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EVALUATION OF BISPHENOL-A RELEASE FROM DIFFERENT FISSURE SEALANT MATERIALS *IN VITRO*

DOCTOR OF PHILOSOPHY THESIS

GÜLFEM ÇAVUŞOĞLU ÇARPAR, DDS

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated .... and numbered ....

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DECLARATION

I declare that this thesis is my own work, that I have not conducted any unethical behavior at any stages from the planning to the writing of the thesis, that I have obtained all the information in this thesis within academic and ethical rules, that I have cited all the information and comments that were not obtained through this thesis study, and that I have included these sources in the list of sources.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
°C	Degrees Celsius
ACP	Amorphous Calcium Phosphate
ADA	American Dental Association
ADHD	Attention Deficit and Hyperactivity
ARBS	Autopolymerized Resin-Based Sealant
ART	Atraumatic Treatment Restorations
BADGE	BPA Diglycidylether
BBB	Blood-Brain Barrier
Bis-DMA	Bisphenol Dimethacrylate
Bis-EMA	Bisphenol Ethoxylated Dimethacrylate
Bis-GMA	Bisphenol Glycidyl Methacrylate
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
CaF_2	Calcium Fluoride
CE	Capillary Electrophoresis
CHX	Chlorhexidine Digluconate
cm	Centimeter
EDC	Endocrine Disrupting Chemicals
EFSA	European Food Safety Authority
EMV	Expected Monetary Value
EPA	US Environmental Protection Agency
EtOH	Ethanol
EU	European Union
FRBS	Fluoride-Releasing Resin-Based Fissure Sealants
FSH	Follicle-Stimulating Hormone
GC	Gas Chromatography
GI	Conventional Glass Ionomer
GIC	Glass Ionomer Cement
GLUT	Glucose Transporter
H_3PO_4	Phosphoric Acid

HEMA	2-Hydroxyl Ethylmethacrylate
HPLC	High Pressure Liquid Chromatograph
HVGIC	High Viscosity Glass Ionomer Cement
KCl	Potassium Chloride
kg	Kilogram
KOH	Potassium Hydroxide
LBRS	Light-Cured Resin-Based Sealants
LC/MS	Liquid Chromatography/Mass Spectrometry
LED	Light Emitting Diode
LH	Luteinizing Hormone
M	Mass
MEKC	Micellar Electrokinetic Chromatography
$MgCl_2 6\ H_2O$	Magnesium Chloride Hexahydrate
MIH	Molar Incisor Hypomineralization
mL	Milliliter
mm	Millimeter
mmHg	Millimeter of Mercury
mW	Megawatt
NaCl	Potassium Chloride
NaF	Sodium Fluoride
ng/mL	Nanogram/millimeter
nm	Nanometer
NTP	National Toxicology Program
NTP-CERHR	National Toxicology Program Center for Evaluation Risk of Human Reproduction
pH	Power pf Hydrogen
PI3K	Phosphatidylinositol 3-kinase
ppb	Parts Per Billion
ppm	Parts Per Million
PRG	Pre-Reactive Glass
PRR	Preventive Resin Restoration
psi	Pounds Per Square Inch
PTFE	Polytetrafluoroethylene

RBS	Resin-Based Fissure Sealant
RMCIS	Resin-Modified Glass Ionomer Cement
rpm	Revolutions Per Minute
SHBG	Sex Hormone Binding Globulin
TCDD	Tetrachlorodibenzodioxin
TDI	Tolerable Daily Intake
TEGDMA	Triethylene Glycol Dimethacrylate
TUTAGEM	Trakya University Research and Developing Center
UDMA	Urethane Dimethacrylate
UPW	Ultrapure Water
UV	Ultraviolet
V	Volt
v/v	Volume/Volume Percentage
µg	Microgram
µL	Microliter
µM	Micromolar

ABSTRACT

Çavuşoğlu Çarpar G. 2024. Evaluation of Bisphenol-A Release from Different Fissure Sealant Materials *In vitro*. Yeditepe University Institute of Health Sciences, Department of Pediatric Dentistry. PhD Thesis, Istanbul.

This study aimed to assess the release of bisphenol-A (BPA) from fissure sealant materials within 24 hours, considering different polymerization times and immersion solutions. In this study, 10x2 mm disks were obtained from Fuji Triage, Fissurit FX, and Clinpro™. Each of the three groups comprised 16 disks, totaling 48 disks examined. The fissure sealant materials used in the experimental groups were as follows: Group 1: Clinpro™, Group 2: Fuji Triage, and Group 3: Fissurit FX. Two polymerization times, 20 and 40 seconds, were employed. The groups were divided into four subgroups: immersion in artificial saliva for 20 seconds, artificial saliva for 40 seconds; ethanol for 20 seconds, and ethanol for 40 seconds (n=3, beta=1, power 80). The disks were then stored in artificial saliva and ethanol solutions for 1 day. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows version 24.0. There was a highly statistically significant difference among fissure sealants regarding BPA release (p=0.000). BPA release was detected in all fissure sealant materials. The lowest BPA release was observed with Fuji Triage at 40 seconds of polymerization in artificial saliva, while the highest release occurred with Fissurit FX at 20 seconds of polymerization in ethanol. Fissure sealants with a high resin-containing filler ratio exhibited low BPA release. Although BPA release occurs following fissure sealant application, the measured amount of BPA release, even after one day, suggests that pit and fissure sealants can be used safely. However, additional measures should be taken to eliminate the oxygen inhibition layer post-polymerization of fissure sealants or to prevent saliva interaction during application.

Keywords: Bisphenol-A, liquid chromatography-mass spectrometry, fissure sealant

ÖZET

Çavuşoğlu Çarpar G. 2024. Farklı Fissür Örtücü Materyallerinde Bisfenol-A Salınımının *In Vitro* Olarak İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Çocuk Diş Hekimliği Anabilim Dalı. Doktora Tezi, İstanbul.

Bu çalışma pit ve fissür örtücü materyallerinin farklı polimerizasyon sürelerinde bir günlük süre içerisinde salınan Bisphenol-A miktarını belirlemek amacı ile gerçekleştirilmiştir. Çalışmada Fuji Triage, Fissurit FX ve Clinpro™ materyallerinden 10x2 mm boyutlarında diskler elde edilmiştir. Her grupta 16 adet disk kullanılmış toplamda 48 adet disk incelenmiştir. Deneyde kullanılan fissür örtücü materyalleri; 1. Grup: Clinpro™, 2. Grup: Fuji Triage, 3. Grup: Fissürit FX'dir. Bu gruplarda 20 saniye polimerizasyon ve 40 saniye polimerizasyon süreleri kullanılmıştır. Bu gruplar 4 alt gruba ayrılmıştır. Polimerizasyon süresi 20 saniye ve 40 saniye olacak şekilde iki farklı polimerizasyon süresi kullanılmıştır. Polimerize edilen diskler yapay tükürük ve etanol solüsyonlari içerisine atılmıştır. (n=3, beta=1, güç 80). Bu diskler yapay tükürük ve etanol solüsyonlarında 1 gün bekletilmiştir. İstatistiksel analiz için Windows 24.0 programında SPSS (Statistical Package for Social Sciences) kullanılmıştır. BPA salınımı açısından fissür örtücüler arasında istatistiksel olarak yüksek derecede anlamlı bir farklılık görülmüştür (p=0,000). Bütün fissür örtücü materyallerinde BPA salınımı gözlenmiştir. En az BPA salınımı yapay tükürük içerisinde, 40 saniye polimerizasyon süresi olan Fuji Triage'da ölçülmüş en fazla BPA salınımı etanol içerisinde 20 saniye polimerizasyon süresi olan Fissurit FX tarafından ölçülmüştür. Rezin içerikli doldurucu oranı yüksek olan fissür örtücülerden BPA salınımının düşük olduğu görülmüştür. Fissür örtücü uyguladıktan sonra BPA salınımının gerçekleşmesine rağmen, bir gün sonra bile salınım miktarında ölçülen değerler içerisnde pit ve fissür örtücülerin güvenle kullanılabileceğini göstermektedir. Bununla birlikte, fissür örtücülerin polimerizasyonu sonrası oksijen inhibisyon tabakasını kaldırmaya veya uygulama sırasında daha az tükürükle etkileşimini önlemeye yönelik ilave önlemler alınmalıdır.

Anahtar Kelimeler: Bisphenol-A, sıvı kromatografi-kütle spektrometri, fissür örtücü

1. INTRODUCTION AND PURPOSE

Dental caries, the most prevalent chronic pediatric disease, are a major concern in society today. In particular, dental caries that begin at a young age and worsen over time, lead to tooth loss as children develop (1). Dental caries is known to develop via a complex process, and studies aimed at avoiding caries recently becoming more prevalent (2). The occlusal surface of the teeth increases with erupting of permanent teeth and interproximal caries decreases (3). The risk of caries on the occlusal surfaces of the teeth is increased by the complicated morphologies of the pit and fissure structures (4).

Studies have established the efficacy of pit and fissure sealants used in the treatment of occlusal caries (4). Pit and fissure sealants are now categorized as resin-containing, glass-ionomer containing, or hybrid compounds with the properties of both resin and glass ionomer dependent on the concentration at use. Due to their simplicity of use, high retention strength, and potent cariostatic effects, resin-based fissure sealants are most popular (1). Similar to composite resins in their composition, resin-based fissure sealants typically comprise dimethacrylates, such as bisphenol-A glycidyl methacrylate (Bis-GMA) or its derivatives (5).

In resin materials, polymerization occurs when monomers are transformed into polymers. Failed restorations are caused by inadequate polymerization of all monomers, which has a negative effect on the material's mechanical qualities. This is because, after being released into the oral environment, unpolymerized monomers produce monomers, such as Bis-GMA, triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), and 2-Hydroxyl Ethylmethacrylate (HEMA). In particular, the resin components found in fissure sealants release Bis-GMA. The release of Bis-GMA and other unpolymerized monomers into the oral environment has been linked to the possibility of allergenic, cytotoxic, genotoxic, and carcinogenic consequences (6).

The examination of unpolymerized monomers released from resin materials is performed using a variety of techniques. The most common technique is high-pressure liquid chromatography, which is repeatable, dependable, quick, affordable, and selective but lately, the use of LC/MS devices has become more popular with new developing technologies (7).

Studies on the release of Bis-GMA from resin-containing materials have been *in vitro*. Because of this, the availability of further studies, particularly those including the most popular fissure sealants on the market now, would be helpful for the clinician when making material choices to treat dental caries.

The purpose of this study was to evaluate bisphenol-A release during specific time periods after the administration of various pit and fissure sealants, which are used primarily as preventive measures for pediatric patients.

The null hypothesis of this study was that there is no significant difference between released bisphenol-A (BPA) values in the fissure sealants. The alternative hypothesis was that there was a significant difference between released BPA values among the fissure sealants.

2. GENERAL INFORMATION

2.1. Dental Anatomy

As a separate component that is crucial for chewing systems that are a part of the occlusion when the jaws and teeth join together, the human body is studied and organized independently in dental anatomy. Although anatomy appears to be a descriptive and static science in general, dental anatomy should be studied separate from other areas of anatomy and analyzed separately because of the dynamic activities of the teeth that need to be explained and explored. Most dental practices primarily focus on periodontal and dental tissues (8).

In oral cavity where the digestive system first develops, are the teeth. The oral mucosa's epithelium is where teeth first develop. The mandible and maxilla contain a variety of human teeth. The oral cavity has numerous roles that include the growth of the skull and face, chewing, swallowing, phonation, aesthetics, and soft tissue defense (9).

There are 20 teeth in primary dentition in humans, ten are located in the mandibular arch, and ten in the maxillary arch. During the permanent teeth period, 16 are located in the maxillary arch, and 16 are in the mandibular arch. The dental crowns have very different properties in terms of size, shape, depth, and appearance. Each crown has a morphologically different structure. Morphological features found in dental crowns include cusps, cusp ridges, ridges, marginal ridges, triangular ridges, tubercules, equatorial line, cingulum, mamelons, fossa, development lobes, pits, and grooves (10).

2.2. Characteristics of Pits and Fissures

Grooves formed between the tubercules of the tooth are defined as fissures, and small pits located in the junction or end areas of developmental grooves are labelled as pits. The susceptibility of pit and fissure surfaces to caries formation is related to the steepness of the tubercule slopes, the depth, and shape of fissures. The depth and narrow structure of pits and fissures, inadequate washing of saliva from these areas, and insufficient mechanical cleaning lead to the formation of an ideal environment for bacteria and plaque accumulation (11). The other reason for caries formation on pits and fissures is because of the thinner enamel thickness in fissures than on a flat surface. The enamel thickness at the base of shallow fissures should be 1.5–2 mm, while the thickness of deep fissures can be 0.2 mm or less. The fine structure of enamel in deep fissures causes caries to progress rapidly to dentin (12).

Early signs of caries in the occlusal pits and fissures indicate that decalcification of caries does not begin simultaneously along the entire length of the fissure. Shallow fissures formed by walls merging at a wide angle of approximately 70–90° show low susceptibility to decay, whereas decalcification of enamel is common in fissures formed at an angle of less than 70° (13).

Nagano classified pits and fissures into five types based on their anatomical structures. Structure of pits and fissures are shown in Figure 1 (14):

- U type, almost the same width from top to bottom
- V type-wide at top, gradually narrowing towards the bottom
- I type, extremely narrow slit
- IK type, extremely narrow slit with a larger space at the bottom
- Inverted Y type

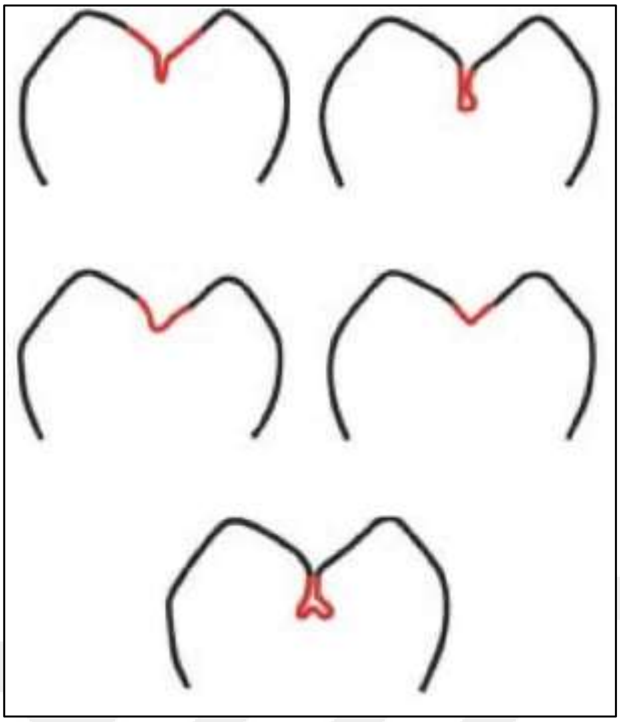

Figure 1: Classification of pits and fissures (14)

2.3. Pit and Fissure Caries

Pits and fissures are most susceptible to caries formation on the occlusal surface. These lesions are also known as pit and fissure caries because the incidence of caries on occlusal surface correlates with the depth and morphology of pits and fissures (15). Moreover, two thirds of caries observed in school-aged children occur on the occlusal surface, despite the fact that the occlusal surface only represents 12.5% of the total surface of permanent teeth (16).

The occlusal surfaces of the teeth are supported by a complex structure, which makes the teeth more resistant to fluoride treatments. Approximately 90% of dental caries in populations using fluoridated water occurs on the occlusal and buccolingual surfaces, of which the majority of these cases are pit and fissure caries (17).

In different countries where fluoride was used, it has been observed that interproximal caries decreased by 60% and buccal, lingual, and occlusal caries decreased by 10% (18) because fluoride does not penetrate the enamel surface of teeth in pits and fissures as much as it does on a flat surface (19).

Fluoride remineralization mechanisms and caries preventive measures function best at high plaque pH values such as 6.7–7.3. Fluoride has the ability to remineralization, but the effect is diminished as a result of nutrients and bacteria building up in pits and fissures (20).

The Nasmyth membrane also forms a barrier that limits remineralization from the topical action of fluoride. It increases the tooth’s susceptibility to caries because it is not autolyzed at the fissure base of erupted teeth (20).

During the earliest stages, pit, and fissure caries can be observed in 20% of children between the ages of 2 and 4 years who have dental caries. Occlusal surface caries account for more than 6.7% of all carious lesions found in these children (21).

Fissures are susceptible to plaque buildup during eruption and are susceptible for caries development at this time (15). Miller found that the incidence of occlusal caries in teeth is highest during eruption, immediately following eruption, and between 1–3 years after the eruption. Furthermore, the incidence of pit and fissure caries on the occlusal surface was reported at 15% and 10% at 8–9 years of age (22).

Pit and fissure caries increased from 4.3% to 6.8% yearly on the occlusal surface of intact permanent first molars in children aged 10–15 years. Also, the interproximal caries rate fell from 2.4% to 0.3%. It was previously thought that caries in pits and fissures started at the base of the fissure before spreading up the wall and tubercule slopes. Today, the earliest histological indicators of pit and fissure caries development are seen at the fissures and typically take the appearance of two distinct independent lesions on opposing tubercule slopes in the enamel. On the occlusal surface of teeth, multiple independent lesions that were previously separate migrate through the fissure walls to form a single caries lesion (23). The caries lesion affects the enamel more at the fissure than at the enamel-dentin border (24).

According to study of different pit and fissure types, V and U type fissures have self-cleaning qualities and a lower chance of developing caries than I, IK, and Y types (25). Grewal and Chopra found that the structure of the fissure is crucial because the fissure sealant materials have the best penetration in V and U types of fissures. The fissure sealant materials had the least penetration in IK and I-type fissures. Y type fissures had stronger penetration than IK and I-type of fissures (26).

2.4. Prevention of Pit and Fissure Caries

Nowadays, pit and fissure sealants are applied for reasons, such as preventing caries that might occur in the pits and developmental grooves of the teeth, and making cleaning easier. Prevent the formation of cavities in the grooves and crevices of teeth, applying fissure sealants alone is insufficient. Proper oral care is essential, involving multiple factors that must be considered. (16).

2.4.1. Oral and dental care

Microbial dental plaque is one of the most significant etiological factors that harm oral and dental health (27). The hazardous substances in dental plaque have a detrimental effect on oral and dental health because it is difficult to remove dental plaque that has built up on tooth surfaces (28).

Preventive dental procedures for children are methods used to protect their teeth from cavities before they form. Children will benefit from better nutrition, clearer speech, and improved overall health. Brushing, flossing, maintaining a healthy diet, applying fluoride, and having regular dental checkups, can give the child a lifetime of good oral and dental health (29).

2.4.2. Using antimicrobial chemical agents

Plaque is associated with dental caries. Chemicals that are effective against the microorganisms in plaque can be used for caries prophylaxis. Antimicrobial agents are used to support mechanical cleaning when brushing teeth in children with high caries risk. Mouthwashes, sprays, toothpastes, gels, gums, chewy lozenges, and long-release fluorides are some examples of oral antiplaque agents (30).

Chlorhexidine digluconate (CHX) is one of the most popular antibacterial agents used in chemical plaque management. It is widely known that when in contact with bacterial cells for an extended period of time, CHX weakens their cell wall and harms their structure. Additionally, CHX is known for its ability to limit the spread of *Streptococcus mutans,* which is a type of bacteria that thrives on dental sucrose or glucose, leading to a decrease in plaque pH. There are two forms of CHX available - gel and mouthwash. Plaque formation is mostly prevented when using a CHX mouthwash twice daily (31). Furthermore, CHX varnish application has been shown to be beneficial for preventing fissures in children whose teeth have not fully erupted by age 6 years (19).

2.4.3. Fluoride applications

Fluoride is an inert element in biology that is present in biological structures in incredibly small amounts, and this ratio is called part per million (ppm). Fluoride is one of the most electronegative and reactive elements found in nature, especially when it is part of a compound (32).

One of the most effective uses of fluorides in general public health is for the prevention of dental cavities. Fluoride decreases the risk of tooth decay, delays the spread of carious lesions, and presents or encourages their remineralization (33).

In dentistry, fluorides are used topically or systemically. Fluoride cariostatic action was originally identified with regard to the naturally occurring fluoride in drinking water. During the development of dental enamel, fluoride is absorbed. As a result, public water supplies were supplemented with fluoride to promote this systemic effect. However, one of the side effects of systemic fluoride use is dental fluorosis, which occurs due to systemic absorption during tooth formation. However, the topical fluoride applications were found to be more effective than systemic ones (34). Topical fluoride administration techniques include fluoride varnishes, fluoride toothpaste, mouthwashes, gels, gums, dental floss, restorative products such as glass ionomer cements, and slow-release methods (30).

Essentially, the beneficial effects of fluoride on dental caries result from its topical activity after teeth erupt in the oral cavity (30). With its low concentration and consistent presence in the mountain during the post eruption, fluoride is more effective at preventing caries when it penetrates the enamel structure in the post-eruptive period (35).

