1.5 mL of methanol, and 1 mL of methanol/water (40:60, v/v) containing 2% formic acid. The target drug was eluted from the cartridge with 1 mL of methanol containing 2% formic acid. The eluate was dried under a gentle stream of nitrogen, and the residual was reconstituted in 1 mL of methanol/water (50:50, v/v) for analysis. For feces samples, the reconstituted solution was diluted with a 100-fold volume of methanol/water (50:50, v/v) before instrumental detection.

2.4. Method validation

Three types of standard calibration curves were prepared for the method assessment: (1) a neat standard curve (plotted using methanol/water (50:50, v/v) dissolved standard solutions from 0.1 μg/L to 100 μg/L), (2) matrix-matched standard curves (plotted using standards spiked in extracts of blank samples that were spiked before LC–MS/MS analysis), and (3) matrix-fortified standard curves (plotted using extracts of blank samples spiked before pretreatment).

2.4.1. Linearity and range

The linearity of the response was studied using the matrix-fortified standard curves (using extracts of blank samples spiked at six concentrations before pretreatment, ranging from the quantification limit to 1000 μg/kg). The peak areas of the selected quantification MRM transitions were used to construct matrix-fortified calibration curves, which were used for quantitative determinations.