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An in-vitro assessment of bisphenol a release from thermoplastic orthodontic appliances exposed to various beverages

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Abstract

Background Thermoplastic orthodontic appliances have been used as retention devices for years and gaining popularity as aligners, alternative to fixed orthodontic mechanics. This study aims to assess Bisphenol-A (BPA) release from thermoplastic orthodontic appliances exposed to different beverages.

Methods The appliances in this study were enrolled from aligners of Invisalign, ClearCorrect, and thermoplastic retention device, Essix ACE. Five different beverages (pure water, whole milk, coke, orange juice, filtered coffee) were determined and 5 glass tubes containing 10 ml of each beverage formed experiment groups. Appliance samples from each brand (with 0.50 ± 0.0082 g weight) were inserted in glass tubes and left in room temperature for 1 h. Later, samples were removed and Flow Injection Liquid Chromatography/Mass-Mass Spectrometry (FIA-LC/MS-MS) analysis was performed to evaluate BPA release. Five glass tubes for each beverage group, which did not contain any appliance, were also analyzed with the same technique to detect the possible pre-existing BPA levels in the liquids.

Results FIA-LC/MS-MS analysis showed no measurable amount of BPA in pure water and any beverage group. BPA was also not detected in samples without appliances.

Conclusion BPA release was not detected from thermoplastic orthodontic appliances after 1 h of incubation in beverages. Within the limitations, different types of beverages were not found to affect BPA release from thermoplastic orthodontic materials. From a clinical perspective, further in-vivo studies will be useful with larger samples and longer term investigations.

Keywords Thermoplastic orthodontic appliances, Clear aligners, Bisphenol-A, Beverage, Orthodontics

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Introduction

Within the technological developments, changes have occurred in the way how orthodontic forces are transferred to dentition in the last decades. Accordingly, clear aligners have recently gained popularity as an alternative to fixed orthodontic appliances [1, 2]. Similarly, thermoplastic retention appliances, which patients can tolerate better due to their aesthetic and usable advantages, are frequently preferred at the end of orthodontic treatment [3].

From a chemical point of view, clear aligners are similar with thermoplastic retention appliances due to their polymer structures [4]. Various chemicals are added to polymers in order to improve their properties, such as durability, hardness and transparency, not only in dentistry but also in many other fields including plastic production [5]. Bisphenol-A (BPA), used as a hardener in plastic products, is one of the chemicals which acts as a synthetic hormone for human reproductive systems, revealing potential risks for nervous, immune, and cardiovascular systems [6, 7]. Potential harms of BPA were mainly reported as estrogenic interactions, proliferative stimulations and toxic effects on human cells and concerns have been raised due to the link of BPA with obesity, diabetes, cardiovascular disease, hormone-sensitive cancers, neurological disorders, and with increased sensitivity in fetuses and newborns [8]. BPA can be detected in saliva, blood, urine and breast milk, thus exposing humans to local and systemic exposure, and may disrupt the normal functioning of the body [9].

Previous studies revealed the BPA release from polymer-based orthodontic materials, such as adhesive composites, chain elastics, chemically polymerized acrylic and thermoplastic retention appliances [10–13]. Although detected BPA levels in these studies were far below the detected daily toxic doses, knowledge about long-term cumulative effects of this chemical has not yet been clearly explained [14]. Considering that orthodontic treatment patients are mostly children in growth period, the seriousness of the situation becomes more evident. Awareness about these chemicals has increased in recent years, and resulted with certain BPA-based products such as food containers and baby bottles have being removed from the market in most countries [15].

Due to their material properties, thermoplastic orthodontic appliances can be deformed by heat or broken as a result of trauma. Therefore, patients are advised not to use them during meals, and not to expose them to direct sunlight and heat [16]. However, some patients still ignore this and consume beverages while their appliances are in their mouth [17]. In-vitro studies showed that coffee, red wine or tea have staining effects on clear aligners [18]. Furthermore, acidic beverages, enzymatic actions and pH changes might increase the surface roughness of aligners [19], and affect BPA release by acting as a solvent [20]. A study previously reported an increase in salivary BPA levels immediately after the first 24 h to 1 week of exposure to thermoplastic retainers [11]. Even though these materials are introduced to the market as BPA-free, it can be seen that safety of these products are still questionable [21]. Thermoplastic orthodontic appliances, either for treatment or retention purposes, are worn by patients for certain times depending on their treatment protocols, and they may be exposed to many factors such as diet, temperature changes and cleaning solutions during this time [22]. The literature knowledge declared the lack of researches evaluating the effect of different beverages on the release of BPA from thermoplastic orthodontic appliances.