The mechanism by which fluoride protects teeth from carious lesions include (35):

- Making the enamel more resistant to acid attacks
- Increasing the maturation level of the enamel
- Contributing to the remineralization of the carious lesion
- Showing an inhibitory effect on microorganisms

Topical fluoride ionizes hydroxyapatite crystals on enamel surface, reacting with calcium to produce calcium fluoride (CaF_2) crystals. Fluoride ions are released from the enamel crystals causing them undergo fluorapatite. The enamel provides adequate resistance to caries when the fluoride content reaches high levels (36).

Fluoride treatment is effective in preventing tooth decay on flat surfaces of teeth but it is not as effective on fissures. This could be because the pH level in fissures is too low, making the plaque fluid unsaturated with fluorapatite. As a result, fluoride therapy may not be as effective in treating fissures (37).

2.4.4. Pit and fissure sealants

Pit and fissure sealants are substances that keep the bacteria and nutrients that lead to the development of caries from sticking to the fissures on occlusal surfaces of the teeth (38).

2.4.5. Features of pit and fissure sealants

Ideal pit and fissure sealants should have the following properties:

- Be suitable for dental and oral tissues
- Be fluid and have low viscosity to easily sink into pits and grooves
- Be simple to use
- Should not change dimensions when hardening
- Should not allow liquids or ions flow through

- Its mechanical and thermal characteristics should match those of tooth tissues
- Must have enough retention and remain in the tooth for a considerable amount of time after application
- Be impervious to all forces involved in mouth function
- Have anti-caries properties (39–47)

2.4.6. Indication of pit and fissure sealants

A patient's medical history, caries activity, family socioeconomic situation, oral hygiene and nutritional habits, and fluoride consumption level should be taken into account when selecting whether to apply a fissure sealant (42). When a patient is at high risk for developing cavities, pit and fissure sealants can be used as a preventive treatment. They can also be used as a secondary preventive treatment to avoid the development of incipient caries. According to ADA 2016 clinical guidelines, considerations for applying pit and fissure sealants include the patient, the tooth, the level of eruption, and the timing of eruption (41).

The clinical guidelines suggest the following indications for applying pit and fissure sealants (41):

- Patients at high risk for caries have primary teeth with fissures and pits
- Children and teenagers who have grooves and pits in their permanent teeth are at high risk for developing caries
- Adult permanent teeth with grooves and pits when the patient is at risk of developing caries
- Children, adolescents, and adults with developing carious lesions
- Children and adolescents with physical, mental, or intellectual problems should have their primary and permanent teeth pits and fissures examined especially if dental illness or the requirement for dental care could endanger their overall health

Pit and fissure sealants should be applied based on patient risk, not age nor the remaining time since the eruption of teeth. There is no need to perform this preventive action at that time if the patient shows no risk factors or has a low probability of developing carious lesions (48).

Some studies suggest that the application of fissure sealants is unnecessary for permanent molars 4 years or more after eruption, though the risk of caries is highest in the first 2 to 4 years after eruption of teeth (49). On the other hand, it is also reported that caries risk exists at all ages and it would be more suitable to assess the variables as opposed to the amount of time that has passed since the fissure sealant's occurrence (50). Children who have caries in their primary teeth are more likely to develop caries in their permanent teeth (51). As soon as the permanent first molars erupt, fissure sealant is advised for all permanent first and second molars if occlusal caries lesions are seen in one of the first molars (52). Children who have a cavity-free primary dentition do not require fissure sealant on their first permanent molars. It has been recommended to periodically examine children who are not at risk for caries and that only deep fissures should have the protective treatment of fissure sealants. However, it is recommended that treatment should be followed up with control appointments (52).

Fissure sealant should be applied to pits and fissures of the enamel before cavitation or caries formation occurs (53). Ensuring the isolation of the tooth and avoiding saliva contamination is an essential factor determining retention in fissure sealant treatments. Due to this, the use of fissure sealant should be delayed until the eruption of all teeth and after full occlusion development (54). Glass ionomer fissure sealants that are less susceptible to moisture, are advised for individuals in the high caries risk group. When the tooth is isolated, a resin-based fissure sealant is also advised (55).

2.4.7. Application of pit and fissure sealants

The process of evaluating and treating teeth starts with a thorough visual examination of the dental area. Due to their complicated morphology, evaluating pits and fissures on occlusal surfaces is challenging. Teeth should be dry and correctly illuminated to identify caries during a clinical examination (56). Plaque and debris should be removed from the tooth surface and fissures before the sealant material is applied on the teeth (38). Numerous techniques have been developed for surface cleaning with this goal in mind. These techniques include hydrogen peroxide, prophylaxis pastes, air polishing, air abrasion, laser treatments, pumice, and brushing (57). The isolation of the tooth is the most important step in the fissure sealant application process (58).

The rubber dam offers the most consistent humidity control during the course of the treatment. However, using a rubber dam clamp on a child’s immature teeth could make them uncomfortable and, in some instances, local anesthetic might be necessary (47).

According the reports, Lygidakis et al. (59) suggests that careful isolation using cotton rolls can produce retention values that are comparable to those of rubber dams. While cotton roll isolation has benefits, such as clamping the teeth and not requiring local anesthesia, its major drawback is that it must be performed by two people together.

The goal of roughening is to achieve a clear, dry, and frosty surface. The most frequently used concentration of orthophosphoric acid is 37%. Small concentration variations have no effect on the quality of the roughened surface. After applying to the tooth for 20–30 seconds, rinse to eliminate acid. Making sure the cleaning procedures last long enough to completely remove etching solution from the tooth surface is particularly important. The teeth must be a rough with a frosty appearance after being air dried (54).

The long-term clinical success of fissure sealants is correlated with their application-related issues. In fissures, tiny air bubbles are a frequently observed. In this situation, it is important to make sure that before polymerization, the fissure sealant material flows toward the pits and fissures, which can be aided by a brush or an applicator with a specialized tip (60). Following the manufacturer’s instructions, polymerization should begin as soon as the light-cured fissure sealant is applied to reduce the possibility of contamination. The polymerization period has been found to be important for micromechanical retention because it allows the fissure sealant to penetrate deeply into the depth of the pits and fissures (61).

2.4.8. Application techniques of pit and fissure sealants

Pits and fissure sealants can be applied by three different techniques on the occlusal surface of tooth (62).

2.4.8.1. Invasive technique

The term invasive technique refers to the use of burs to deepen and expand fissures while removing plaque, organic material, and thin superficial prism-less enamel. With this technique, the surface area is increased while the acid and sealant can reach deeper into the cavities (62).

According to a study, invasively applying acid and fissure sealant to both primary and permanent teeth allow for deeper fissure penetration. Additionally, they showed an increase in the surface area required to retain the fissure sealant materials (63). Burrow et al. (64), Salama and Al-Hammad (66) reported that when organic material, plaque, and the prismatic layer in the enamel are removed using an invasive approach, the penetration of fissure sealant increased. Additionally, this method enables more difficult in fissures where clinical caries detection is challenging.

2.4.8.2. Non-invasive technique

A non-invasive fissure application procedure is applied to teeth with suspected caries without removing material from the tooth surface (67).

Pit and fissure caries can be prevented effectively by applying sealants using non-invasive methods. It has been reported to be efficient and numerous clinical investigations have proven both its efficiency and retention (69). In comparison to invasive procedures, mechanical preparation of fissures has been shown to increase the rate of sealant retention and decrease the risk of microleakage (70). In a study, comparing invasive and non-invasive techniques to test the microleakage and penetration of resin and glass-based fissure sealants, the invasive method showed 80% success in terms of penetration and was found to be marginally superior to non-invasive in terms of microleakage (68).

Additionally, simply increasing non-carious tooth fissures using an invasive technique involves removing the causes of exposure (71). Choosing between invasive and non-invasive techniques remains a controversial issue (65).

2.4.8.3. Preventive resin restoration

The prevention and treatment of non-carious pits and fissures frequently involves the use of fissure sealants. Additionally, sealants can be used to cure fissures and pits that have suspectable carious lesions (72). Simonsen introduces preventive resin restoration (PRR) as a method to both treat early-stage carious lesions with the least amount of tooth structure removal to protect teeth against future caries development (38). PRR is a conservative technique that solves issues including the risk of secondary caries in fissures and margins that have not been prepared, edge leakage, and repeated caries formation (73). PRR can be used to both diagnose and treat shallow pit and fissure caries and determine the condition of suspected caries or fissures. It can also be used in deep fissures and pits that might prevent the penetration of the filling material or result in caries and in opaque, chalky-appearing fissures and pits that are signs of early-stage caries. However, it should not be used on deep and wide surfaces that have caries on more than one surface (74).

2.5. Pit and Fissure Sealant Materials

Fissure sealant materials are divided into three groups according to the contents. These are resin-based fissure sealants (RBS), glass ionomer fissure sealants, and polyacid-modified fissure sealants (75). The classification of fissure sealant materials is shown in Figure 2.

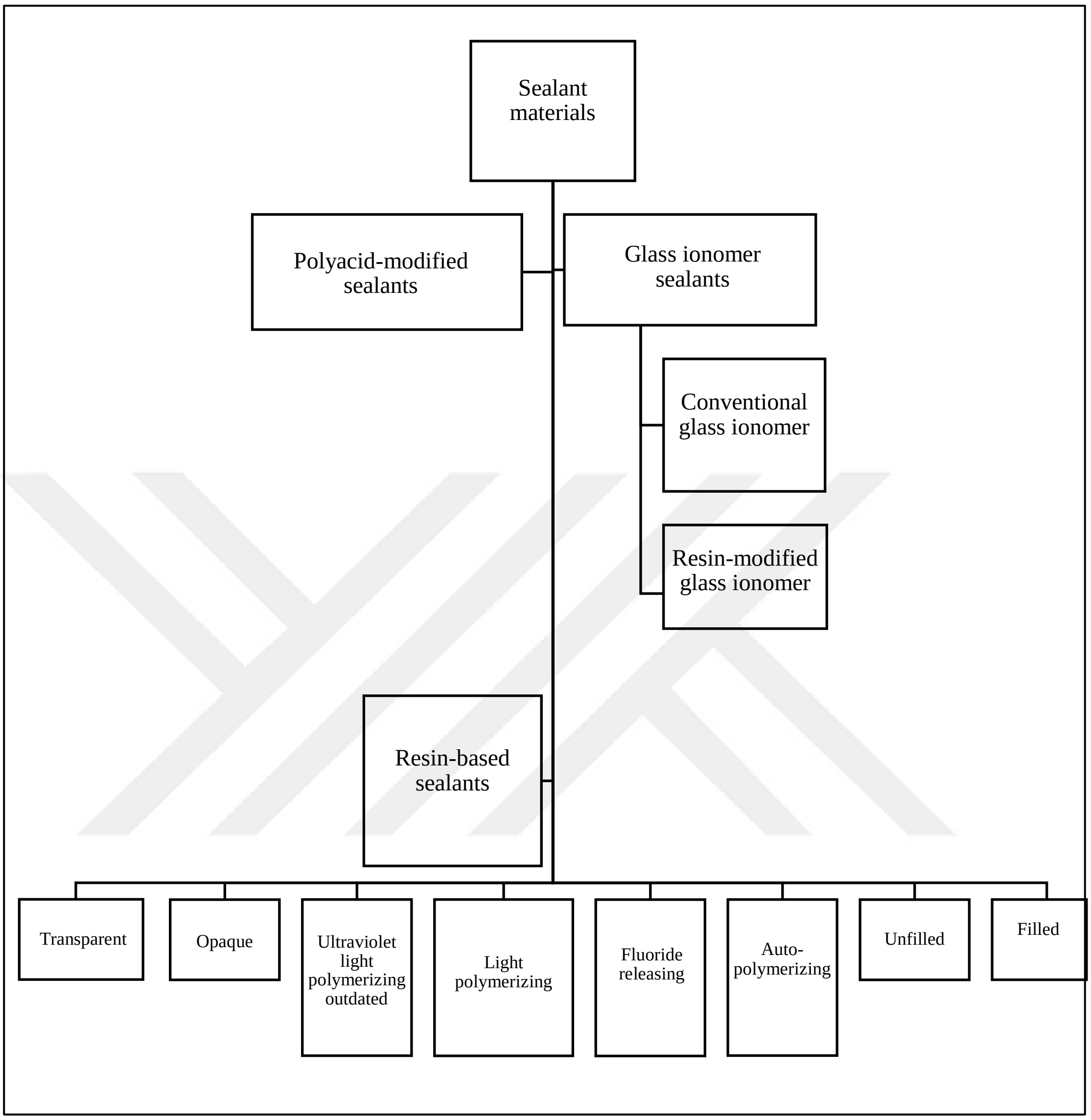

Figure 2: Classification of fissure sealant materials

2.5.1. Resin-based fissure sealants

According to the different forms of polymerization, fissure sealants with resin content are categorized into four categories (75).

Ultraviolet (UV) photons initiate polymerization upon exposure to the substance causing the first generation of RBS to polymerize. Fissure sealants from the first generation are no longer used (76).

The second generation of fissure sealants includes self-curing and chemically polymerizing fissure sealants called auto-polymerized resin-based sealant (ARBS). Activating tertiary amine is introduced to one chemical before being combined with another molecule. These components combine to make free radicals, which then trigger the polymerization of the RBS (76).

Third generation fissure sealants called light-cured resin-based sealants (LBRS) are now replacing second generation ones. Visible light is used to cure these fissure sealants and activates the photo initiators. The photo initiators are sensitive to visible light at a wavelength of 470 nm (77). Comparing LBRS to ARBS, LBRS polymerizes in 10–20 seconds, while it can take 1–2 minutes for ARBS to polymerize. LBRS has a longer working time because it does not polymerize unless exposed to light (77).

Fluoride-releasing resin-based fissure sealants (FRBS) are the fourth generation of fissure sealants. FRBS was formed by adding fluoride-releasing materials to third generation sealants (LBRS) (38,78,79).

RBS are also classified as either filled or unfilled. Clinical results appear to be only minimally affected by the use of filler particles in the fissure sealing material. Filled sealants are less able to reach fissures, despite having better wear resistance. Typically, the filled sealants call for occlusal changes, which extend this procedure unnecessarily. The unfilled resin sealants, on the other hand, have lower viscosity and offer better retention and more penetration into fissures (79).

RBS can also be classified as either opaque or clear according to their transparency. Transparent sealants might be clear, pink, or amber while opaque materials can be either white or tooth colored (38). If compared with tooth colored, opaque, or transparent sealants; white opaque fissure sealants are easier to recognize during application and can be detected clinically at follow-up. The sealant substance selected is typically a matter of personal preference (76).

2.5.2. Glass ionomer fissure sealant

Fissure sealant with glass ionomer cement (GIC) was first introduced by Mclean et al. (80). Conventional glass ionomer (GI) has been used for pit and fissure sealants.

By an acid–base reaction involving aqueous-based polyacrylic acid solution and fluoroaluminosilicate glass powder, GI sealants chemically attach to enamel and dentin (81).

GI sealants are divided into two groups: low viscosity and high viscosity types. It is important to note that the majority of research on GI sealants uses low-viscosity, older-generation GI, which has poor physical characteristics. A more recent version, such as Fuji Triage (GC, Tokyo, Japan), has since taken its place with improved physical characteristics and is designed to release more fluoride (82).

High viscosity glass ionomer cements (HVGIC) were subsequently released on the market. Studies on these materials have included atraumatic treatment restorations (ART) regarding their use as sealants and for restoration. The preventive element of ART sealant uses the finger-press technique to apply HVGIC to pits and fissures (83).

When resin is mixed with GI, it is altered to a resin-modified GI cement (RMCIS). Additionally, RMCIS serves as a material for pit and fissure sealant materials. There are initiator photo activators within this type of resin-based fissure sealant material, also ionomer molecules that undergo an acid–base reaction. The physical characteristics of RMCIS are better than GI, but GI are less sensitive to humidity and have a longer working time (84).

GI sealants have continuous fluoride release and fluoride recharge capabilities, which is one of its key benefits. Due to the possibility that some sealant material might still be present deep within the fissures, its preventive function might continue after the substance has been visibly lost. RBS cannot be applied due to moisture control, which is important for partially erupted permanent teeth (84). They can be used as a transitional sealant, particularly when the operculum is covering the distal part of occlusal surface. Due to a child’s lack of cooperation, extensively fissured primary molars that are difficult to isolate might benefit from GI sealant. When better isolation is achievable, it should be replaced with RBS. GI sealant is only temporary (85).

2.5.3. Polyacid-modified resin-based sealants

Fissure sealants can be made using polyacid-modified resin-based composite materials, also known as compomers. These combine the positive features of visible light polymerized RBS with the fluoride abilities of GI sealants. In comparison to GI sealant material, a polyacid-modified RBS has better adhesive properties to enamel and dentin, but are also less water soluble and technique sensitive (86).

Martin et al. (87), state that compomers are primarily hydrophobic and have a low ability to bind to oral tissues. Despite being substantially lower than GI cements, fluoride release levels are still better than typical composite resins (88). Additionally, they are not able to recharge fluoride. GI and RMGIC cannot compete with the mechanical qualities of compomers, which include higher abrasion resistance, resistance to shrinkage, and resistance to bending. However, they completely capture the material characteristics of composite resins. For the best adhesion, the enamel and dentin surfaces should first be treated with a bonding agent, unlike glass ionomer cement (GIC), which is chemically linked to the surface (89).

In comparison to compomers and RMGIC, compomers perform better to be used as fissure sealants, compomers were developed and then made in flowable form (90).

2.5.4. Ormocer fissure sealant

Ormocers are the latest class of materials that are still being researched and developed for use in dental applications. They were introduced to the market in 1998. Ormocer means organically modifies ceramics, often referred to as ormosils in the literature. Their chemistry is similar to that of organic polymers and silicones (91).

Ormocers are advantageous because they offer acceptable adherence to enamel and dentin, are biocompatible, condensable and simple to manipulate, greatly reduce polymerization shrinkage, and produce pleasing aesthetic outcomes (92).

2.5.5. Giomer fissure sealants

The development of giomer materials is a recent advancement. Pre-reacted glass ionomer filler is used in the resin matrix of these materials. After undergoing a pre-reaction with polyacid to create a glass ionomer matrix structure, the fluoro-aluminosilicate glass is combined with resin (93). Giomers are fluoride-released adhesives containing pre-reactive glass (PRG) fillers (94).

Beautisealant was the brand name used when giomer-based fissure sealants were first released on the market. If used with its own primer, this material (which is applied using the self-etch technique) does not require acid etching or washing processes. Eliminating this phase shortens the length of the clinical procedures, shortens the length of the clinical procedures, and saves both the patient and dentist time. It also removes potential issues that could arise during the acid etching process. Furthermore, this process is easy to apply to pediatric patients (95).

2.5.6. Glass carbomer fissure sealants

Glass carbomer, a material created from glass ionomer, contains fluorapatite and nano-powder particles. Better mechanical qualities and heat-activated command settings are advantages of glass carbomer over GIC (96).

Glass carbomer cements, which are created from a common GI substance, are monomer free, carbonized, and are nano glass restorative cements that incorporate hydroxyapatite and fluorapatite nanoparticles (97). The benefits of glass carbomers over GIC are that it has superior chemical and mechanical qualities. Other improved features include resistance, flexural strength, wear, remineralization power, and command setting with an light emitted diode (LED) curing devices (98).

In one study, it was reported that the shear bond strength of glass carbomer was on par with or greater than that of traditional GIC. Furthermore, glass carbomer release fluoride at levels higher than traditional GI (99). According to a study, the level of fluoride seen in glass carbomer was also higher than in GIC in terms of fluoride uptake behavior; however, even this increased fluoride uptake does not appear to result in a high fluoride release (100).

Glass carbomer sealants retain moisture during application, which is a significant benefit in pediatric dentistry particularly for non-cooperative children. However, organic solvents and BPA monomers, which are a risk to children have been eliminated from glass carbomer fissure sealants (101).

2.5.7. Amorphous calcium phosphate fissure sealant

Biologically active restorative materials that release calcium and phosphates can promote tooth structure regeneration and prevent cavities. These substances often referred to as smart composites, contain amorphous calcium phosphate (ACP) as a bioactive filler enclosed in a polymer binder (102). ACP was first described in the 1958, by Posner et al. (103).

In response to disturbances in the oral environment caused by bacterial plaque or acidic foods, calcium and phosphate ions released from ACP composites build-up in dental structures as an apatite mineral (likely hydroxyapatite) that is naturally present in teeth and bone (104). ACP composites are used in the treatment of mild cavitation caries and in the pits and fissures of teeth to stop the build-up of plaque. Additionally, when used as orthodontic adhesives, they can stop enamel demineralization that frequently occur around tapes and brackets. These remineralized and anti-demineralized ACP composites can also be used in individuals with caries-prone salivary dysfunction as temporary restorations or as a basis material for amalgam or resin composites. Kishor et al. (105), compared ACP and fluoride-based resin fissure sealants used on the first molars of children aged 6–9 years and found no statistically different results, but stated that ACP fissure sealants show better retention on tooth surfaces. They reported that the combination use of two remineralization agents was extremely successful in preventing demineralization in another study looking at the caries preventive benefits of ACP and fluoride-based resin fissure sealants (105).

