



Detection of Bisphenol-A in Dental Materials by High Pressure Liquid Chromatography –Electrochemical Detector

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ABSTRACT

The xenoestrogenic substance bisphenol-A (BPA) is an important chemical building block that is used primarily to make polycarbonate plastic and epoxy resins, both of which are used in a wide variety of applications for example, eyeglass lenses, digital media, (electronic and electrical equipment housings), food, and dental materials. Increasing the risk of human exposure to BPA, mainly through food and dental materials (sealants or composites)

results in endocrine disrupting chemicals (EDCs). Exposure to BPA and its potential effect on human health are receiving increase attention, as studies on laboratory animals have shown that BPA can cause genetic damage. BPA stimulates cell proliferation and induce expression of estrogen-responsive gene in vitro. BPA was detachable from diglycidyl methacrylate, which is frequently utilized as the chief dimethacrylate monomer in polymerized materials utilized as a part of this study were polluted with BPA. Quantitative examination utilizing bisphenol-F as internal standard. BPA substance in business dental materials going from < 1 microgram/g material to around 20 micrograms/g material. The polymerized dental materials discharged up to 100 ng /g material into phosphate saline 24-h hatching. These outcomes show that BPA can be discharged from dental materials.

KEYWORDS : Bisphenol A , endocrine disorder , dental material

Introduction

BPA is a monomer used to make polycarbonate plastic and epoxy resins.

Traces of BPA can leach out of this polycarbonate plastic surfaces under various environmental conditions such as heat or pH changes and eventually are consumed by humans. BPA was detected in various matrices such as urine, dental materials² ground water and plasma.

Taking into account the suspicion that a limit exists above which poisonous impacts are seen the U.S Environmental Protection Agency (US EPA) has built up 50 µg/kg body weight/day as the reference measurement (RfD) for BPA¹. In this research we used a structurally similar commercially available compound bisphenol F, (BPF), along with BPA to determine separation efficiency. We utilized a comparative monetarily accessible compound bisphenol (BPF) (Figure 1).

The HPLC-ED (ECD) method offers significant advantages over other analytical instruments including selectivity, sensitivity needed to monitor BPA in dental material. Ethyl acetate hplc grade used for BPA and BPF (liquid/liquid extraction).

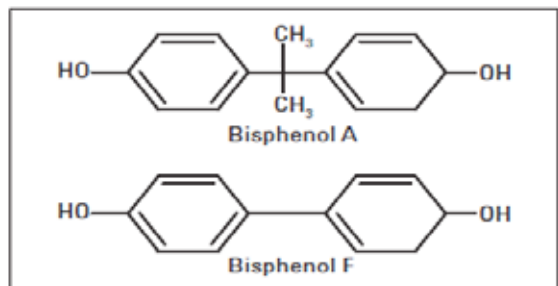


Figure1 Molecular structures of bisphenol A and bisphenol

Reagents and materials

All chemicals and solvents used were HPLC grade. Acetonitrile and ethyl acetate were purchased from Carlo Erba. Standards of bisphenol A and bisphenol F and potassium phosphate monobasic was purchased from Sigma-Aldrich

Preparation of standards

1 mg of analytical grade BPA (>99%, Sigma-Aldrich, USA) in 1 ml of

acetonitrile From this, 3, 5, 10, 25 and 50 ng /ml BPA and BPF were prepared freshly by diluting the stock solutions using the dilution buffer of 5% acetonitrile and 95% 10 mM monobasic potassium phosphate in water. Both the standards and the unknown samples (in triplicate) were finally analyzed using Jasco HPLC system, equipped With EC (Antec-Decade).

Chromatographic parameters

The chromatographic parameters for reverse phase Dr.Maisch (C8 150mm x4.6 mm) liquid chromatography Using Jasco PU 1580-i and Antec (Decade ECD) figure 2

Column 4.6x150 mm 5 µm

Temperature: 40 °C

EC cell 0.9 mv oxidation mode

Mobile phase A: 10 mM monobasic potassium phosphate in water

Mobile phase B: 35% Acetonitrile

Gradient: Time (min) %B

0 5

2 5

12.5 35

Flow: 0.9 mL/min

Injection volume: 20 µl

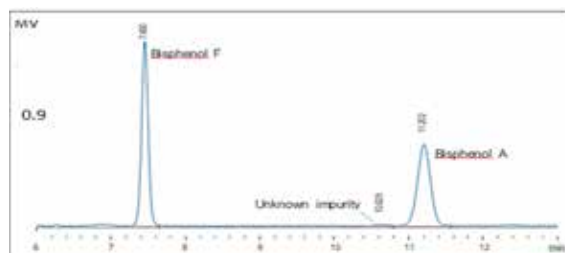


Figure2 Separation of 3ng/ml Solution of bisphenol F and bisphenol A using hplc with C8 column, ECD.

Sample preparation

BPA was extracted following the schematic shown in Figure 3

70 mL HPLC grade hot water thermostatically controlled into 10 g sample in borosilicate beaker for 60 minutes, the water was transferred into a 250 ml separatory funnel, to which 10 ml of hplc grade ethyl acetate, was added; the contents were swirled gently for 30 sec and finally left undisturbed for the formation of clear and distinct organic and aqueous layers.