Therefore, this study aimed to assess BPA release from aligner and retention appliances exposed to whole milk, coke, orange juice and filtered coffee in comparison to pure water. The null hypothesis was that different beverages would not affect the BPA release from thermoplastic orthodontic appliances.

Materials and methods

Thermoplastic orthodontic appliances used in this study included as-received aligner materials from Invisalign (Align Technology, Santa Clara, Calif), ClearCorrect (Straumann Group, Basel, Switzerland) which were obtained for treatment purpose, and thermoplastic retention plate with 1 mm material thickness (Essix ACE; Dentsply International, Sarasota, Fla). The retention plate was formed according to the manufacturer's instructions with an Essix™ Machine (Raintree Essix, New Orleans, La) through softening with heat and vacuuming. Then, aligners and Essix plates were cut by scissors to small pieces of 0.50 ± 0.0082 g to fit in glass tubes. Weight measurement of each sample was achieved by using a scale (Radwag WAA 220/C/2, Radom, Poland) (Fig. 1.a). Thermoplastic appliance brands and their contents are given in Table 1.

Five beverages, each forming an experiment group were determined as the liquid media in which the aligner samples would be stored: pure water (pH = 7.0), whole milk (pH = 6.0), coke (pH = 2.7), orange juice (pH = 3.5), and fresh filtered coffee (pH = 4.9) from a local coffee house. Pure water was kept at the room temperature, and other beverages except coffee were kept in the refrigerator at + 4 °C. Coffee was stored in a stainless-steel thermos until it was used in experiment and its temperature was measured as + 56 °C before it was added in glass tubes.

A statistical power analysis was conducted using G*Power software version 3.1.9.7 to determine the required sample size based on a 95% confidence level, an effect size of 0.42, and 80% power. The results indicated that five replicates were necessary for each of the

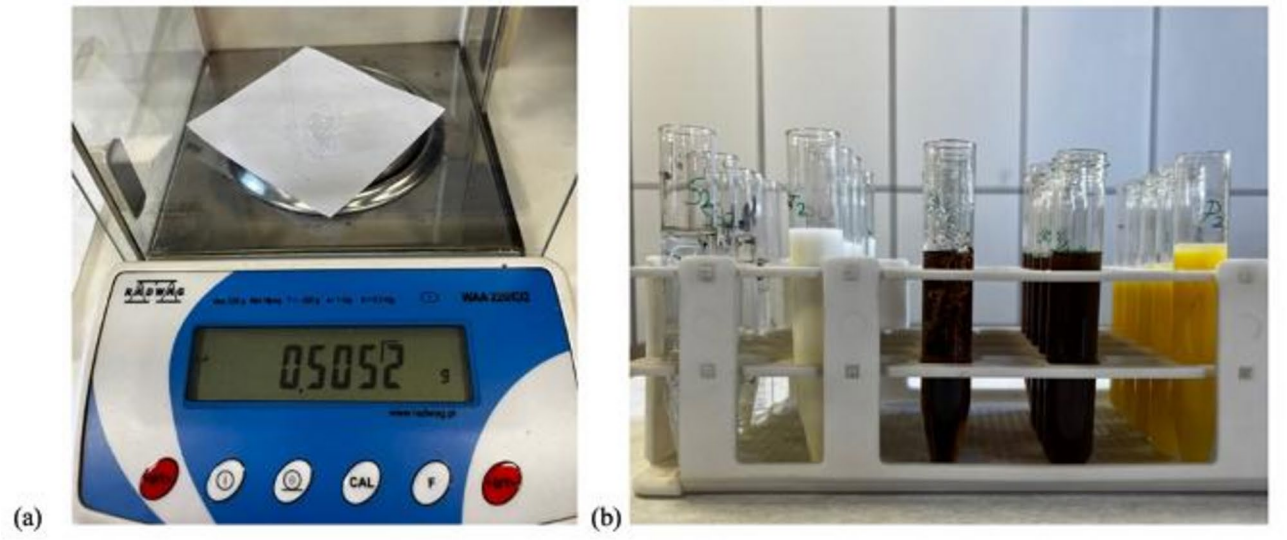


Fig. 1 (**a**) Weight measurement of each sample with scale, (**b**) Glass tubes with samples inside