2.6. Polymers and Polymerization

Polymers are long-chain, high molecular weight compounds made of several identical or dissimilar atomic groups joined by chemical bonds in an irregular pattern.

Monomers are the building blocks that make up polymers. Multiple monomers can number in the thousands or millions per polymer chain (106).

Polymerization describes when monomers undergo polymerization processes and form polymers. Condensation polymerization and chain polymerization are the two main methods used to create polymers. The two subgroups of condensation polymerization are further separated. The terms radical chain polymerizations are used if the active centers promoting chain growth are of a radical nature, while those of ionic nature are defined as ionic chain polymerization (107). The three main structural types of polymers are linear, branching, and cross-linked. A networked polymer results from an excessive cross-linking (107).

Atoms are joined to one another in linear and branching formations by light physical connections. Covalently bound atoms create a network structure, which leads to the formation of cross-linked structures. Since they increase the endurance of resin materials and have superior mechanical qualities to those of linear form polymers, cross-linked polymers are crucial (108).

The number of monomers that are converted to polymers throughout polymerization is referred to as the degree of polymerization or conversion, which represents the number of carbon double bonds being converted to single bonds as a percentage. The remaining monomers that are not converted to polymers weaken the material’s structure and cause the restoration to fail. The amount of unreacted residual monomer reduces and the physical characteristics of the resin material improve if the degree of polymerization is high. This is why a resin material must have a high degree of polymerization, meaning that the monomers should be fully polymerized (109).

2.7. Exposure of Monomers from Fissure Sealant

Adhesive systems and composites both utilize similar resin monomers, which form a polymer-containing matrix structure after polymerization, acting as a support structure similar to a skeleton. Monomers are crucial components of adhesive systems because of this (110). The biological safety of these materials has come under increased scrutiny due to the growing usage of resin-based materials in dentistry. Resin-based materials can contain monomers such as Bis-GMA, TEDGMA, HEMA, and UDMA, which are the most commonly used in resin-based fissure sealants (111).

All the carbon double bonds in monomers are expected to react and form a polymer chain during polymerization, but the reaction does not occur at full efficiency. When molecules are added to the polymer chain during polymerization, the viscosity rises and the conversion of monomers to polymers is slowed. Additionally, free radicals react with oxygen to create non-reactive peroxy radicals because they are more likely to do so when oxygen is present. On surfaces that come into contact with oxygen, peroxy radicals form and polymerization does not take place completely. The layer that forms on the surface is known as an oxygen inhibition layer, and the phenomenon is known as inhibition of polymerization by oxygen (112). The solvents in glue and the liquid flow can both have a negative effect on the polymerization process (113).

Unpolymerized monomers that are still present in the resin substance are released. Over time, as a result of deformation of the surface and internal structure of the material, residual monomers are released from resin materials. The polymer matrix contains distributed, unreacted monomers. The amount of residual monomers released from a resin material can vary depending on several factors. These factors include the type, size and combination of monomers that make up the material, the level of abrasion on the material's surface, and changes in the composition of saliva. (114).

In comparison, composites and resins containing fissure sealants have less filler material and a higher resin matrix. In relation to this circumstance, they contain more monomers (115).

According to Eliades et al. (116), and Komurcuoglu et al. (117), there are potential dangers to biological tissues when these unreacted residual monomers are released into the oral environment.

2.7.1. Unpolymerized residual monomers

During the process of polymerization, it is theoretically assumed that all carbon double bonds of the monomers should be completely incorporated into the polymer chain by reaction. However, in reality, this does not happen. As more molecules are added to the polymer chain through reaction, the rate of conversion of monomer to polymer slows down. This results in a reduced starting moment and higher viscosity. As a consequence, there is a greater amount of unreacted monomer in the substance. Due to this, the substance becomes less mobile and spreads more slowly in the polymerization zones. As a result, some monomers react while waste monomers are formed because no more monomers can be added (114).

The amount of conversion of carbon double bonds depends on certain factors. These are (117):

- Resin composition (monomer structure, filler type, and ratio)
- Types and amounts of photosensitive catalysts and inhibitors
- Light wavelength, intensity, and exposure period
- Ambient temperature
- Level of oxygen in the surrounding environment

2.7.1.1.Bisphenol-A glycidyl methacrylate (Bis-GMA)

Bowen (118) discovered a way to mix two moles of glycidyl methacrylate with one mole of BPA while exploring the genesis of BPA. To catalyze the joining of the phenolic hydroxyl groups to the epoxide groups, a tertiary amine was added. A test to ascertain if the reaction was performed involved combining monomers with silica powder that contained approximately 2% benzoyl peroxide. This paste was inserted between two polyethylene films and heated to 90°C. The third Bowen process can produce impurities such as glycidyl methacrylate and BPA in Bis-GMA resins, which can cause allergic and estrogenic reactions if not properly purified.

Numerous additives and various monomers are present in these materials, which can either encourage or impede the polymerization reaction. Bis-GMA, BPA ethoxylated dimethacrylate (Bis-EMA), and BPA dimethacrylate (Bis-DMA) are the major monomers used in the process. Secondary monomers include UDMA and TEGDMA (119).

2.7.1.2. Triethylene glycol dimethacrylate (TEGDMA)

Dimethacrylates, such as Bis-GMA, and its derivates are polymerizable matrix base monomers used to create the polymeric matrix for pit and fissure sealants. Because Bis-GMA is so viscous, it requires significant dilution to function. TEDGMA, one of the diluents with lower molecular weight, is co-polymerized with this monomer (117).

2.7.1.3. Urethane dimethacrylate (UDMA)

UDMA is a polymer matrix and the reaction product of 2,4,4-trimethylhexamethylenediisocyanate and 2-hyroxyl methacrylate, which serves the same function as Bis-GMA (111). UDMA has an analogous molecular weight to Bis-GMA, but with less viscosity. To create resin materials, UDMA is either used alone or in conjunction with other molecules such as Bis-GMA and TEDGMA (120).

The UDMA molecule has a considerable number of double bonds due to its ability to create hydrogen bonds with its aliphatic core architecture and two urethanes. The durability of a resin material including UDMA also improves as a result of its elastic urethane bond (121).

2.8. Human Health Risk of BPA

BPA is primarily made using the acetone process, which involves the condensing of acetone with phenol under the influence of an acid or cation exchanger. The dried crystals are a premium item with a purity of over 99.8% for the manufacturing of polycarbonates and epoxy resins following the recovery and purifying methods, including freezing and centrifugation (122).

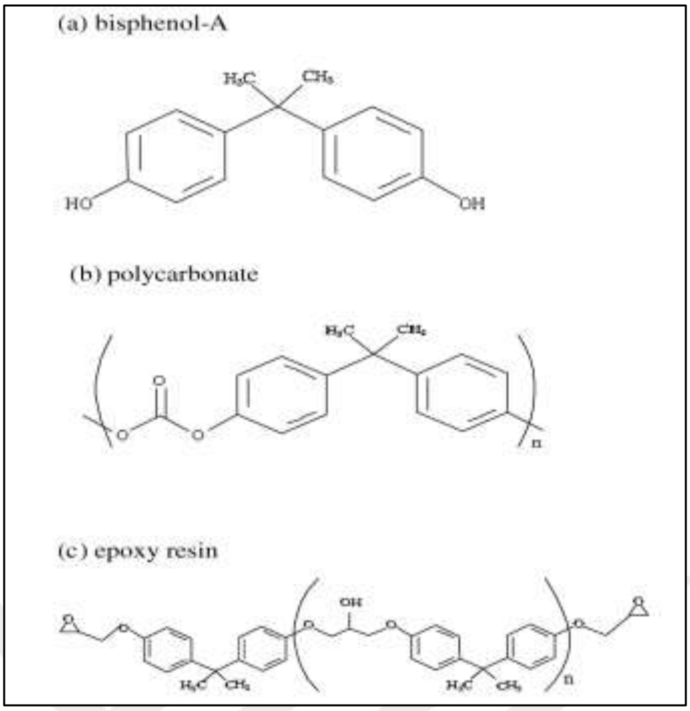

Figure 3: (A) Bisphenol-A formula array. (B) Polycarbonate formula array. (C) Epoxy resin formula array (123).

In the manufacture of plastic goods -particularly in the fabrication of polycarbonate water bottles bottle caps and epoxy resin lacquer linings for metal food can- BPA is a synthetic, chemical resin that is used as a monomer. BPA formulation array is shown in Figure 3A, polycarbonate formula array shown in Figure 3B, and epoxy resin formula array shown in Figure 3C (123).

Resin-based dental sealants and composites, commonly used in restorative materials and oral health care, contain derivatives of BPA. Concern about the possibility of neurotoxic and nephrotoxic consequences of mercury-containing amalgam fillings, and the need for aesthetically pleasing restorations have led to an increase in the use of composite resin dental materials for fillings. Although contact with mercury has been observed, the effects have been deemed to be negligible, and the few meticulous prospective epidemiological studies using resin-based dental materials as standards have not discovered any adverse effects on neurodevelopment or kidney function (125,126).

The sustainability of these materials with regards to biological compatibility has been questioned due to the widespread use of resin-based materials in dentistry. When a material comes into touch with living tissues, biological compatibility is the feature that the material does not have any local systemic, toxic, allergenic, mutagenic, or carcinogenic effects (127).

BPA is currently manufactured in excess of 2 million tons per year, with a 6% to 10% increase in demand per year is anticipated in the future (124). The European Food Safety Authority (EFSA) has established a daily tolerable dose limit of 4 µg/kg to 0.2 µg/kg for BPA intake (166). The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) conducted a risk analysis on BPA, primarily based on animal studies, to evaluate the potential risks to human health for neural and behavioral effects in fetuses, infants, and children at current human exposures. This analysis was done in response to increased complaints from researchers, leading to the recognition that concerns do exist. (128).

2.8.1. Cytotoxic and estrogenic activity of BPA

The hazardous effects on live cells are referred to as cytotoxicity. Toxicology requires careful control over the migration of oligomers, monomers, indicators of synthetic polymers, and various other low-weight molecules from polymer chains to prevent harmful interactions between these molecules and biologically significant molecules (129).

Chemical curing procedures or photoactivation is required for the in-situ polymerization of monomers in resin-based dental sealants. However, this process is not entirely complete. Some residual monomers could still be present and might bleed out of the cured resin into the surrounding media (130). Ratanasathien et al. (131), researched the cytotoxic effects of Bis-GMA, UDMA, TEGDMA, and HEMA in terms of TC50 values (a marker of cytotoxicity) in mice, and reported Bis-GMA having the most cytotoxic effect.

In 1960, BPA was first identified as a cancer-related chromosomal abnormality reported using cytogenetic methods (132). According to studies, women with breast cancer had median serum levels of BPA that were higher than those of women without the disease. Concomitant blood BPA concentrations might not accurately reflect the etiologically important exposure period for breast cancer growth years to the years before clinical detection since BPA has a short half-life (133).

BPA has been linked to diseases, such as recurrent miscarriages, aberrant karyotypes, polycystic ovarian syndrome, reproductive problems. Recent researches have investigated the harmful consequences of BPA linked to diabetes, cardiovascular disease, neurobehavioral disorders, and cancer (134,138, 139, 146). In addition, BPA can cause an increased risk of obesity in humans (136).

BPA competes with estradiol for binding to the estrogen receptor and induces the expression of both progesterone receptor and the proliferation of MCF-7 breast cancer cells (135). Furthermore, BPA can cause increased cardiovascular disease, hypertension risk, systolic blood pressure and diastolic blood pressure, and also decrease systolic blood pressure during pregnancy (137). According to Hwang et al. (138), found that exposure to BPA was associated with an increased risk of type 2 diabetes mellitus whereby both urine and serum BPA levels were positively associated with increased risk of the condition.

A major cytotoxic monomer in the endocrine system is BPA. Problems can be caused by a number of endocrine systems, including glucose homeostasis, obesity, insulin resistance, β-cell dysfunction, inflammation, and diabetes. BPA plays a part in the production of epoxy resin and polycarbonate plastic goods, such as food packaging, beverage vessels, and dental sealants (138). Research on BPA in the endocrine system has increased as a result of extensive studies that have shown it to be a potential endocrine disruptive chemical (EDC). If EDCs enter and are stored in the body, they can act as hormone agonists and antagonists, interfering with the physiological effects of thyroid, androgen, and estrogen hormones. EDCs block cell signaling pathways involved in glucose and weight homeostasis (138).

Numerous studies have examined ovarian response, sexual function, and sex steroids as potential targets of BPA exposure. Adult epidemiological researchers have found links between urine BPA levels and serum levels of the hormones that control reproduction. Some evidence indicates that BPA exposure and higher serum concentrations in men can be seen in the hormones FSH, LH, SHBG, and testosterone (139–141).

Early BPA exposure can increase the likelihood of disrupted puberty, which is considered a breast cancer risk factor (142). Also, early BPA exposure can hasten puberty development and raise the chance of breast cancer, according to several animal studies (143). The timing of pubertal development in girls might be affected by BPA exposure. More research is required to determine the connection between male puberty development and BPA exposure (144). Farabollini et al. (145), noted that in males, there was a decrease in sexual activity in postnatally treated rats. However, they also saw the modest changes detected as a hint to an influence of prenatal BPA in promoting more defensive tactics during agonistic interactions and in shortening the copulatory sequence, delayed puberty, and a rise in aggressiveness.

According to research, the mammary gland's epithelial cell cycle is altered by BPA as an ambient estrogen-like substance (146). On the assumption of the proliferative effect and the degree of cell cycle perturbation, these findings might also serve as markers for assessing differences and similarities in early alteration in growth and development of the mammary gland from exposure to estrogen-like chemicals (147). According to Ashby et al. (148) BPA affects the development of male reproductive organs in rats and mice, and in women causes premature vaginal opening.

BPA is a synthetic estrogen analog. Research has shown that it seriously harms both human and mammalian hormone systems, having an effect not just on reproduction, but also on child physical growth and behavior. Other research on the estrogenic effects of BPA suggest that it damages the prostate and mammary glands, promotes early puberty in females, and induces neurological and behavioral abnormalities in fetuses, infants, and children (150). It has been shown that prolonged exposure to chemicals, such as BPA and BPA derivates in women decreases productivity (151).

According to a study by Olea et al. (135), BPA can disrupt the reproductive system by having estrogenic effects on the body. This can lead to an increase in the number of breast cancer cells in humans and a reduction in sperm count in male mice.

These changes in cellular function are attributed to the presence of BPA and its derivatives. It has also been linked to unexpected and recurrent miscarriages in pregnant women (152). Also, girls with high urinary BPA concentrations experienced delayed breast growth (153).

TEGDMA cytotoxicity and genotoxicity have been discovered in many studies during the past 10 years. It is well known that the co-monomer TEGDMA causes in vitro gene alterations. The suppression of the phosphatidylinositol 3-kinase (PI3K) cell-survival signaling pathway, which mediates cell death, genotoxicity, and a delay in the cell cycle, has been linked to the activation of apoptosis by TEGDMA in human pulp cells (154).

Bandarra et al. (155), found that compared to TEGDMA, which should not be present in the cranial nerve system, both Bis-GMA and UDMA were discovered to be able to penetrate the blood-brain barrier (BBB). A thorough assessment of the permeability capability of prospective substances is provided by the BBB permeability model. Although prediction models cannot replace experimental methods, this method enables passive transport across the BBB to be predicted with 90% accuracy, and the study identifies Bis-GMA and UDMA resin-based monomers as possible neurotoxic substances (155).

2.9. Prenatal Exposure of BPA

BPA has a pleiotropic effect on the body and when present during pregnancy, can have an effect on a number of tissues and mechanisms involved in controlling bodyweight, such as the development and growth of adipocyte tissues, pathways and circuits crucial for glucose uptake and homeostasis, and energy homeostasis. The pathways and processes affected by perinatal exposure to BPA might react differently according to endogenous hormone levels at the time of exposure (156).

Prenatal exposure to BPA examined by Rubin et al. (156) who founded a shift in bodyweight that lasts both during pregnancy and after birth. Furthermore, BPA affects adipocyte cell metabolism (156). BPA initially disrupts normal glucose homeostasis. An increase in baseline and insulin-stimulated 3T3-F442A adipocytes is induced by an increase in the glucose transporter 4 (GLUT4) protein (157). Adipocyte cells begin to differentiate after exposure to BPA (158).

When exposed to BPA from foods or the environment, the first permanent molars and incisors are at a higher risk of developing hypoplasia and hypomineralization. Salmela et al. (159) conducted an in vitro study to validate the combined effects of sodium fluoride (NaF) and tetrachlorodibenzodioxin (TCDD) on rat tooth germ. The study used immune iso-chemical analysis and found that the combination of NaF and TCDD led to problems in pre-dentin secretion and enamel matrix mineralization.

Numerous studies have linked BPA exposure to poor prenatal development (160,161). A disorder known as molar incisor hypomineralization (MIH), which affects the first molars and permanent incisors, has been linked to postnatal BPA exposure, which may be related to a predisposition to BPA exposure as it corresponds to the time of ameloblast development (160). During this time, BPA might affect amelogenesis and cause MIH. A study on rats and human enamel found that BPA increases the organic content, albumin, and enamelin content of enamel, which might cause amelogenesis and MIH when a tooth erupts (161).

The immune system, allergies, and asthma are all promoted by prenatal exposure to BPA. According to Zhou et al. (162) maternal urinary BPA concentrations are positively correlated with the risk of newborn allergic reactions during the first 6 months of life, but only in female infants and not in male infants.

Furthermore, in vivo studies show low-dose BPA release in the uterus can expose future generations to behavioral abnormalities in social interactions (163). Environmental variables have the potential to cause attention deficit hyperactivity disorder (ADHD). In animal studies, exposure to substances, such as lead, alcohol, or cigarettes while the fetus is developing has been linked to ADHD or its symptoms, thus, BPA exposure could be a factor in ADHD (164).

BPA and its derivates can cross the placental barrier. In fact, BPA has been identified in human milk. If BPA is detected in mature milk, it indicates the mother's most recent exposure. If present in colostrum, it suggests the mother's cumulative exposures during the second half of pregnancy. Newborns that are breastfed will be exposed to BPA if their mothers are exposed to environmental contaminants (165).

2.10. BPA in Fissure Sealants

Dental sealants act as a barrier that keeps bacteria and nutrients from growing in cracks and pits on teeth that are more likely to develop caries. According to statistics, between 5% and 10% of sealants need to be repaired, replaced, or restored each year (167). There was no preventive treatment for molars in dental clinics before the development of dental sealants. Almost all preventive measures either failed or resulted in irreparable or irreversible damage to the teeth (168). Sealants nowadays include primary monomers Bis-GMA, Bis-EMA, Bis-DMA, and Bis-BADGE and secondary monomers UDMA and TEGDMA (169).

2.10.1. Exposure of BPA after fissure sealant treatment

Dental sealants were observed to release BPA into saliva, either immediately or after a specific amount of time (170). However, when Bis-GMA comes in contact with water, a strong acid, base like hydrochloric acid or sodium hydroxide, it undergoes a hydrolysis reaction that leads to the production of BPA. The process happens due to the catalytic effect of the acid or base on the ether bonds between the BPA and glycidyl methacrylate (GMA) units, which causes the release of BPA and GMA molecules (171). Instead of pure BPA, dental resins typically contain BPA derivatives. Bis-GMA can hydrolyze to BPA under acidic or alkaline conditions, such as oral cavity or the environment (170).

According to Rathee et al. (172) resin-based products might additionally release BPA as a result of the salivary enzymatic hydrolysis of BPA variants, such as Bis-GMA and Bis-DMA. These monomers can release from resins during the very first setting phase and concurrently with fluid adsorption and desorption over time.

Furthermore, the placement of Bis-GMA-based restorations in children and adolescents can momentarily raise the level of BPA in urine; however, at low levels, BPA might be more harmful than at large doses (175). Studies have reported that the amount of BPA released can be influenced by how long light-curing is extended after polymerizing the dental sealant on the outside of the tooth (176).

The pH levels of oral environment can be different from one patient to another. The effectiveness of BPA release at various pH levels piqued researchers' interest. A study founded that the amount of BPA released from dental sealants varied depending on the pH level, and as a consequence, it was concluded that a low pH level had a detrimental effect on BPA release. Consuming various beverages often after receiving a sealant treatment can significantly affect the chemical stability of the sealant in the oral cavity (180).