The organic phase was collected in a 50 ml glass test tube and the aqueous phase was drained out into a beaker for the second and third time extractions, each with 10 ml of ethyl acetate hplc grade

The collective 30 ml of the organic phase (ethyl acetate) extract) was evaporated to dryness at 60 C using a water bath. The dried residue was re dissolved in 1 ml of acetonitrile,

Filtered through 0.2 m, PTFE(polytetrafluoroethyleneNupore Filtration syringe filter using a glass syringe. The filtered extracts were transferred into 5 ml amber-colored glass vials and stored at 4C for further analyses

Figure 3 Extraction of BPA from dental material and sample preparation

Recovery

To check the recovery rate of BPA achieved in the extraction method employed in this study, BPA aqueous solution in the concentration of 10, 25 and 50 ng/ml was made and the solutions extracted with ethyl acetate, similar to that of the samples. In figure 2. Finally, chromatograms of the BPA extracts prepared from the known concentrated BPA aqueous solutions compared to those of the external standards (3, 5, 10, 25 and 50 ng/ml) and percentage efficiency of BPA extraction procedure was calculated. Finally in three different brands of dental materials analyzed to determine the leaching concentration of the two bisphenols using the standards and HPLC method. The method was then transferred to Jasco 1580i system and run on C8 column using the same experimental conditions to test resolution and sensitivity of this method figure 4

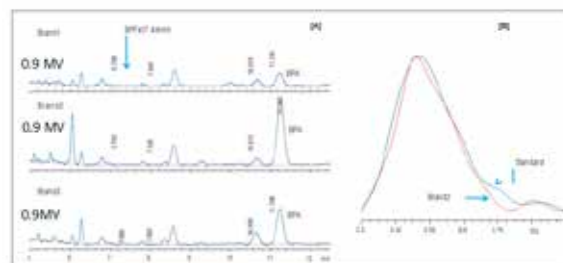


Figure 4[A] the overlay of chromatogram from three different dental material analyzed for BPA and BPF. [B] The overlay of the oxidation spectrum of BPA from standard and that obtained from sample 2

Results and discussion

The concentration of BPA derived using the peak area of external standards versus BPA concentration by fitting into the linear curve equation. The results show using hot water, provides evidence for BPA leaching from the samples to the water stored at minimum temperature (70 C for 1 h).figure 5

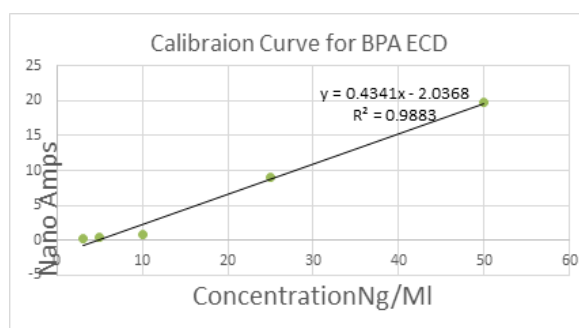


Figure 5 Calibration curve for BPA

Conclusion

The bisphenols show trademark oxidation designs over the cluster that is of quality while testing these compounds in complex matrix. HPLC-ECD is one of the most sensitive analytical procedures available today. In spite of the fact that it is a prevalent view that moderately few compounds are electrochemically dynamic this assumption is wrong. Numerous compounds, for example, endogenous metabolites, drugs, poisons, and contaminants are capable of HPLC-ECD.3,4 The amperometric anode offers unrivaled sensitivity and selectivity as well as offers unparalleled stability and is maintenance free without routine operation. As analysts are recognized, both by retention time and oxidation behavior across the array compound misidentification and co elution are minimized.

From the perspective of the safety of dental materials, it is necessary to assess the

Contribution of dental materials to the overall body burden of BPA, and whether or

not individual patients could be influenced by exposure to dental materials that contain BPA or BPA-based compounds.

Concerns have been expressed about the possible exposure of patients to BPA from dental materials, in particular some fissure sealants. Bisphenol glycidyl dimethacrylate (Bis-GMA) is one of the most commonly used monomers for both anterior and posterior resin restorative materials, fissure sealants and a range of other dental products. Bis-GMA derived from the reaction of bisphenol A and glycidyl dimethacrylate. This resin is commonly referred to as Bowen's resin, after its inventor. There are also several composites that use a urethane methacrylate resin (UDMA) rather than Bis-GMA. Bis-GMA and urethane. It has been demonstrated that BPA may be released from some resin composite restorative materials and fissure sealants (5). In the event If BPA is observed to be leached from dental restorative materials at that point this might be for one to two reasons.

The release may be due to residual BPA being available in the resin as part of the production process of Bis-GMA as a contaminant. The polymerized dental materials released up to 91.4 ng bisphenol-A/g material into phosphate buffered saline during 24-h incubation. These results indicate that bisphenol-A can be released from dental materials, however the leachable amount would be less than 1/1000 of the reported dose (2 micrograms/kg body weight/day) required for xenoestrogenicity in vivo.⁶

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