Table 1 Thermoplastic appliance brands used in the study and their contents

Thermoplastic appliance brand name	Material content	Commercial manufacturer
Invisalign	SmartTrackTM Material; Multilayer polyurethane/co-polyester	Align Technology Inc, San Jose, USA
ClearCorrect	ClearQuartz Material; Zendura Polyurethane Tri-layer proprietary material (Two outer layers of polyethylene terephthalate-glycol, and a polyurethane inner layer)	Institut Straumann AG, Basel, Switzerland
Essix Ace	Single layer polyethylene terephthalate-glycol	Dentsply GAC, York, Pa

appliance-beverage combinations (three appliances × five beverages), resulting in a total of 75 observations. Accordingly, for each beverage type, five glass tubes were used, with each containing 10 mL of the respective liquid. One piece of thermoplastic appliance was placed in one glass tube, and left at the room temperature for 1 h (Fig. 1.b). After that, appliances were removed from samples. This process was made 3 times for 3 different appliance brands. Due to Liquid Chromatography/Mass-Mass Spectrometry's (LC/MS-MS) delicate device design and properties, there was a possibility that its columns could be blocked by particles in the liquids. Because of this reason, beverages which could include particles (milk, orange juice, filtered and coke) were diluted and passed through a polyvinylidene fluoride (PVDF) 0.45 µm syringe filter to eliminate solid particles. Between each process, glass tubes were shaken gently by hand to prevent sedimentation and ensure homogeneity in samples. In order to determine if there was already BPA in the beverages and exclude its possible interference effect on the results, beverage samples (5 glass tubes for each) without any appliance sample were also prepared and analyzed with the same concept. 100 samples of liquids were analyzed as total. The flowchart of the study was given in Fig. 2.

Schimadzu 8040 (Shimadzu Corporation, Kyoto, Japan) branded Flow Injection Liquid Chromatography/Mass-Mass Spectrometry (FIA-LC/MS-MS) analysis was performed for BPA detection samples. During FIA-LC/MS-MS analysis, device parameters were set as follows: Mobil phase (A) 5mM Ammonium acetate / Mobil phase (B) Acetonitrile (10:90), total flow 0.4 mL/min, Oven Temperature 30 °C, Nebulizing Gas Flow 3 L/min, Drying Gas Flow 15 L/min, Interface ESI Interface Voltage 3.5 kV, Interface Current 1 µA, DL Temperature 250 °C, Heat Block Temperature 400 °C, Conversion Dyno 6 kV, Detector Voltage 1.8 kV, IG Vacuum 1. 6e-3 Pa, PG Vacuum 8.6e + 1 Pa, CID Gas 230 kpa, Precursor m/z 227, Product m/z 212.1; 113.1.

Results

In FIA-LC/MS-MS analysis, the sample called blank was first analyzed and no peak belonging to BPA was seen in the chromatogram (Fig. 3.a). The retention time and peak of BPA in LC/MS-MS was given in Fig. 3.b. It was determined that the BPA peak was quite distinct and clearly