As a result, following is a list of the variations in BPA release that depend on the environment (160):

- Temperature: increased BPA release is seen in a warmer oral environment
- Acid–base shifts in pH cause an increase in BPA release
- Degree of conversion: the light energy density should be adequate for improved polymerization of free radicals
- The ideal polymerization duration is 20 seconds; however, it is crucial to alter the polymerization period according to the manufacturer's instructions
- Distance from light source: as the length of the light source tip approaches its surface, increase the BPA release
- Light curing apparatus: LED light sources provide the best polymerization results
- Storage environment: acetonitrile and methanol are strong organic compound solvents that are chosen over ethanol to enhance the release of monomers

2.10.2. Recommended application techniques

Studies or randomized controlled trials have shown that BPA or its derivatives are exposed during the application of composites or sealants (181,182). After curing, monomers are still unpolymerized, and have the potential to contaminate saliva. As exposure to oxygen prevents polymerization, these unpolymerized monomers are often present in a liquid layer on the material's outer surface. The amount of accessible monomer is decreased if this unpolymerized layer is removed immediately after sealant application (181). Researchers have recommended a number of procedures to be followed after or during treatment to try to reduce the release of BPA from dental products because of this. These recommendations are (182):

- All fissure sealants do not release or have the same amount of BPA in ingredient; therefore, while purchasing fissure sealants, consideration should be given to the resin compounds used
- Restoration should be performed with a rubber dam to stop un-polymerized monomers from dissolving in saliva
- Apply glycerine gel before polymerization; alternately surface polish with a pumice or cotton applicator or wash with an air-water spray for 30 seconds to eliminate the oxygen inhibition layer after the fissure sealant has been applied
- The patient should gargle with water for 30 seconds after the treatment
- When making orthodontic appliances for patients, photopolymerized resins should be selected over self-curing resins
- BPA has substantial estrogenic and cytotoxic effects, so it is important to treat children, adolescents, and pregnant women with extreme caution. For these patients, nearly all of the suggested application methods listed above should be used. Treatments for expectant mothers might be postponed during the first trimester.
- Follow the principle of one procedure per visit in order to minimize BPA release. For either composite restoration and fissure sealant, more than four treatments should be applied in a single session.

According to Rueggeberg et al. (183) the best way to remove the air-inhibited layer from sealants after they have been applied is to use a mild abrasive along with some mechanical force such as a hand scrub with a cotton roll or an air-driven prophylactic cup. The uncured, hydrophobic monomers found on the newly cured sealant surface cannot be removed using an air water syringe. Furthermore, simply rubbing off the surface with cotton tends only removes a tiny amount of these materials, whether they are wet or dry.

Azarpazhooh et al. (184) reports information that can help dental professionals reduce BPA exposure after applying dental sealants. By applying a mild abrasive or pumice to a cotton applicator or prophylaxis cup can reduce the possibility of unpolymerized BPA remaining on the surface. Washing the outer layer of the sealant for 30 seconds with an air water syringe with suction to remove fluids and debris from the child's mouth is recommended, as is instructing older children to rinse with water after curing.

Additionally, CAD/CAM composite restorations and indirect restorations enhance conversion rates while minimizing monomer release. Certain techniques, such as the use of rubber dams, longer curing, or a second curing process after coating the restoration with glycerin, limit exposure to free monomers caused by direct composite restorations. Additionally, the majority of the aforementioned free monomers are found on the material surface, where exposure to oxygen prevents polymerization (181).

Unreacted monomers can be eliminated from the mouth using a variety of techniques. According to study, the chemicals included in resin components only partially lower the amounts of Bis-GMA compounds released following application (117). Komurcuoglu et al. (117) found that prophylactic paste treatment reduced residual monomers by up to 90% after applying to a dental sealant.

2.11. Methods used to determine BPA

Nowadays, a variety of methods are used to ascertain the depth of polymerization or the degree of polymerization in resin-based materials. Several of these methods include (111):

- Separative thermal analysis
- Fourier transform spectroscopy
- Solid phase nuclear magnetic resonance
- UV spectrophotometer
- Multiple internal reflection spectroscopy

- Surface hardness measurement
- Chromatographs
  - Gas chromatography (GC)
  - Liquid chromatograph/mass spectrometry (LC/MS)
  - Micellar electrokinetic chromatography (MEKC)
  - High pressure liquid chromatography (HPLC)

2.11.1. Gas chromatography (GC)

Martin and James (185) developed the current gas chromatography method in 1952. These innovations produced some of the most significant and popular analytical methods in contemporary chemistry (185). To produce separations using GC, a mixture is injected into a narrow band, followed by an assortment of partitions among the moving gas phase and an immobile phase of liquid kept in a tiny diameter tube (the column). Then, as the gas flow exits the column containing separated components, a detector monitors its composition and the outcomes of these signals serve as the starting point for data acquisition. The examination of mixtures, including chemicals with boiling points ranging from close to 0 to over 700 K or those that can be sufficiently heated without decomposing to produce a vapor pressure of a few mmHg, can be performed using GC. This range is expanded by derivatization to boost volatility. The sample size can be as low as pg scale, however, tens of grammes are manageable in preparative applications as opposed to analytical ones. In several sectors, especially petrochemical production, GC is now a common analytical technique that supports study, development, and quality control. It is also used in the environment, food contamination, drug residue, and forensic investigation. The interior system of gas chromatography is shown in Figure 4 (186).

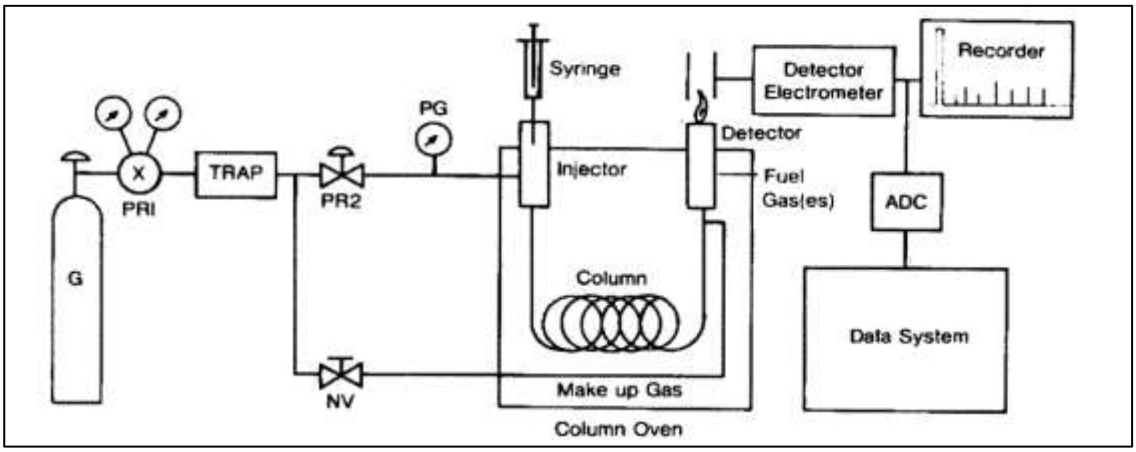

Figure 4: The interior system of gas chromatography (185)

2.11.2. Liquid chromatography/mass spectrometry (LC/MS)

The liquid chromatography/mass spectrometry (LC/MS) interface system was used to produce effective results in the research that performed. Modifications that reduce the challenges of handling increasing percentages of fluids have enhanced the status of this interface. The release of the solvent, the elimination of the solvent in a state of vacuum, and the process of evaporation of the specimen to the ion source are the three stages that make up the moving tape's operating principle (187).

LC/MS was created by Hayes et al. (189) based on the spray purifying principle that discovered. Thus, reports of ion flow profiles that were challenging to construct were made (189).

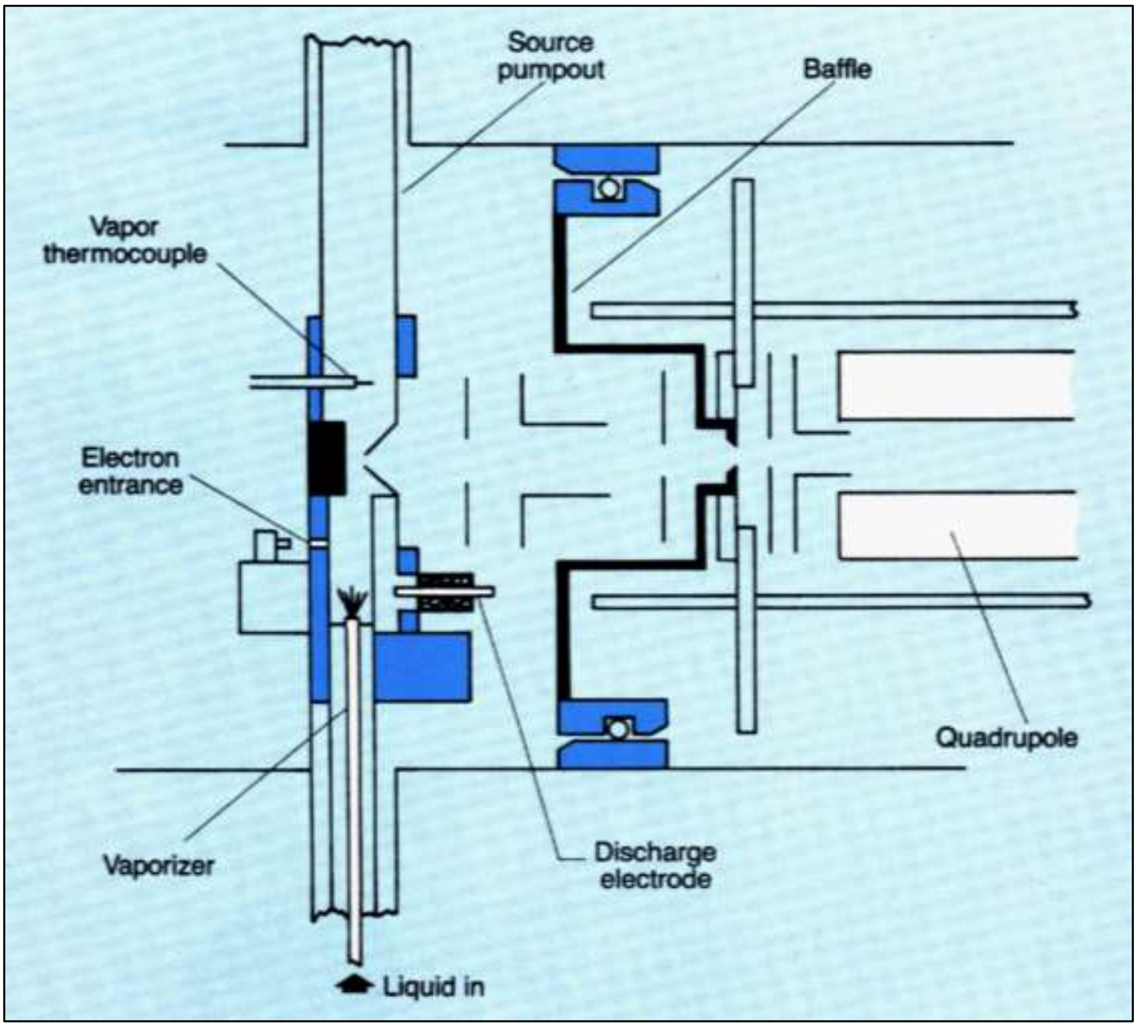

Figure 5: The mechanic system of LC/MS (190)

Liquid removal and sample volatilization are made simpler by reduced sample mixing within the film despite the belt's presence and the fact that the fluid is uniformly distributed as small drops on the belt surface. Direct deposition produces a crooked film with liquid heads on the belt edge that are challenging for the vacuum system to remove. The spray deposition adjustment allows for the introduction of total effluents with higher water content (189).

Eluents with extremely high-water content might still need to have their chromatographic flow rates lowered or partially separated from the interface. Games et al. (190) revealed the other key development to the moving-belt LC/MS interface, which is the mechanic system of LC/MS shown in Figure 5 (190).

These experts compared the belt's two earlier iterations with an updated version that had the belt's terminal located near the electron beam, inside the ion source. Chemicals can be heated and desorbed off the surface of the belt in this manner and then volatilized right into the electron beam. Ion flows are shown in Figure 6. For instance, this method has demonstrated some of the most spectacular transportation LC/MS results on the notoriously challenging drug digoxin. Volatilization of polar samples is facilitated by deactivating the belt surface with Carbowax 20M, and medium MS resolution can differentiate specimen ions from the background, further improving performance (191).

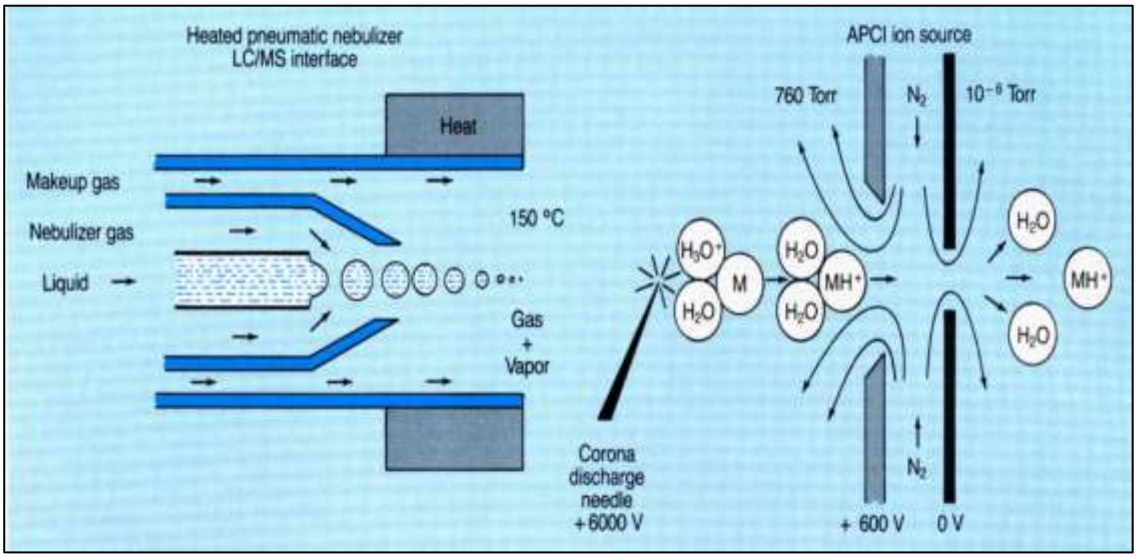

Figure 6: Ion flow in LC/MS (190)

2.11.3. Micellar electrokinetic chromatography (MEKC)

Capillary electrophoresis (CE) was first published in the early 1980s. The strong discriminatory power of CE piqued the interest of many chromatographers, but few academics were able to use or study CE. Because CE had features that needed to be improved in use, and since the capillaries used a delicate glass tube and needed extra care for detection, handling them was challenging. The CE needed a high voltage current to function. The experiment's most challenging component—the glass tube—was discovered and replaced in 1983 with fused silica capillaries, which the researchers had the greatest trouble with. Finally, CE established itself in the marketplace in 1989 in accordance with the work completed (192).

In theory, CE could only examine ions or charged variables since the basis for its separation mechanism is the variation in the electrophoretic mobilities of analytes. The separation principle of micellar electrokinetic chromatography (MEKC) is shown in Figure 7. To create micellar electrokinetic chromatography, Nakagawa et al. (193) added an ionic micelle to a CE solution. Electrically neutral analytes can be separated using MEKC without modifying the CE equipment, and the separation performance is nearly identical to that of CE, allowing MEKC to broaden the application range of CE (194). Zone separation of MEKC is shown in Figure 8A and a chromatogram of MEKC is shown in Figure 8B (193).

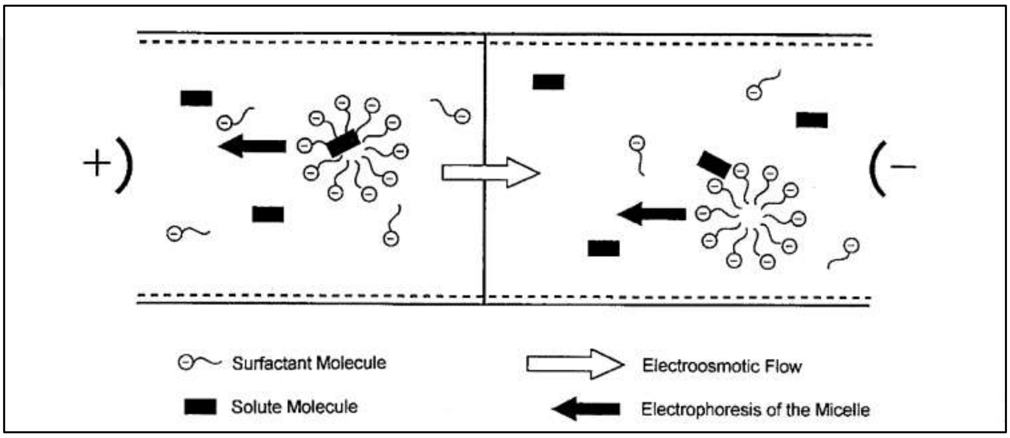

Figure 7: Separation principle of MEKC (193)

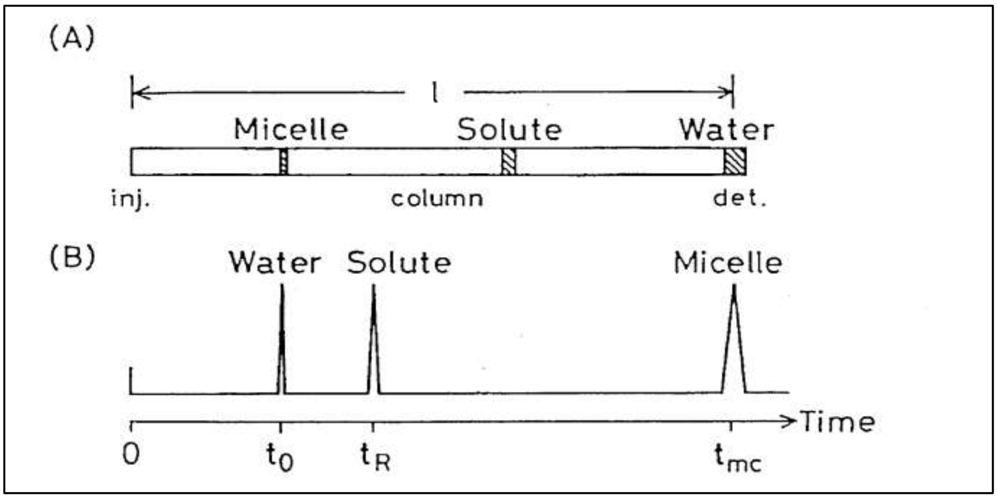

Figure 8: (A) Zone separation (B) Chromatogram of MEKC of hypothetical mixtures of water solute and micelle (193)

2.11.4. High pressure liquid chromatography (HPLC)

In recent years, biological fluids, medications, and metabolites have been separated and catalytically determined using high pressure liquid chromatography (HPLC) technology. Considering that the components are not required to be volatile, HPLC has become one of the most common methods for chromatographic separation, particularly for the separation of macromolecules (195).

The development and operation of an HPLC require four key components: mobile phase, stationary phase, pump, and detector (195).

2.11.4.1. Mobile phase

This is made up of combinations of solutions or solvents with different physical and chemical characteristics that transport the sample's constituent parts through the column for analysis. Numerous factors, including the characteristics of the material being studied, the stationary phase that will be used, and the characteristics of the detector should be taken into account when choosing the mobile phase to be used in the measurement.

Before advancing to the mobile phase, gases that are dissolved in the solution itself must be eliminated. If not, air bubbles will form in the column and the solvent flow rate will slow down, producing inaccurate findings. The gases that dissolve in the mobile phase are removed using a variety of technologies, including vacuum pumps, distillation equipment, heating systems, and ultrasound (196).

2.11.4.2. Stationary phase

With HPLC, stationary phases are referred to as columns. With a specific amount of pressure and speed, this phase interacts with the elements from the mobile phase, allowing them to slow down and be retained to some extent, allowing them to move forward later. Retention time refers to this delay. The retention time shows how long it takes for the material under analysis to exit the stationary phase. This allows for the determination of each molecule's retention time under certain, steady analytical conditions. Depending on the procedure, several columns of different diameters and lengths are used in HPLC applications. A typical HPLC column, however, is constructed of stainless steel and has a length of 10–40 cm with a diameter of 3–4 mm. It is designed as a metallic tube with substances for coating on its inner surfaces that have highly varied properties, making it appropriate for the components being studied. The column’s filler particles have diameters that range 3–20 µm. The efficiency of the column increases as the diameter of the filler particles decreases, although using very small particles could result in higher pressures back in the column (196).

2.11.4.3. Pump systems

Pump systems are used in HPLC applications to enable the solvent mixtures that make up the mobile phase flow to along the column at a specific rate and pressure. Pneumatic, screw (syringe type), and piston pumps are the three different types of pump systems (195).

2.11.4.4. Detector

A crucial piece of equipment in contemporary liquid chromatography is a sensitive detector that allows the parts of the substance moved through the column to be measured. The physical and chemical characteristics of the substance to be tested are used to define the type of detector applied in the analysis (195).