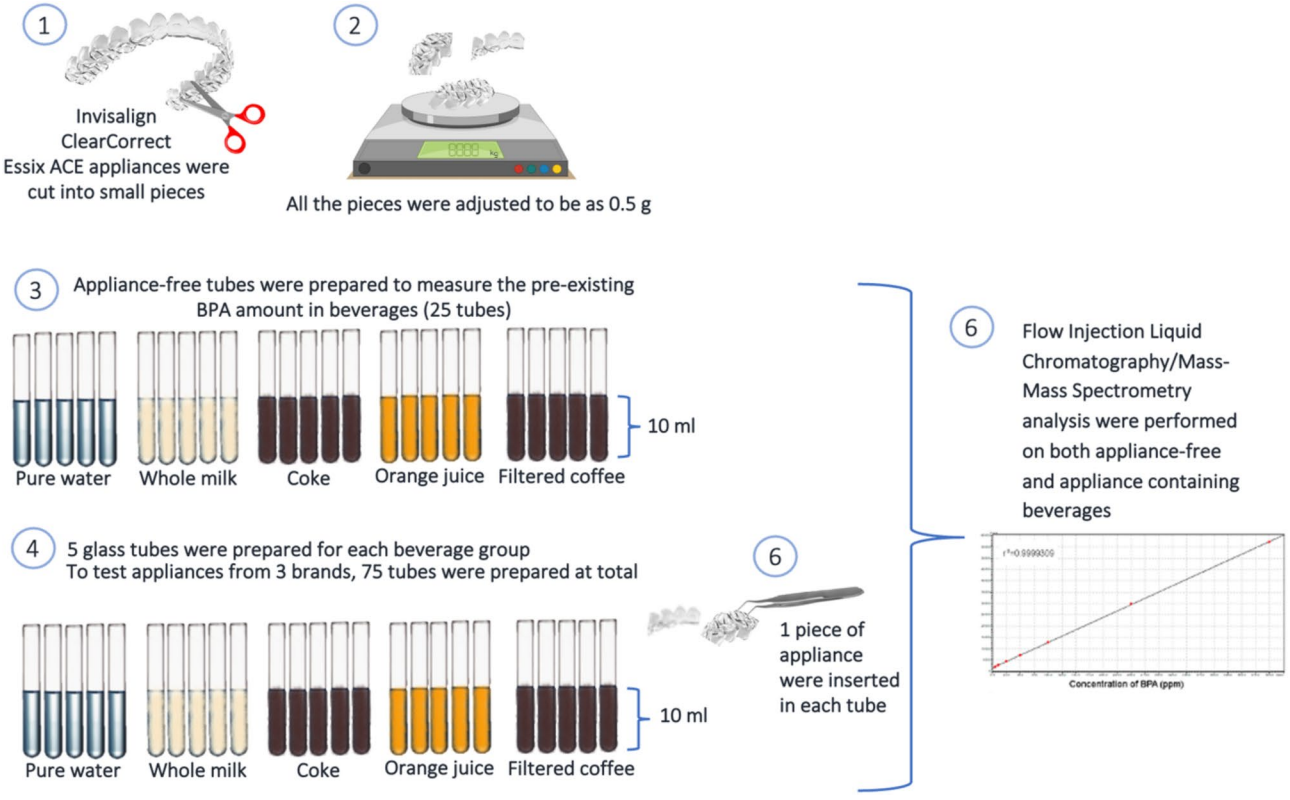


Fig. 2 Flowchart of the study

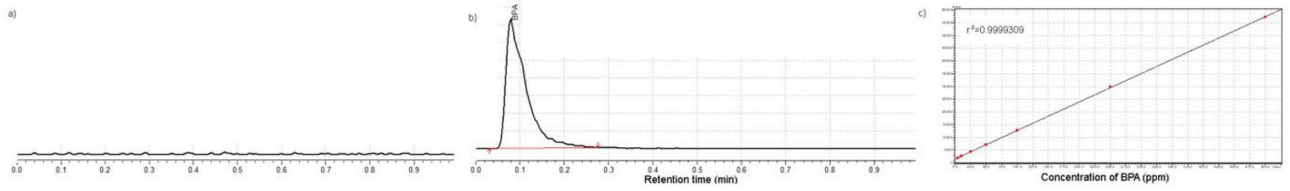


Fig. 3 (**a**) Chromatogram of blank sample, (**b**) Chromatogram of 5 ppb BPA, (**c**) Calibration curve of BPA

visible during the retention time. Limit of Detection (LOD) and Limit of Quantification (LOQ) values were determined as 0.06 ppb and 0.1 ppb, respectively. A calibration chart was drawn for BPA analysis in five different types of beverages (Fig. 3.c). The fact that the R^2 value of the calibration graph (0.9999309) was close to one proved the accuracy of the calibration graph drawn.

Chromatograms of BPA peak (Fig. 4b) were obtained from thermoplastic orthodontic appliance samples in pure water and other 4 beverages were also shown in Fig. 4.a, 4.b. and 4.c. When the entire chromatograms were examined, no peaks belonging to BPA were seen as a result. The very low intensity peaks seen were thought to be noise which can be explained as a fluctuation or deviation caused by the sensitivity of the device and measurement errors since they were below the detection limit and not assumed as a true analytical signal. Beverages without appliance also did not show any BPA release.