Some of the factors need optimizing before the experiment to help obtain the significant statistical results that HPLC will provide (197). This is crucial since it backs up the researchers’ assessments of them and makes it easier to create mathematical models in this way (198).

3. MATERIALS AND METHODS

The experiments of this study were performed together with Yeditepe University Faculty of Dentistry and Trakya University Faculty of Health Sciences TUTAGEM Laboratory.

In this study, the number of samples was determined by G power analysis. To determine the number of samples, power analysis was performed using the G*Power (v3.1.9.2) program. The strength of the study is expressed as 1-β (β=probability of type II error) and in general, research should have 80% power, based on the publication study conducted by Eun-Deok Jo (215). BPA (ng/mL) measurements were calculated based on the changes in follow-ups according to the groups, and the effect size was found to be d=0.7045 in the evaluation, and was calculated to obtain 99% power at the level of α=0.05, a total of 39 cases that is at least 13 subjects per group. Considering that there might be losses during the working process, in this study took this number as 16 subjects per group.

In this study, evaluated the amount of BPA release of different fissure sealant brands under different polymerization times (20 seconds and 40 seconds) and in different solutions (artificial saliva and ethanol) with LC/MS.

3.1. Materials

Experimental groups were formed from the fissure sealants where is used in Yeditepe University Pediatric Dentistry clinic. In this study, fissure sealant with three different features was used. All fissure sealants were applied in accordance to the manufacturer’s instruction.

These materials were:

- Resin-based Clinpro™ Sealant (3M, St. Paul, MN, USA) which is light-cured, has the ability to change color during polymerization, contains organic filler, and has a low filler content is shown in Figure 9. Ingredients of the Clinpro™ are shown in Table 1.

- Chemically hardened containing GI-containing organic filler GC Fuji TRIAGE (GC Fuji TRIAGE, White, GC US) is shown in Figure 10. Ingredients of the GC Fuji Triage are shown in Table 2.
- Fissurit FX (VOCO, Hamburg, Germany) a light-cured, resin-based product with a high filler content is shown in Figure 11. Ingredients of the Fissurit FX are shown in Table 3.

Figure 9: Clinpro™ (3M, St. Paul, MN, USA) fissure sealant

Table 1: Ingredients of Clinpro™

Brand name	Clinpro™ Sealant
Manufacturer	3M ESPE, St. Paul, MN, USA
Chemical content	Bis-GMA, TEGDMA, tetrabutylammonium tetrafluoroborate, and titanium dioxide
Filler particulate powder	6% reinforced inorganic filler (silanated amorphous silica)

Figure 10: GC Fuji Triage (GC Corporation, Tokyo, Japan) capsule fissure sealant

Table 2: Ingredients of GC Fuji Triage

Brand name	GC Fuji TRIAGE
Manufacturer	GC Corporation, Tokyo, Japan
Chemical content	Polyacrylic acid, polycarboxylic acid, alumina-fluoro-silicate, and distilled water
Filler particulate powder	Alumina-fluoro-silicate Liquid: polyacrylic acid

Figure 11: Fissurit FX (VOCO, Hamburg, Germany) fissure sealant

Table 3: Ingredients of Fissurit FX

Brand name	Fissurit FX
Manufacturer	VOCO, Hamburg, Germany
Chemical content	Bis-GMA, TEGDMA, UDMA, and 2% NaF
Filler particulate powder	55% inorganic and GI filler

3.1.1. Preparation of sample discs

Fissure sealants were placed on BPA-free 10 mm wide and 2 mm thick custom-made plates, with each sample was 0.12 mL. Fissurit FX and Clinpro™ were directly squeezed into custom-made discs are shown in Figure 12. Since Fuji Triage has a capsule structure, it was mixed with an amalgamator as shown in Figure 14 at 400 rpm for 15 seconds, and placed into custom-made discs with a capsule gun. For discs to be a flat surface, they were polymerized by pressing them with flat glass tables from the front and back. Ivoclar Vivadent Bluephase G4 (Ivoclar, Vivadent, Liechtenstein) is shown in Figure 13, and was used for polymerization. A wavelength spectrum of 385–

515 nm was used and emitted range up to 2000 mW cm². In this study, LED source's emission rate of 600–700 mW cm² with a wavelength spectrum of 468 nm was used. 24 discs were polymerized for 20 seconds, and the other 24 discs were polymerized for 40 seconds. Polymerization was ensured by keeping a distance of 1 mm between the polymerization device and the samples. The sealants were given in disk form, the rough edges were sanded with a sanding cutter and the disks were all of equal size and weight. Each disk was adjusted to weight 1.2 g. 48 sealant disc samples were created and there were 16 samples per group.

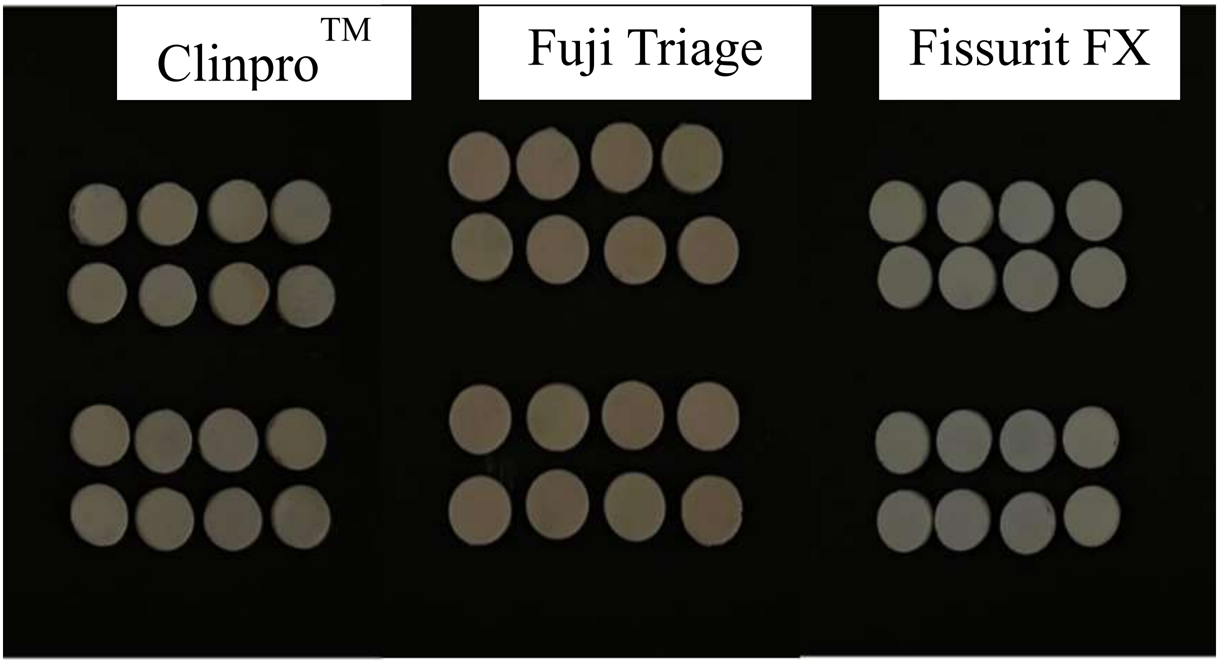

Figure 12: Fissure sealant specimens

Figure 13: Polymerization device Ivoclar Vivadent Bluephase G4 (Ivoclar, Vivadenti, Liechtenstein)

Figure 14: Amalgamator

3.1.2. Preparation of solutions

Ethanol (Sigma-Aldrich) is shown in Figure 15, and artificial saliva solutions were prepared to store the fissure sealant discs. Artificial saliva solution was prepared by the Yeditepe University Faculty of Pharmacy Analytical Chemistry Department.

The ingredients in artificial saliva are shown in Table 4. The solution was consisted of 250 mL of artificial saliva, and then adding 750 mL of an ethanol–distilled water mixture (75:25 v/v). The mixture was incubated (Heidolph, GERMANY) at 37°C for 24 hours as shown in Figure 16, centrifuged (Sigma, GERMANY) at 1100 g for 19 minutes as shown in Figure 17, then directly divided into 24 cups with 5 mL in each. Likewise, the ethanol solution (Sigma-Aldrich) was divided into 24 cups of 5 mL. Samples were left in their solution for 24 hours at room temperature. The discs were then stored at -20°C until the day of the experiment. Samples of experimental groups are shown in Figure 18.

Table 1: Ingredients of artificial saliva

Artificial saliva
0.03 g $MgCl_2$ 6 H_2 O
0.08 g $CaCl_2 \cdot 2H_2O$
0.3 g KCl
0.9 g KOH
472 µl H_3PO_4
1.3 g NaCl
pH: 7

Figure 15: Ethanol (Sigma-Aldrich, Germany)

Figure 16: Incubator

Figure 17: Centrifuge device

3.1.3. Preparation of experimental groups

There were three main experimental groups and four subgroups. Experimental groups are shown in Table 5. Group 1 (n=16) was prepared with Clinpro™ (3M, St. Paul, MN, USA) fissure sealant. The first subgroup (n=4) was polymerized for 20 seconds and immersed in artificial saliva solution, second subgroup (n=4) was polymerized for 40 seconds and immersed in artificial saliva solution, third subgroup (n=4) was polymerized for 20 seconds and immersed in ethanol (EtOH) solution, and the fourth subgroup was polymerized for 40 seconds and immersed in ethanol solution.

Group 2 (n=16) was prepared with Fuji Triage (GC Corporation, Tokyo, Japan) fissure sealant. The first subgroup (n=4) was polymerized for 20 seconds and immersed in artificial saliva solution, second subgroup (n=4) was polymerized for 40 seconds and immersed in artificial saliva solution, third subgroup (n=4) was polymerized for 20 seconds and immersed in ethanol (EtOH) solution, and the fourth subgroup was polymerized for 40 seconds and immersed in ethanol solution.

Group 3 (n=16) was prepared with Fissurit FX (VOCO, Hamburg, Germany) fissure sealant. The first subgroup (n=4) was polymerized for 20 seconds and immersed in artificial saliva solution, second subgroup (n=4) was polymerized for 40 seconds and immersed in artificial saliva solution, third subgroup (n=4) was polymerized for 20 seconds and immersed in ethanol (EtOH) solution, and the fourth subgroup (n=4) was polymerized for 40 seconds and immersed in ethanol solution.

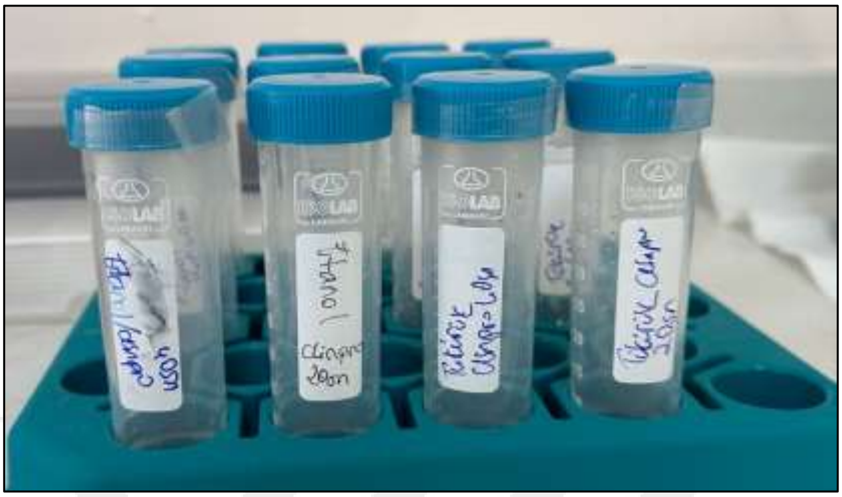

Figure 18: Samples of experimental groups

Table 2: Experimental groups

	1st group: Clinpro™	**2nd group: Fuji Triage**	**3rd group: Fissurit FX**
S1: Immersed in artificial saliva; polymerization time 20s	n=4	n=4	n=4
S2: Immersed in artificial saliva; polymerization time 40s,	n=4	n=4	n=4
S3: Immersed in EtOH: polymerization time 20s	n=4	n=4	n=4
S4: Immersed in EtOH; polymerization time 40s	n=4	n=4	n=4

S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time N: number

3.2. Methodology

The BPA analysis was performed at TUTAGEM, the research laboratory of Trakya University. Samples kept at -20°C were transferred to this laboratory in dry ice. Samples were stored at -20°C until the time of analysis.

3.2.1. Features of the LC/MS device

LC/MS device was used for the analysis of the experimental samples. The LC/MS device used in the experiment is shown in Figure 19. The features of the LC/MS device used in the experiment are listed in the Tables 6 and 7.

Table 3: LC conditions

Instrument	Agilent 1290 Infinity LC System
Column	Agilent ZORBAX Eclipse Plus C18 2.1 mmx50 mm 1.8 µm (p/n 959757-902)
Column temperature	30 °C
Mobile phase	Methanol: water acidified with 0.1 % acetic acid (70:30/v:v) isocratic elution
Flow rate	0.3 mL/min
Injection volume	10 µl

Table 4: MS conditions

Instrument	Agilent 6400 Series Triple Quadupole LC/MS System
Ion mode	AJS-ESI, negative ionization
Capillary voltage	3500 V
Drying gas	10 L/min
Drying gas temperature	250°C
Nebulizer	45 psi
Sheat gas heater	300°C
Sheat gas flow	10 L/min

3.2.2. Preparation of the standard

The BPA standard (Sigma-Aldrich) shown in Figure 20 was weighed on a precision balance shown in Figure 21. 5 ppb, 10 ppb, 25 ppb, 50 ppb, and 100 ppb solutions were prepared with a precision balance as shown in Figure 22. The ingredients of BPA are shown in Table 8.

Table 5: BPA ingredients

Product name	Bisphenol-A
Synonym(s)	2,2-Bis (4-hydroxyphenyl) propane, 4,4′-isopropylidenediphenol
Brand	Sigma-Aldrich
CAS-No.	80-05-7
Molecular weight	228.29
Percentage purity	>99%

Figure 19: LC/MS device at Trakya University, TUTAGEM Laboratory

Figure 20: BPA standard (Sigma-Aldrich)

Figure 21: Precision scales

Figure 22: Prepared BPA standards

3.2.3. Ion flow in the LC/MS device

Optimization studies were performed on LC-MS/MS (Agilent Technologies 6460 Triple Quadrupole Jet stream) using the BPA main stock standard. MS/MS parameters (main ion, fragmentation ion, polarity, fragmentor voltage, and collision energy) and LC parameters (flow gradient, injection volume, column selection, and column temperature) were determined. MS/MS parameter, optimization source parameter, and LC parameter are shown in Tables 9, 10, and 11.

Table 6: BPA optimization MS/MS parameters

Component	Ion	Fragmentation ions	FV	CE	Polarity
Bisphenol-A	227	211.9;132.9	80	12;22	Negative

Table 7: BPA optimization source parameters

Gas temperature	275°C
Gas flow	10 L/min
Nebulizer	45 psi
Sheat gas temperature	375°C
Sheat gas flow	10 L/min
Capillary	4500 V
Nozzle voltage	1500 V
Delta EMV (-)	400

Table 8: BPA optimization LC parameters

Time (min)	A (%)	B (%)
0.00	98	2
2.00	98	2
4.00	0	100
8.00	0	100
8.10	98	2
12.00	98	2
Mobile phase	UPW; 5µM ammonium acetate Acetonitrile; 10µM ammonium acetate	
Column	Zorbax SB-C8, 3.0X150 mm, 3.5 micron	
Column temperature	30°C	
Injection volume	10 µl	
Flow	mL/min	

3.2.4. Preparation of the LC/MS device

During the preparation of the components and the device, the samples, which were stored at -20°C, were kept in a shaker for 30 minutes and in an ultrasonic bath (Daihan, Wiseclean, South Korea) for 15 minutes to ensure that the phthalate components were transferred to the solvent phase. The samples were filtered through a PTFE filter with 0.22-micron pore diameters.

The samples taken from the groups were distributed equally into 1.5 mL glass containers by distillation method. Then, each standard solution representing the calibration step was injected into the device and a six-point calibration curve was drawn with three repetitions for each calibration step is shown in Figure 23. The correlation coefficient found in calibration studies for target compounds was over 0.99. Additionally, five repeated recovery studies were performed at concentrations of 50 and 250 µg/L to validate the accuracy of the applied method.

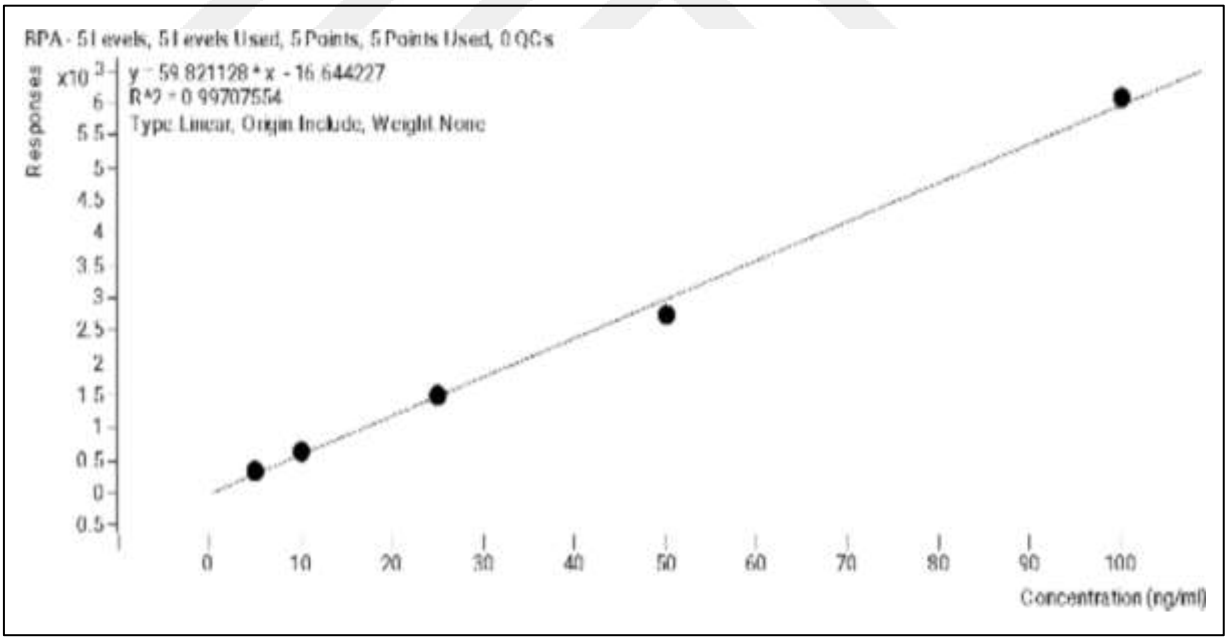

Figure 23: Calibration curve

Figure 24: Samples before entering the devices

Figure 25: Placing the test samples into the LC/MS machine

3.2.5. LC/MS analysis

The samples were analyzed after the machine was run empty for 12 minutes to clear all residual monomers. The samples were placed in the machine with one blank sample between each group are shown in Figure 24 and Figure 25. The previously developed methodology was defined to the machine. The device was set so that the analysis time for each sample was 12 minutes. A total of 88 samples, including blank samples, were scanned with the device. Total analysis time was determined as 44 hours.

3.3. Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 24.0 was used for statistical analysis. The results were evaluated at 95% confidence interval with the significance at $p<0.05$ level. Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of BPA values. Mann–Whitney U test was used to determine which groups were different. Whether there is a statistically significant difference between the BPA values between the polymerization times within the groups was examined with the Wilcoxon test. Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the measurements of peak values between the groups. Mann–Whitney U test was used to determine which groups were different. One sample t-test was used to determine whether the BPA values of the groups were statistically different from the normal values.

4. RESULTS

Statistical program used for the findings obtained in the study, SPSS (Statistical Package for Social Sciences) for Windows 24.0 program was used for statistical analysis. The results were evaluated at 95% confidence interval and significance at $p<0.05$ level.

Table 9: Comparison of BPA (ng/mL) release after 24h with different polymerization times and different solutions

		N	Mean	Sd.	p
S1	G1	16	7.4435	0.0006	**0.000***
	G2	16	1.8416	0.0004	
	G3	16	3.8877	0.0003	
S2	G1	16	3.3163	0.0004	**0.000***
	G2	16	1.6714	0.0004	
	G3	16	4.3840	0.0008	
S3	G1	16	17.8223	0.0000	**0.000***
	G2	16	12.1223	0.0000	
	G3	16	41.8348	0.0000	
S4	G1	16	14.5723	0.0004	**0.000***
	G2	16	2.3208	0.0004	
	G3	16	40.1852	0.0006	

G1: Clinpro™, G2: Fuji Triage, G3: Fissurit FX; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s; ng/mL: nanogram per microliter
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Kruskal–Wallis test, Mann–Whitney U test

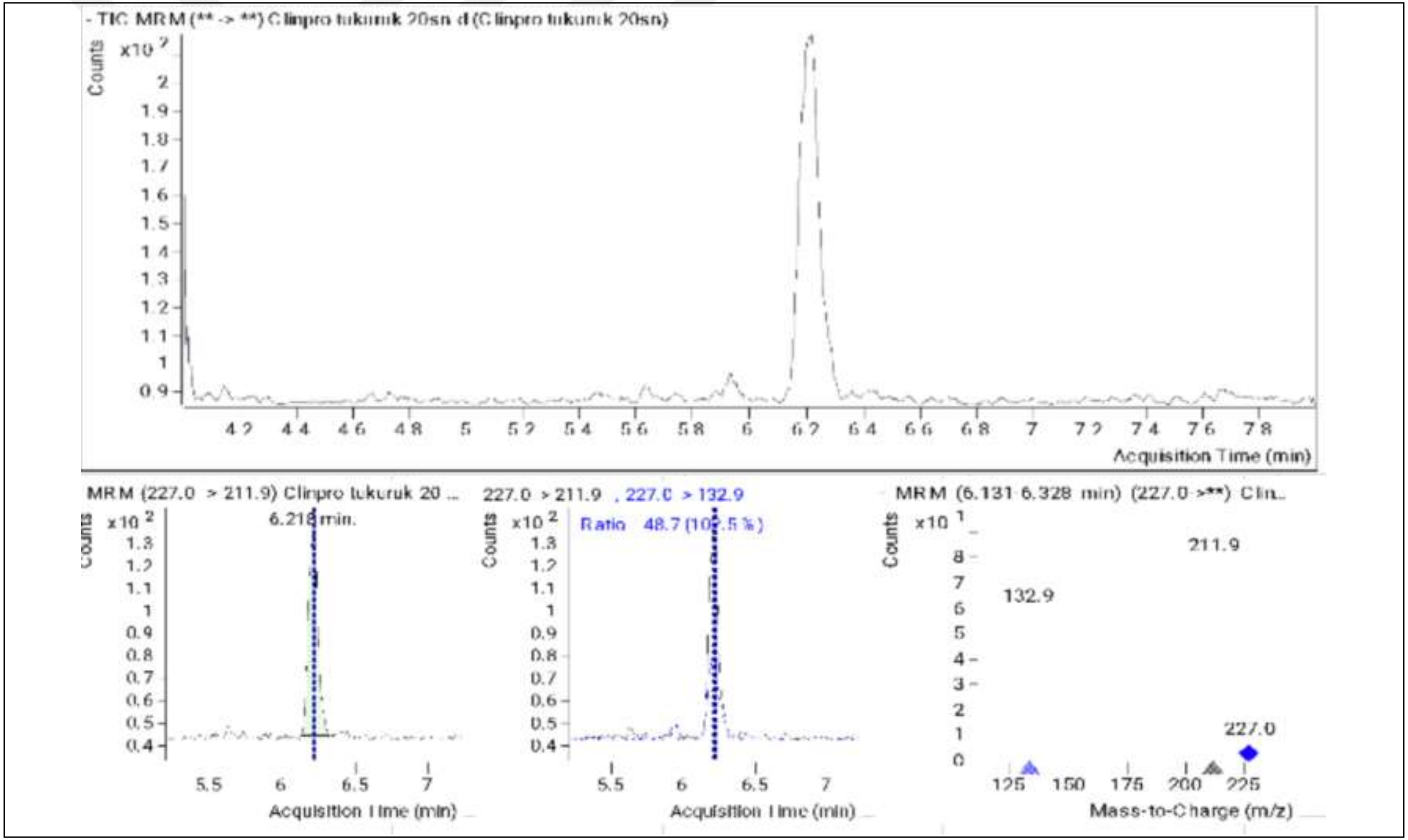

Figure 26: Chromatograph of Clinpro™ immersed in artificial saliva after 24 h and 20 s polymerization time

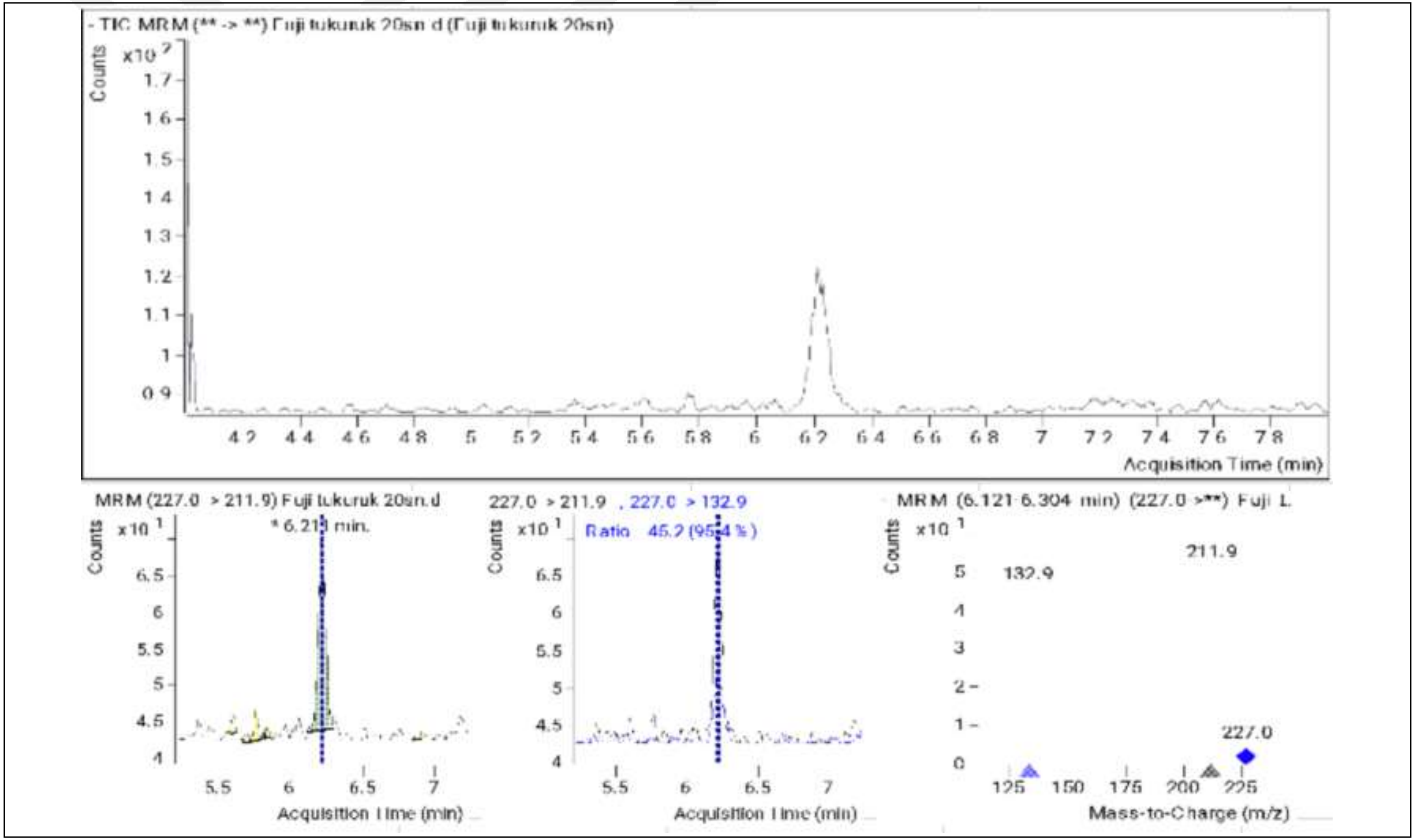

Figure 27: Chromatograph of Fuji Triage immersed in artificial saliva after 24 h and 20 s polymerization time

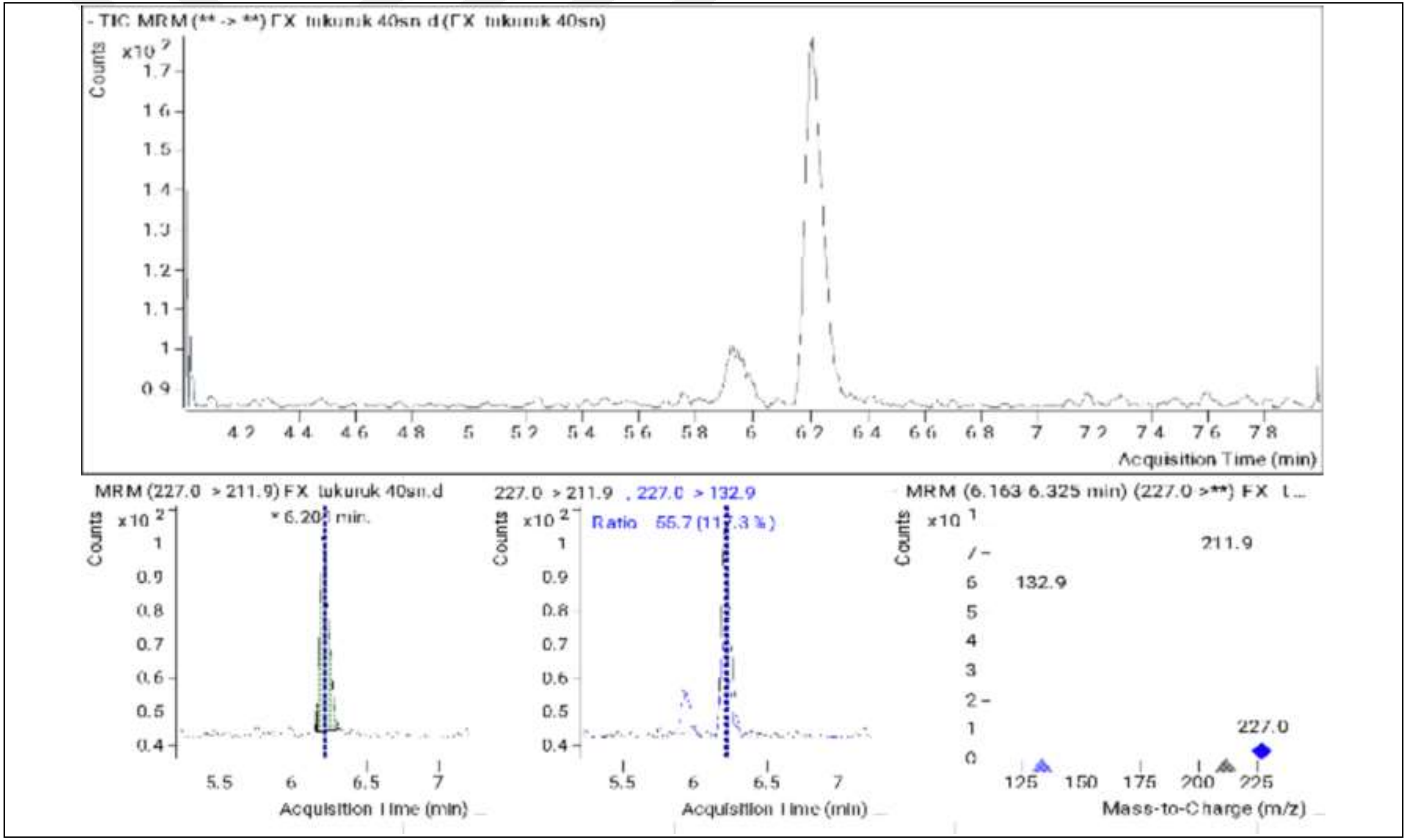

Figure 28: Chromatograph of Fissurit FX immersed in artificial saliva after 24 h and 20 s polymerization time

Immersed in artificial saliva with polymerization time at 20 s (S1): The Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of BPA values. Table 12 shows a statistically significant difference between group 1 (G1), G2, and G3 ($p<0.05$). The Mann–Whitney U test was used to determine which groups were involved in the difference. S1 BPA chromatographs are shown in Figures 26, 27, and 28. The G1 (mean=7.4435) value was higher than the G2 (mean=1.8416) value ($p<0.05$) ($p=0.000$), G1 (mean=7.4435) was higher than G3 (mean=3.8877) ($p<0.05$) ($p=0.000$), and G2 (mean=1.8416) was lower than G3 (mean=3.8877) ($p<0.05$) ($p=0.000$).

Immersed in artificial saliva with polymerization time of 40 s: The Kruskal–Wallis test showed a statistically significant difference in S2 BPA measurements between groups ($p<0.05$). S2 BPA chromatographs are shown in Figures 29, 30, and 31. The G1 (mean=3.3163) value was higher than G2 (mean=1.6714) ($p<0.05$) ($p=0.000$), G1 (mean=3.3163) was lower than G3 (mean=4.3840) ($p<0.05$) ($p=0.000$), and G2 (mean=1.6714) was lower than G3 (mean=4.3840) ($p<0.05$) ($p=0.000$).

Immersed in EtOH; polymerization time 20s: The Kruskal-Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of BPA values. A statistically significant difference was found between S3 and BPA measurements between groups ($p<0.05$). The Mann-Whitney U test was used to determine which groups were involved in the difference. BPA measurements chromatographs are shown in Figure 32, Figure 33 and Figure 34. The G1 (mean=17.8223) value was found to be higher than the G2 (mean=12.1223) ($p<0.05$) ($p=0.000$). G1 (mean=17.8223) value was lower than the G3 (mean=41.8348) value ($p<0.05$) ($p=0.000$). The G2 (mean=12.1223) value was found to be lower than the G3 (mean=41.8348) value ($p<0.05$) ($p=0.000$).

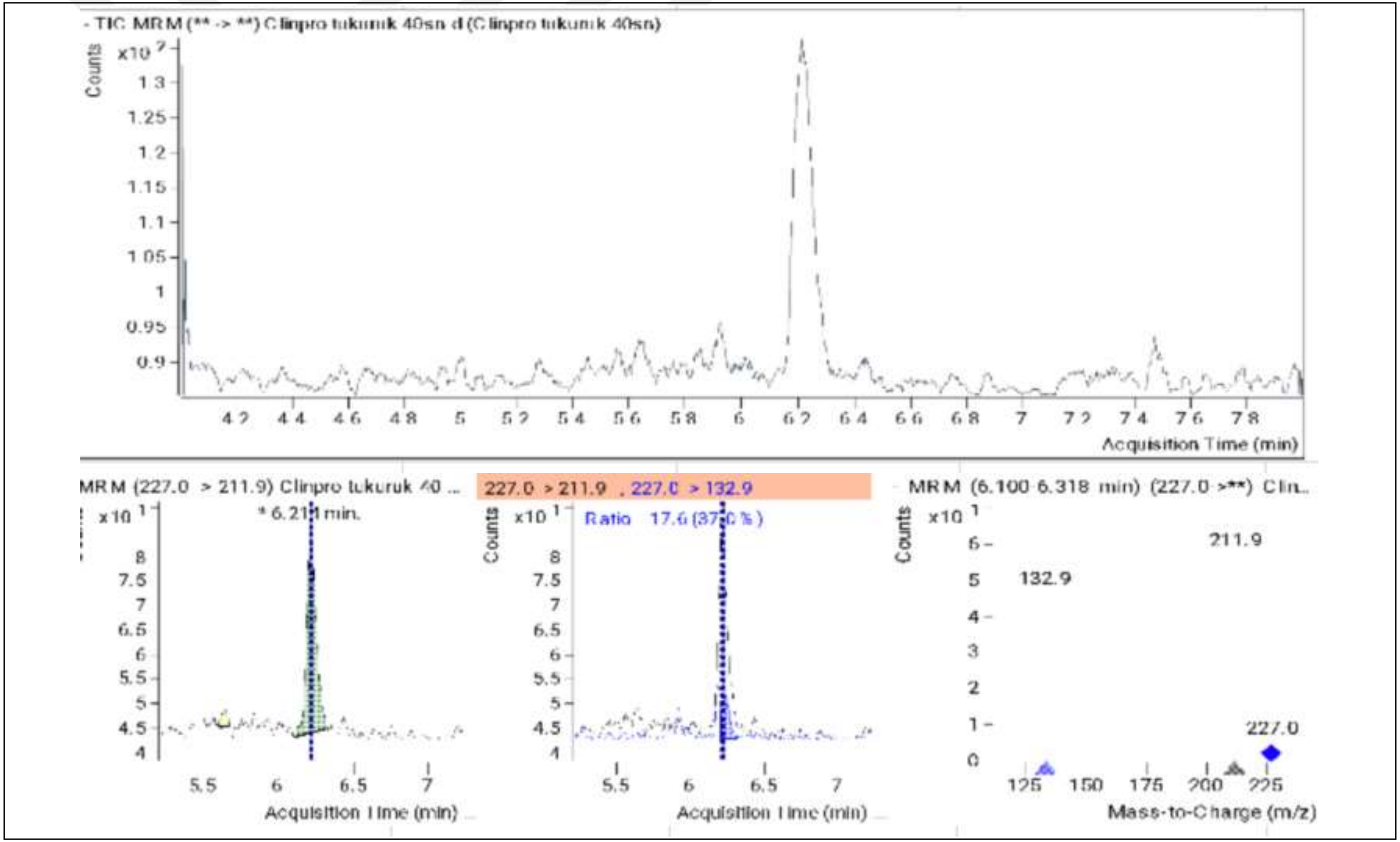

Figure 29: Chromatograph of Clinpro™ immersed in artificial saliva after 24 h and 40 s polymerization time

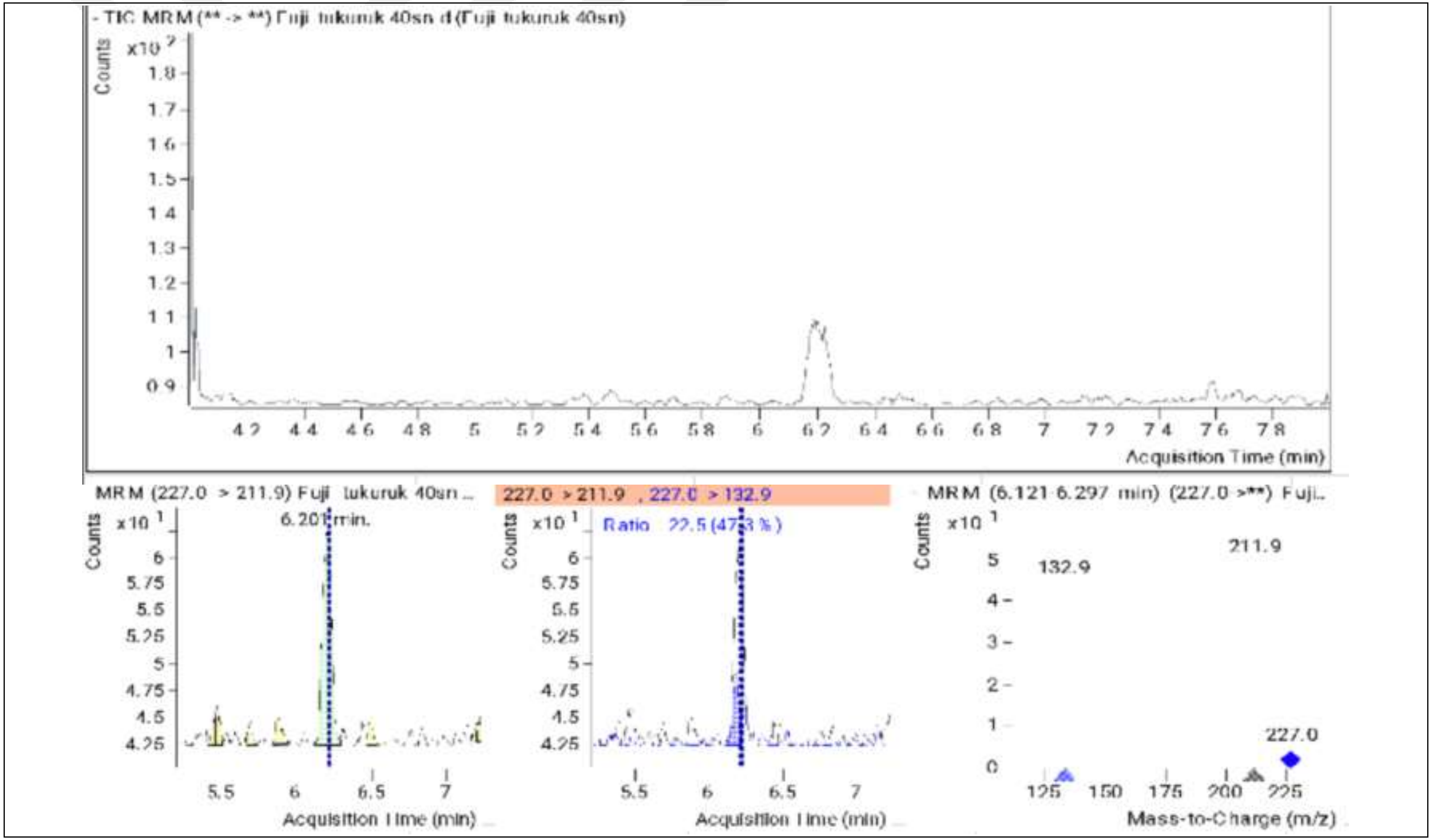

Figure 30: Chromatograph of Fuji Triage immersed in artificial saliva after 24 h and 40 s polymerization time