Discussion

Patients' aesthetic expectations from orthodontic treatments have increased due to developments in materials. However, it is one of the duties of clinicians to not only treat them well but also to provide safe solutions during treatment processes. Clear aligners are subjected to mechanical stresses, temperature changes, moisture, chemical stresses of saliva and beverages, and the thermal stress associated with the aligner creation process [23]. The aim of this study was to present data on BPA release from thermoplastic orthodontic materials, and the results revealed no significant leaching from samples soaked in different beverages. Therefore, the null hypothesis was accepted.

Even though polymer-based products were emphasized to be safe and being used for many years, harmful effects of these synthetic chemicals on human body are being questioned recently, and the increasing demand

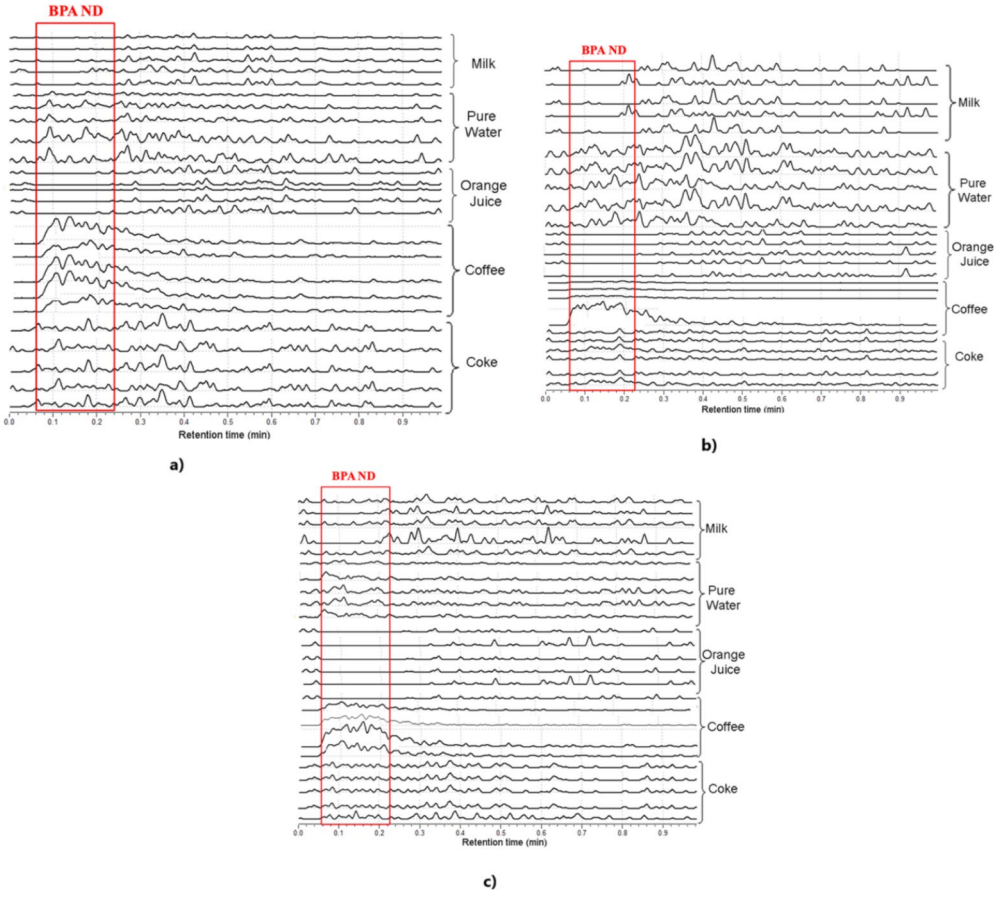


Fig. 4 Chromatograms of BPA peak from (**a**) Invisalign, (**b**) ClearCorrect and (**c**) Essix ACE thermoplastic appliances. BPA ND: Bisphenol A not detected

for aligners, which have similar chemical structures, led concurrent researches in this field. Materials used in the production of thermoplastic orthodontic appliances are primarily polymers. Manufacturers have been working on to promote new advancements to explore the best material combinations for better outcomes. Invisalign revised the initial material and used a multi-layer aromatic thermoplastic polyurethane/co-polyester material, named as SmartTrack™ (LD30) in 2013 to achieve more gentle and constant force through long-term action, greater elastic recovery and chemical stability. The ClearCorrect aligners used ClearQuartz material, a Zendura polyurethane tri-layer proprietary material, which incorporates an elastomeric layer between two resilient low-porosity shells. Two outer layers comprised polyethylene terephthalate-glycol (PETG), and a polyurethane-based layer characterized the inner layer [24]. Different from aligner materials, Essix ACE had a single-layer structure composed of PETG [5]. In addition to known chemical contents, companies' patented specialized formulas may also cause differences between materials. When chemical resistance properties are considered, thermoplastic orthodontic appliances are sometimes exposed to beverages other than water, which may adversely affect the chemical composition of thermoplastic polymers over time. For this reason, it is desirable that the polymers used in aligners be resistant to hydrolysis and water degradation [25]. Even though chemical components of thermoplastic appliances were different, no difference was found in terms of BPA release as a result of this experiment.