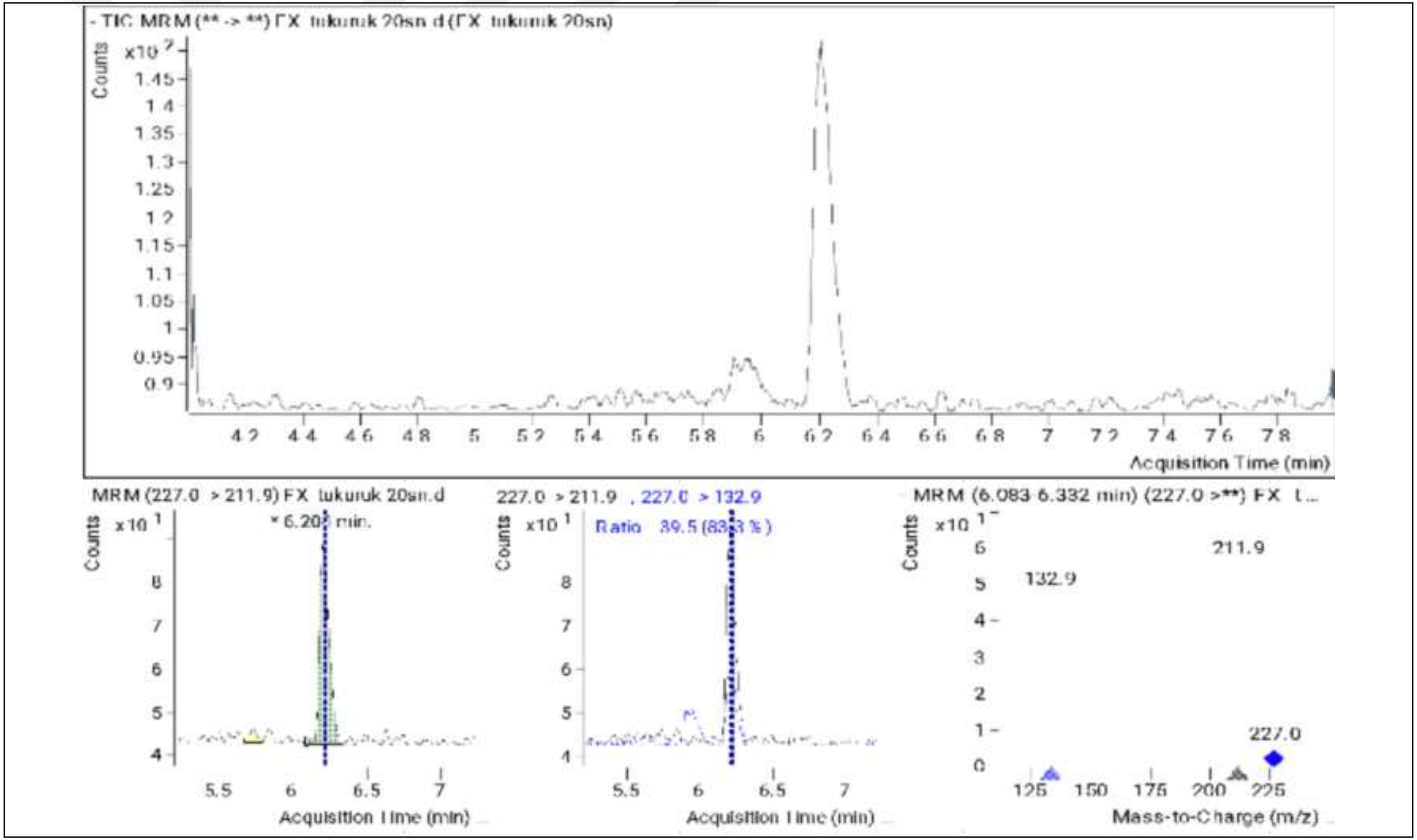

Figure 31: Chromatograph of Fissurit FX immersed in artificial saliva after 24 h and 40 s polymerization time

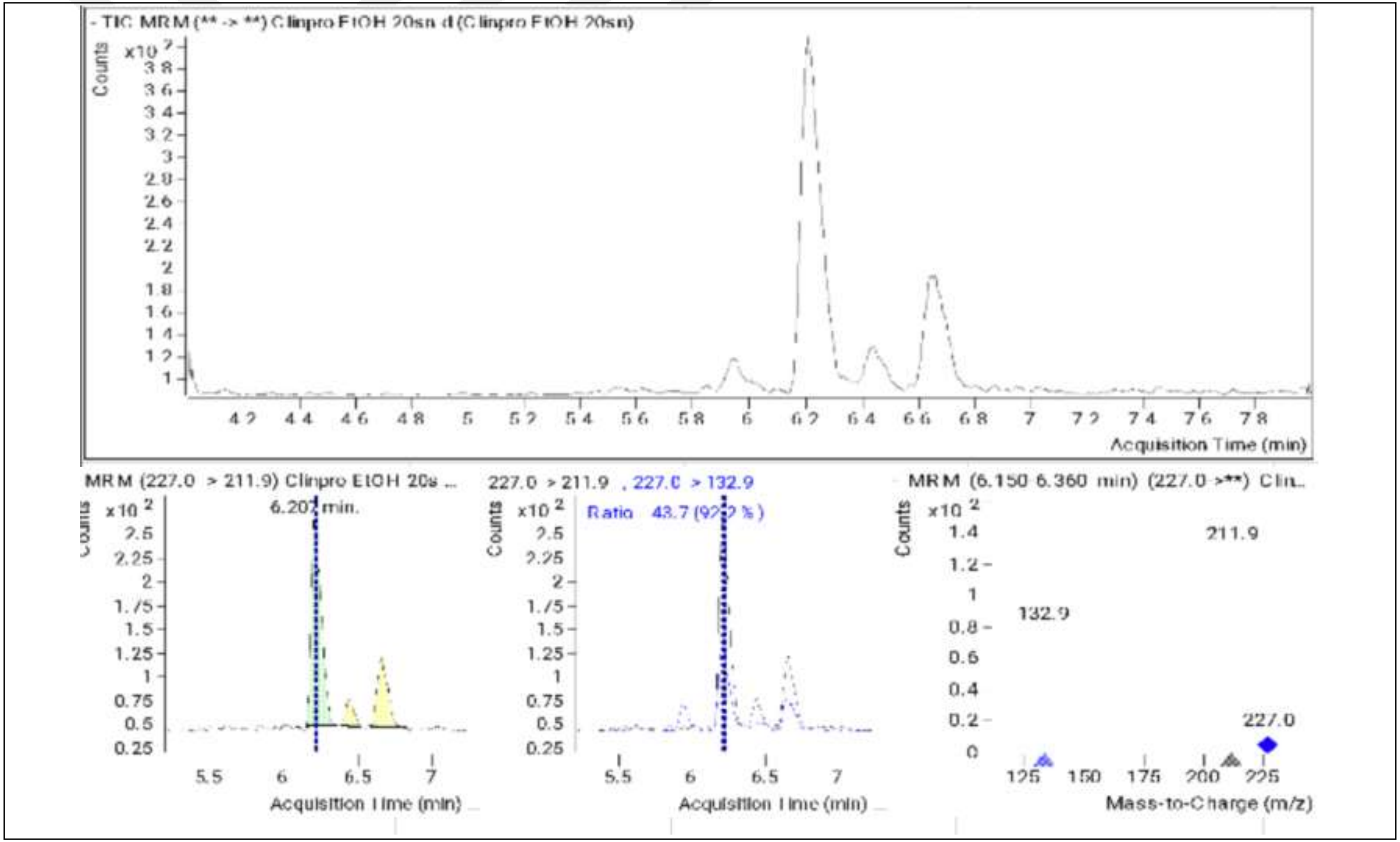

Figure 32: Chromatograph of Clinpro™ immersed in EtOH after 24 h and 20 s polymerization time

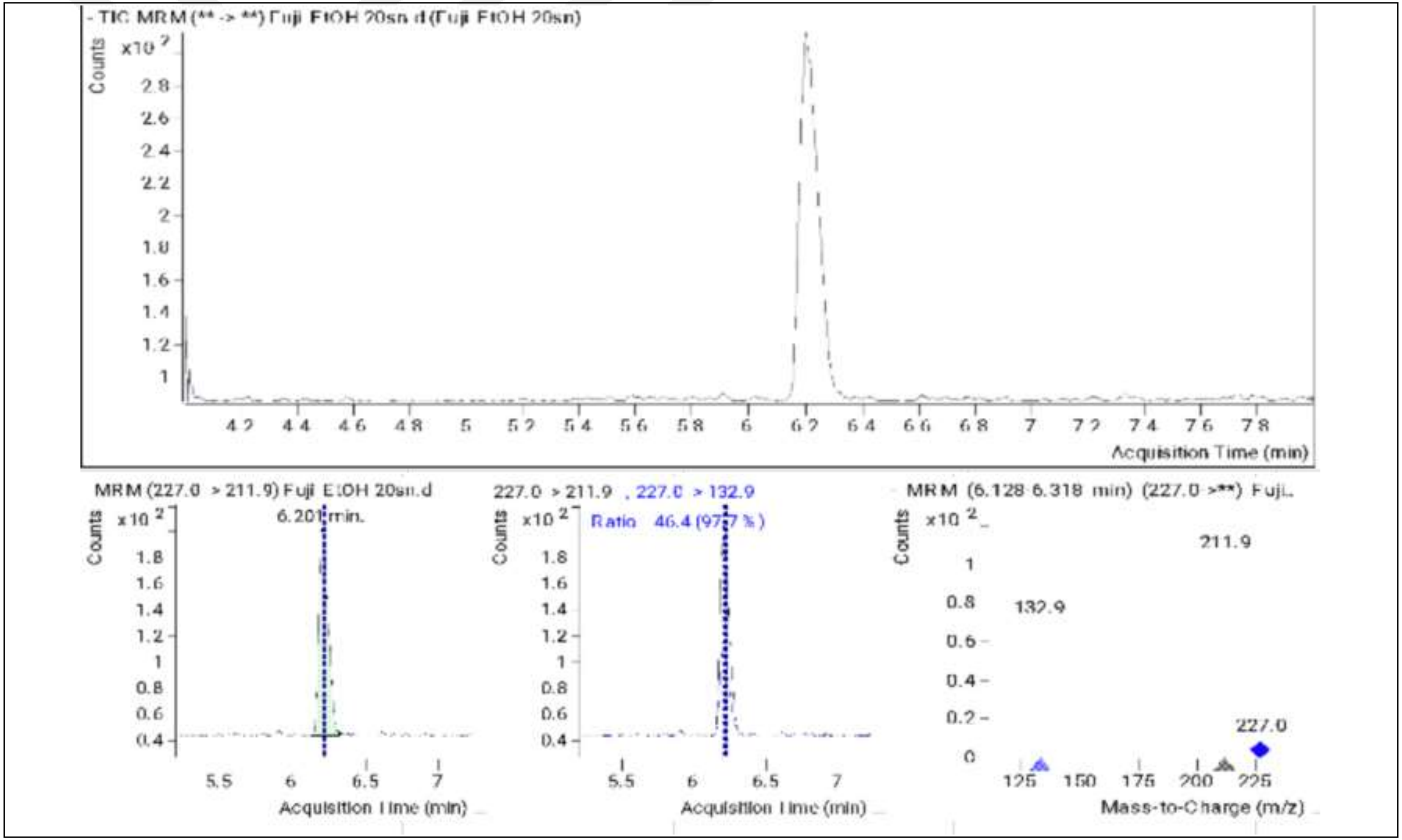

Figure 33: Chromatograph of Fuji Triage immersed in EtOH after 24 h and 20 s polymerization time

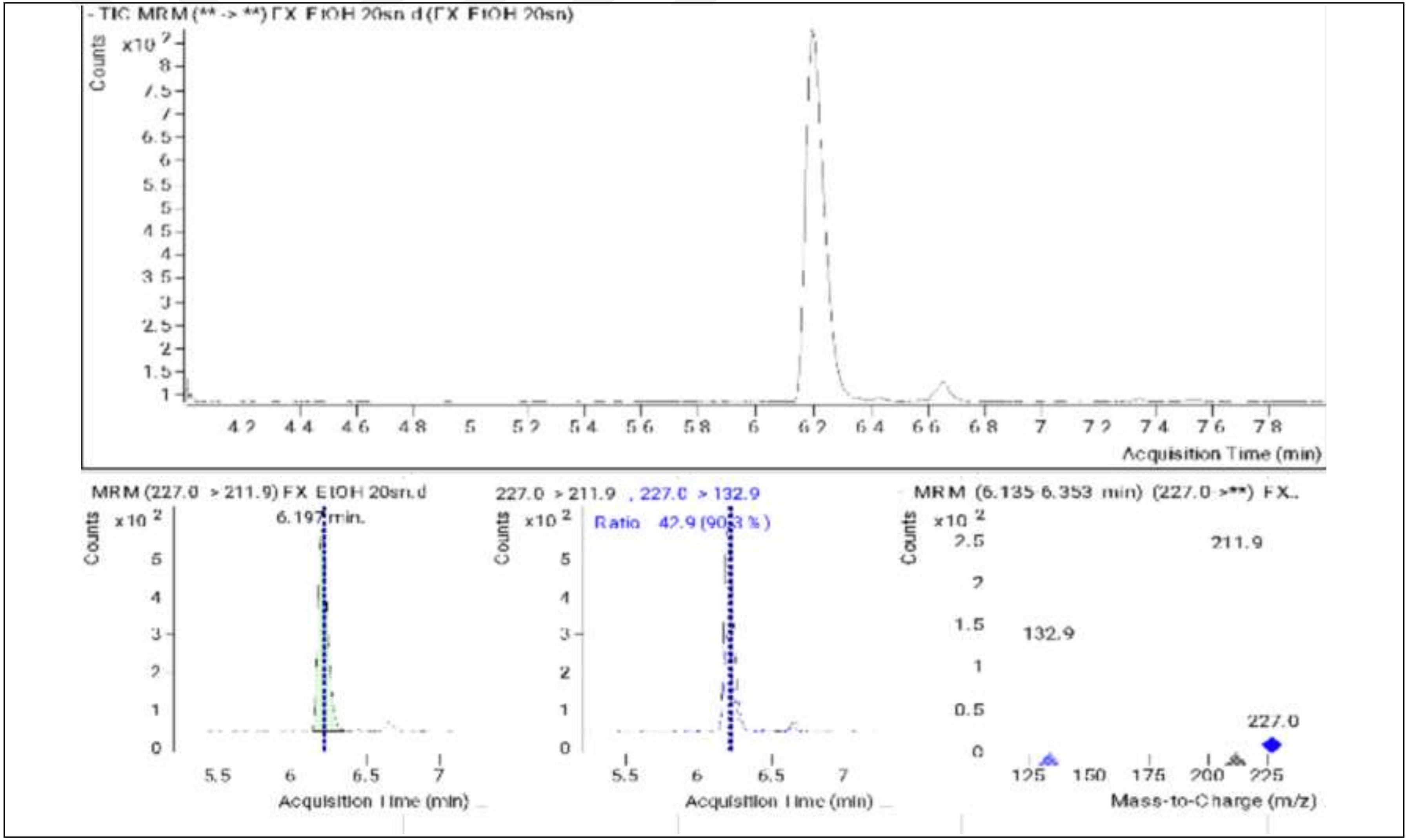

Figure 34: Chromatograph of Fissurit FX immersed in EtOH 24 h after 20 s polymerization time

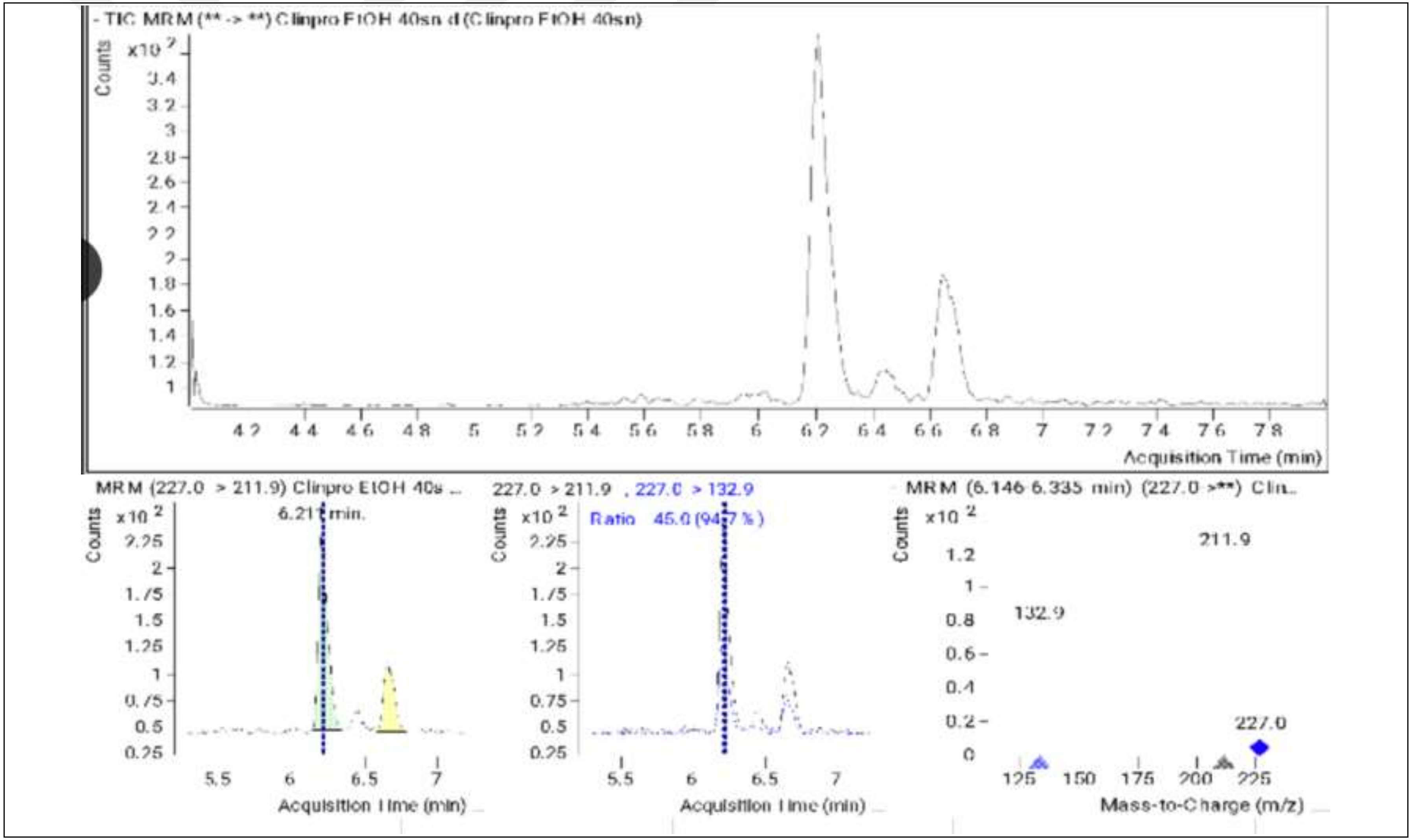

Figure 35: Chromatograph of Clinpro™ immersed in EtOH after 24 h and 40 s polymerization time