As stated above, the thermoplastic materials used to manufacture orthodontic aligners are primarily polymers with variable characteristics. In this study, two frequently preferred aligner brands and a popular thermoplastic retainer made of polyethylene glycol terephthalate glycol were investigated in terms of BPA release. Katras et al. [26] compared Essix ACE, Smile Direct Club and Invisalign regarding BPA release in-vitro, and found out that all samples revealed BPA release at different times. In a similar study, BPA was detected in Biocryl material (Scheu-Dental GmbH, Iserlohn, Germany), but no measurable BPA was found in Essix A+ (Dentsply International, Sarasota, Fla) showing that BPA release may differ between brands [10]. Literature search also showed that samples were mostly kept in artificial saliva [10], artificial gastric juice [26], saline solution [13] or ethanol [20] in previous studies, and no research was conducted with

different beverages in terms of evaluating BPA release. Worldwide known and mostly consumed beverages were used in our study to align the findings with real life more easily and to ensure international validity of experiment by avoiding cultural differences regarding beverage consumption [27]. It was also thought that commercial products usually included chemical additives different from previously preferred liquids such as pure ethanol or saline solution, which could give different results. Samples were kept in beverages for 1 h, to simulate average time spent on a meal or considering 10 min of daily beverage consumption per day in a short break with 1 week aligner use, total contact time of 70 min. Beverages in glass bottles were obtained from a local market to minimize external BPA contamination. In addition, beverages without any samples were also analyzed to detect the preexisting BPA amount which was thought to be a significant factor that could affect the results.

Khan et al. [28] suggested that substance release may occur from dental materials due to polymer structures, surface characteristics, solvent types, or fillers since they are exposed to various chemicals by consumption of different food and beverages in the oral environment. Polyurethane, forming the polymeric component of aligners is not an inert material and may be affected by factors such as heat, moisture and oral enzymes [29, 30]. Retrieved aligners showed adsorption of integuments, localized calcification areas of biofilm and changes in material stiffness [19]. Lira et al. [31] reported that their mechanical properties were significantly reduced, surface roughness tended to increase similar to nanocomposites and microcracks were appeared after oral exposure. Due to such surface changes, possible BPA release into the oral environment might be questioned. The current results revealed no detectable amounts of BPA release in neither of the beverages with and without samples, similar to previous studies [20, 32, 33]. Again, a recent study found no BPA release from unused and used ClearCorrect aligners assessed by high performance liquid chromatography [34]. Our findings were in contrast with the findings of Katras et al. [26] and Raghavan et al. [11]. Katras et al. [26] examined BPA release from Essix ACE and Invisalign samples in different time intervals ranging from 24 h to 20 days, by submerging the samples in artificial saliva, artificial gastric fluid and ethanol, and noted significant increase of BPA release in the first 24 h. In this sense, their method and the assessment interval were different from our study, which might have caused the difference in results. Raghavan et al. [11] examined the salivary BPA levels in patients using vacuum-formed retainers before placement of the appliances, within 1 h, 1 week and 1 month after placement. They concluded that thermoplastic retainers could release BPA. Differences between studies may be due to their incubation of samples in ethanol solution, which is known as a solvent for BPA and could be the reason for BPA release [35]. Again, thermoformed Biocryl retainers immersed in artificial saliva declared BPA leaching, where simulated intraoral conditions were performed by subjecting the samples to abrasion, thermal shock involving temperature cycling from 60 °C to 4 °C water baths [10].

Beverages in the current study also had different physical and chemical properties such as temperature and pH values, which might have caused a possible BPA release due to aligners' thermoplastic nature. It is known that high temperatures could increase BPA release from plastic products [36]. Based on this idea, an increased BPA release was expected in the filtered coffee group, however the current result that did not reveal any change in BPA content was compatible with the reported findings [19, 31, 33]. Hot beverages can be balanced by the intraoral temperature when they enter the oral cavity, which can make them safer in terms of BPA release from a clinical point of view.

The pH value of the materials is also an important factor, and it has been stated that low acidity can cause physical deterioration and chemical degradation of the resin composite materials. In a study examining the residual monomer leakage of three different restorative composite resins according to acidity and immersion time, it was reported that leakage increased significantly as the immersion time increased at pH 4 [37]. In another study, effect of different pH levels on the release of BPA was assessed from dental pit and fissure sealants under three different pH conditions. The authors immersed the samples in solutions of pH 3.0, 6.5, and 10.0 at 37 °C for 10 min, 1 h, and 24 h periods, and concluded that more BPA was detected with longer duration of acidic drinks. In conclusion, it was emphasized that long-term acidic drinks could increase the risk of BPA release [38]. In addition, there was also a possibility to find increased levels of BPA in beverages with acidic pH values, since low pH might cause minor degradations on the plastic surfaces. The effects of artificial saliva, orange juice, coke and milk on the surface topography of dental nanocomposites were examined and significant level of deterioration and difference in surface topography in the coke and orange juice groups (orange juice being more prevalent) were noted, when compared to artificial saliva [28]. However, in this study no apparent differences were detected in terms of BPA release. In this regard, it is considered that, the limited observation period in our study may have affected the fact that different pH values in beverages did not cause a difference in BPA release. In addition, BPA release was not present in no-aligner sample group, which might depend on preferring beverages in glass tubes. This result also showed the reliability of our study.

The lowest dose at which BPA can cause toxic effects on the human body has been reported as 50 µg/kg bw/day by United States Food and Drug Administration [39], and 0.2 ng/kg bw/day was announced by European Food Safety Authority lately [40]. In this study, ready-to-use dental arch-shaped appliances were cut into small pieces in order to be able to fit the samples into the test tubes, similar to the literature [10, 13]. Therefore, when considered in terms of quantity, the samples in test tubes were smaller than the appliances used in real life. Since thermoplastic appliances are generally used in both arches for long periods, caution should still be exercised when interpreting the results due to the increase in mass and indented morphological surfaces during clinical use of real appliances. The fact that no BPA release was found within the limitations of this study still reveals the necessity of studies on the long-term accumulation effect of orthodontic treatment.

For chemical analysis of BPA, gas chromatography/mass spectroscopy (GC/MS) [10, 20], high performance liquid chromatography (HPLC) [11, 41] and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) [13] methods were used in previous studies. In this study, FIA-LC/MS-MS method was performed for BPA determination since it is known to be more sensitive and accurate in detecting low concentrations of substances and had short running time when compared to HPLC [42]. It also has advantages such as absence of derivatization step and may give higher throughput than GC/MS method [43, 44].

The in-vitro design of the study, beverages being at different temperatures, the evaluation being made only after 1 h, and the examination of only 3 brands of thermoplastic appliances with a limited number of samples can be highlighted as the limitations of the study. A previous study showed that aligners might also release bisphenol F and bisphenol S [13], yet only BPA could be investigated in this study. Still, further studies could be designed to further assess the release of BPA derivatives. Experimental conditions may not fully mimic the real-life situations and therefore, patients should still be warned about the potential effects of contact of these materials with beverages and cariogenic effects should also be considered. In the future, in-vivo studies evaluating BPA release from diverse brands including more beverages through longer examination periods will be beneficial to improve the findings.

Conclusion

BPA is a harmful chemical compound for human body and it is crucial to detect its presence which may have a direct impact on systemic health. In this study, BPA release were not detected from thermoplastic orthodontic appliances after 1 h incubation in pure water, milk, orange juice, coke and filtered coffee. Within the limitations, beverages did not cause BPA release from the thermoplastic orthodontic appliances in the short term.

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Author contributions
BAA: Conceptualization; Data curation; Investigation; Roles/Writing - original draft.OCB: Investigation; Methodology.AO: Investigation; Methodology; Project administration; Roles/Writing - original draft.EY: Investigation; Methodology; Project administration; Roles/Writing - original draft; Supervision.BBT: Conceptualization, Supervision, Writing - review & editing.CT: Supervision, Writing - review & editing.

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Data availability
The datasets used and/or analyzed during the current study are available from thecorresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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