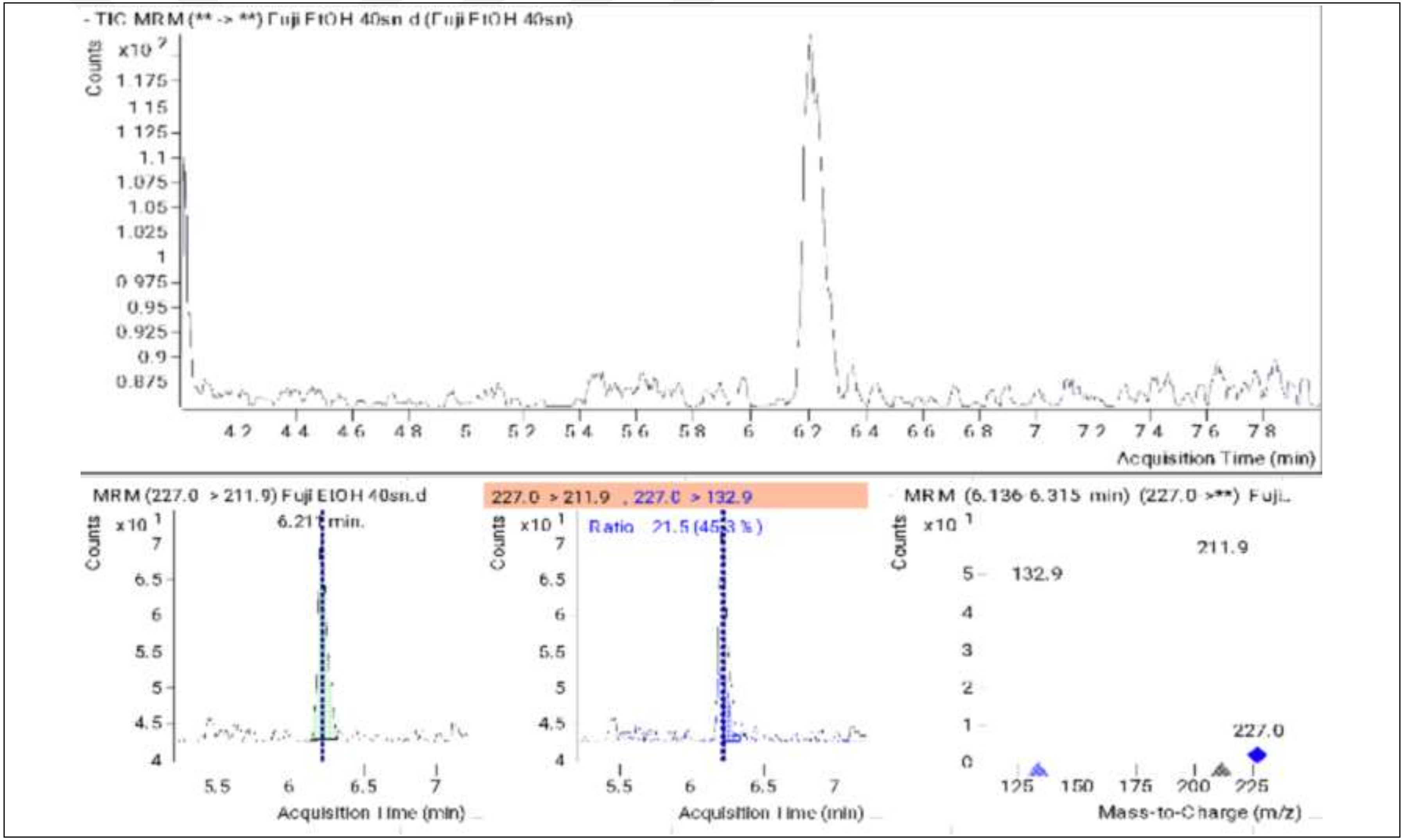

Figure 36: Chromatograph of Fuji Triage immersed in EtOH after 24 h and 40 s polymerization time

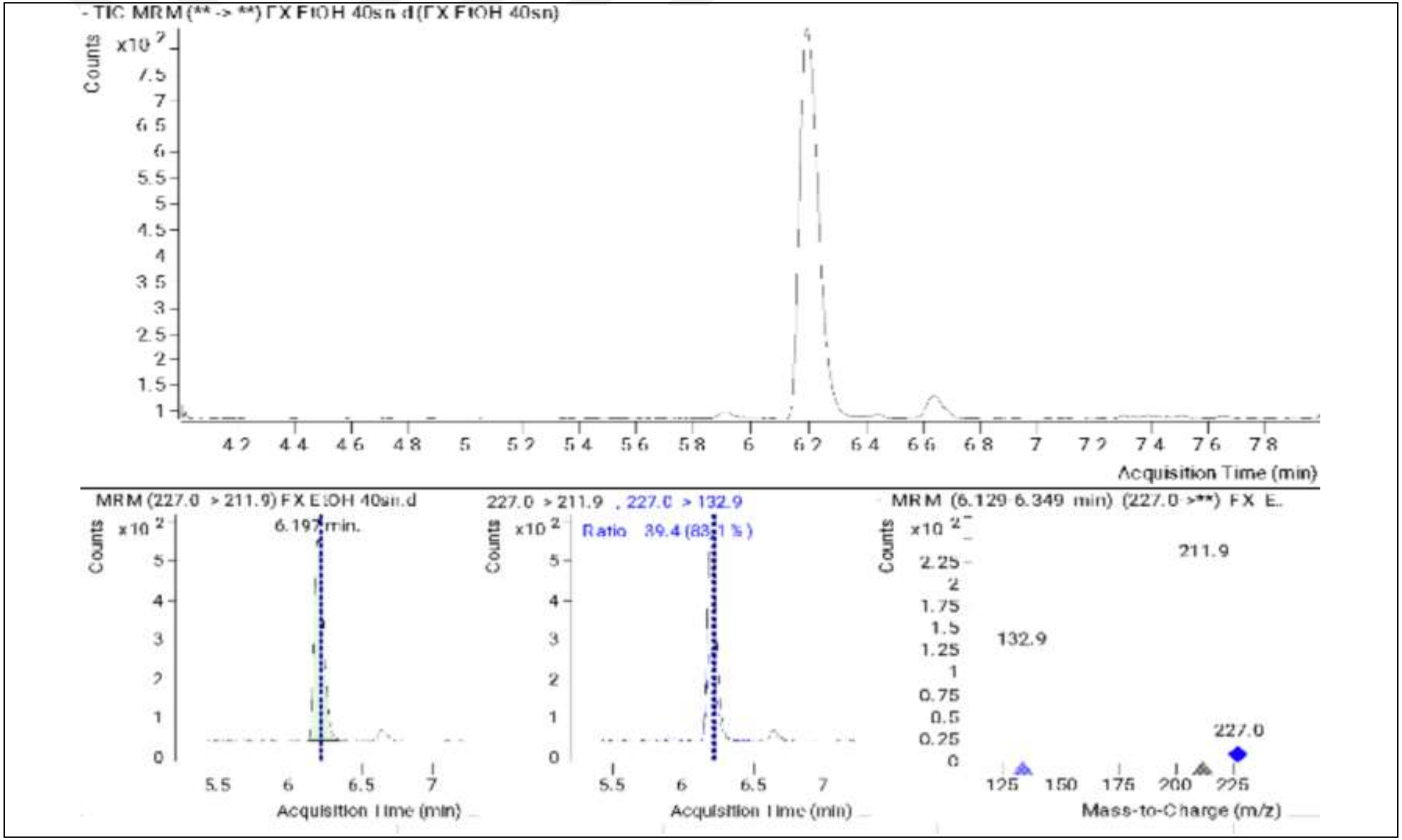

Figure 37: Chromatograph of Fissurit FX immersed in EtOH after 24 h and 40 s polymerization time

Immersed in EtOH with polymerization time of 40 s: a statistically significant difference was found between S4 and BPA measurements between the groups ($p<0.05$). S4 chromatographs are shown in Figures 35, Figure 36, and Figure 37. The G1 (mean=14.5723) value was higher than G2 (mean=2.3208) ($p<0.05$) ($p=0.000$), G1 (mean=14.5723) was lower than G3 (mean=40.1852) ($p<0.05$) ($p=0.000$), and G2 (mean=2.3208) was lower than the G3 (mean=40.1852) ($p<0.05$) ($p=0.000$).

Table 12 shows the mean values of the release of fissure sealants at different polymerization times and in different solutions after 24 hours. With 20 s of polymerization of artificial saliva, the groups ranked as G1> G3> G2 from highest to lowest BPA release. Clinpro™, released significantly more BPA than the other groups ($p=0.000$). With 40 s of polymerization time, the ranking was G3> G1> G2. Fissurit FX released significantly more BPA than the others ($p=0.000$). With 20 s of polymerization in EtOH, the ranking was G3> G1> G2. Fissurit FX released significantly more BPA than the others groups ($p=0.000$). With 40 s of polymerization, groups ranked as G3> G1> G2. Fissurit FX released significantly more BPA than the others groups ($p=0.000$).

Table 10: Comparison of BPA (ng/mL) released by Clinpro™ after 24 h with different polymerization times and different solutions

G1	Mean	N	Sd.	p
S1	7.4435	16	0.0006	**0.000***
S2	3.3163	16	0.0004	
S3	17.8223	16	0.0000	**0.000***
S4	14.5723	16	0.0004	

G1: Clinpro™; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s; ng/mL: nanogram per microliter
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Wilcoxon test

Wilcoxon test was used to determine whether there was a statistically significant difference between subgroup 1 (S1), S2, S3, and S4. BPA release values in G1 are given in Table 13. A statistically significant decrease was observed between S1 and S2 BPA release values ($p<0.05$). A statistically significant decrease was observed between S3 BPA and S4 BPA release values ($p<0.05$).

Table 13 shows the mean values of the release of Clinpro™ after 24 h for different polymerization times. The BPA release values were ranked as S1>S2 and S3>S4 and a highly significant difference was found between the groups (p=0.000). Clinpro™ was found to release less BPA in artificial saliva when polymerized for 40 s.

Table 11: Comparison of BPA (ng/mL) release by Fuji Triage after 24 h with different polymerization times and different solutions

G2	Mean	N	Sd.	p
S1	1.8416	16	0.0004	**0.000***
S2	1.6714	16	0.0004	
S3	12.1223	16	0.0000	**0.000***
S4	2.3208	16	0.0004	

G2: Fuji Triage; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s; ng/mL: nanogram per microliter
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Wilcoxon test

Wilcoxon test was used to determine whether there was a statistically significant difference between S1, S2, S3, and S4. BPA release values in G2 are given in Table 14. A statistically significant decrease was observed between S1 BPA release values and saliva S2 BPA releasing values (p<0.05). A statistically significant decrease was observed between S3 and S4 BPA releasing values (p<0.05).

Table 14 shows the mean values of BPA release of Fuji Triage after 24 h with different polymerization times. The subgroups were ranked as S1>S2 and S3>S4. A highly significant difference was found between the groups (p=0.000). Fuji Triage was found to release less BPA in artificial saliva when polymerized for 40 s.

Table 12: Comparison of BPA (ng/mL) release by Fissurit FX after 24h in different polymerization times and different solutions

G3	Mean	N	Sd.	p
S1	3.8877	16	0.0003	**0.000***
S2	4.3840	16	0.0008	
S3	41.8348	16	0.0000	**0.000***
S4	40.1852	16	0.0006	

G3: Fissurit FX; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s; ng/mL: nanogram per microliter
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Wilcoxon test

BPA release values in G3 are shown in Table 15. A statistically significant increase was observed between S1 BPA and S2 BPA release values ($p<0.05$). A statistically significant decrease was observed between S3 BPA and S4 BPA release values ($p<0.05$).

Table 15 shows the mean values of BPA release from Fissurit FX after 24 h at different polymerization times. BPA release was S2>S1 and S3>S4. A highly significant difference was found between the groups ($p=0.000$). Fissurit FX was found to release less BPA in artificial saliva when polymerized for 20 s.

Table 13: Comparison of peak values (ng/mL) of the groups in different solutions and with different polymerization times

Peak Value		N	Mean	Sd.	p
S1	G1	16	429	0.00	**0.000***
	G2	16	94	0.00	
	G3	16	216	0.00	
S2	G1	16	182	0.00	**0.000***
	G2	16	83	0.00	
	G3	16	246	0.00	
S3	G1	16	1050	0.00	**0.000***
	G2	16	709	0.00	
	G3	16	2486	0.00	
S4	G1	16	855	0.00	**0.000***
	G2	16	122	0.00	
	G3	16	2387	0.00	

G1: Clinpro™, G2: Fuji Triage, G3: Fissurit FX; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s; ng/mL: nanogram per microliter
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Kruskal–Wallis test, Mann–Whitney U test

Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of S1 peak values are shown in Table 16. A statistically significant difference was found between the peak values of S1 and the other groups ($p<0.05$). Mann–Whitney U test was used to determine which groups were involved in the difference. For S1 peak measurement, G1 (mean=429) higher than G2 (mean=94) ($p<0.05$) ($p=0.000$), G1 (mean=429) was higher than G3 (mean=216) ($p<0.05$) ($p=0.000$), and G2 (mean=94) was lower than G3 (mean=216) ($p<0.05$) ($p=0.000$).

Kruskal–Wallis test showed a statistically significant difference between S2 peak values between groups ($p<0.05$). Mann–Whitney U test was used to determine which groups were involved in the difference. For S2 peak measurements, G1 (mean=182) was higher than G2 (mean=83) ($p<0.05$) ($p=0.000$), G1 (mean=182) was lower than G3 (mean=246) ($p<0.05$) ($p=0.000$), and G2 (mean=83) was lower than G3 (mean=246) ($p<0.05$) ($p=0.000$).

For S3 peak values, a statistically significant difference was found between S3 and the other groups ($p<0.05$). For S3 peak measurements, G1 (mean=1050) was higher than G2 (mean=709) ($p<0.05$) ($p=0.000$), G1 (mean=1050) was lower than G3 (mean=2486) ($p<0.05$) ($p=0.000$), and G2 (mean=709) was lower than G3 (mean=2486) ($p<0.05$) ($p=0.000$).

For S4 peak values, a statistically significant difference was found between S4 and the other groups ($p<0.05$). For S4 peak measurements, the G1 (mean=855) was higher than G2 (mean=122) ($p<0.05$) ($p=0.000$), G1 (mean=855) was lower than G3 (mean=2387) ($p<0.05$) ($p=0.000$), and G2 (mean=122) was lower than G3 (mean=2387) ($p<0.05$) ($p=0.000$).

Table 16 shows the peak values of the fissure sealant materials when introduced into the LC/MS device. For S1, the peak values were measured as G1> G3> G2 and a highly significant difference was found between the groups ($p=0.000$). For S2, G3> G1> G2 and a highly significant difference was found between the groups ($p=0.000$). In S3, the measurements ranked G3> G1> G2 and a highly significant difference was found between the groups ($p=0.000$). For S4, the ranks were G3> G1> G2 and a highly significant difference was found between the groups ($p=0.000$). The highest peak value was measured for Fissurit FX with polymerized in EtOH for 20 s and the lowest value was measured when Fuji Triage was polymerized in artificial saliva for 40 s.

Table 14: Comparison of peak times (min) of the groups in different solutions and different polymerization times

Peak time		N	**Mean**	**Sd.**	**p**
S1	G1	16	6.2180	0.0000	**0.000***
	G2	16	6.2110	0.0000	
	G3	16	6.2040	0.0000	
S2	G1	16	6.2140	0.0000	**0.000***
	G2	16	6.2010	0.0000	
	G3	16	6.2040	0.0000	
S3	G1	16	6.2070	0.0000	**0.000***
	G2	16	6.2010	0.0000	
	G3	16	6.1970	0.0000	
S4	G1	16	6.2110	0.0000	**0.000***
	G2	16	6.2110	0.0000	
	G3	16	6.1970	0.0000	

G1: Clinpro™, G2: Fuji Triage, G3: Fissurit FX; S1: Immersed in artificial saliva; polymerization time 20 s, S2: Immersed in artificial saliva; polymerization time 40 s, S3: Immersed in EtOH; polymerization time 20 s, S4: Immersed in EtOH; polymerization time 40 s
Min: minute
*p<0,05, p=0,000 highly significant, N: number, Sd: Standard, p: Kruskal–Wallis test, Mann–Whitney U test

Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of S1 peak times. A statistically significant difference was found between the peak times of S1 between groups (p<0.05) S1 peak times are shown in Table 17.

Mann–Whitney U test was used to determine which groups were involved in the difference. For S1 peak measurements, G1 (mean=6.2180) was higher than G2 (mean=6.2110) (p<0.05) (p=0.000), G1 (mean=6,2180) was higher than G3 (mean=6,2040) (p<0,05) (p=0,000), and G2 (mean=6,2110) was higher than G3 (mean=6,2040) (p<0,05) (p=0,000).

Kruskal–Wallis test was used to determine whether there was a statistically significant difference between the intergroup measurements of S2 peak times. A statistically significant difference was found between the peak times of S2 between groups (p<0.05).

Mann–Whitney U test showed that G1 (mean=6.2140) was higher than G2 (mean=6.2010) (p<0.05) (p=0.000), G1 (mean=6.2140) was higher than G3 (mean=6.2040) (p<0.05) (p=0.000), and G2 (mean=6.2010) was lower than G3 (mean=6.2040) (p<0.05) (p=0.000).

Kruskal–Wallis test showed a statistically significant difference in peak times of S3 between groups (p<0.05). The Mann–Whitney U test was used to determine which groups were involved in the difference. For S3 peak measurements, G1 (mean=6.2070) was higher than G2 (mean=6.2010) (p<0.05), G1 (mean=6.2070) was higher than G3 (mean=6.1970) (p<0.05), and G2 (mean=6.2010) was higher than G3 (mean=6.1970) (p<0.05) (p=0.000).

Kruskal–Wallis test showed a statistically significant difference in the peak times of S4 between groups (p<0.05). For S3 peak measurement, G1 (mean=6.2140) was higher than G3 (mean=6.1970) (p<0.05) (p=0.000), and G2 (mean=6.2010) was higher than G3 (mean=6.1970) (p<0.05) (p=0.000).

Table 17 shows the peak times of fissure sealants. Measurements were taken from the sixth minute in all groups. In S1, peak times were measured as G1> G2> G3 and a highly significant difference was found between the groups (p=0.000). In S2, G1> G3> G2 and a highly significant difference was found between the groups (p=0.000). In S3, G1> G2> G3 and a highly significant difference was found between the groups (p=0.000). In S4, G1> G3 and G2> G3 and no significant difference was found between G2 and G1, but a highly significant difference was found between the other groups (p=0.000).

5. DISCUSSION

The null hypothesis that there was no statistically significant difference between the BPA values in the fissure sealants was rejected. The alternative hypothesis that there was a statistically significant difference between the BPA release among the fissure sealants was accepted considering the results of this study.

BPA is a common commercial chemical, which is largely used in the manufacture of plastic goods, and its use is on the rise. Production in Asia and the USA has risen by 13% yearly. In an effort to increase awareness, this increase in use has raised worries and prompted greater research on BPA (211).

Previous researchers have found daily BPA exposure to be less than 50 μg/kg bodyweight/day, despite the EPA's recommended limit for safe substances (160). However, in 2023, the EFSA and the EPA computed a tentative tolerable daily intake (TDI) for BPA, which was reduced from 50 μg/kg/day to 4–0.2 μg/kg/day. Resin-based fissure sealants have BPA release a lot lower than this above-mentioned value (166).

BPA use has been identified in dental products since resin is a component of composites and sealants. Pit and fissure sealants are now widely used because pediatric preventive care is becoming more important and common. Due to this, in this study the composition and biocompatibility of the newly produced fissure sealant materials were investigated. The use of resin-containing light-curing pit and fissure sealants is relatively prevalent among the sealant materials available on the market. These substances might fail to fully polymerize and facilitate the monomer-to-polymer conversion if inadequate polymerization occurs after application, releasing excess monomers into the oral environment. BPA is an especially well-known residual monomer among these monomers, and it is well recognized that when BPA is released into the oral cavity, it can have detrimental biological effects (135).

Fissure sealants made of resin also have a high concentration of monomers, a minimal amount of filler particles, and a resin matrix. According to reports, when the material's filler content drops, the total amount of monomers will rise (115). Fissure sealant materials with different filler ratios are offered in the market.

Fissurit FX, Clinpro™ and Fuji Triage are among the various fissure sealant materials used at the Yeditepe University Faculty of Dentistry Department of Pediatric Dentistry. In this study, Fissurit FX fissure sealant was the preferred material due to its resin content and the highest filler rate. Kucukyilmaz et al. (100) claimed that Clinpro™ had a lower filler rate with resin content and also released fluoride, and Fuji Triage that is mainly GI-based without resin content claims to be BPA-free.

The solutions where the fissure sealant disks were immersed to assess BPA release were artificial saliva and EtOH in this study. Artificial saliva was preferred as it is a more representative model of oral conditions. Since artificial saliva has not been previously preferred for in vitro conditions, EtOH was also used to analyze the experimental results. The reason for selecting EtOH solution was that BPA is soluble in EtOH under in vitro conditions (160). Artificial saliva was preferred because it is closer to oral conditions with its enzyme and cytokine content, and dilution with EtOH was applied before analysis in the LC/MC devices.

Nowadays, halogen and LED light sources are most commonly used for the polymerization of resin-based fissure sealants. Halogen bulbs have a limited working life of approximately 40 to 100 hours and the bulb, reflector, and filter equipment of these systems can deteriorate over time due to high operating temperatures and subsequent heat generation (214). In addition, LED light sources have the advantages of having short polymerization times and not generating heat during this process. In this study, an LED light source (Bluephase G4, Ivoclar, Vivadent, Liechtenstein) in standard power mode was used considering all of its positive features. All light-polymerized pit and fissure sealants were polymerized with the same light source to standardize the negative effects that could arise from the polymerization source.

As the quantity of filler inorganics in dental materials increase, the amount of polymerization of monomers is expected to decrease (115). At the same time, as more monomers are polymerized for a longer period of time, BPA release is expected to decrease, but whether the decrease in the amount of BPA released can create a significant difference, is a controversial issue. Therefore, the polymerization times were tested at 20 s and 40 s to determine whether complete monomer to polymer polymerization would improve with increased polymerization time. However, there is a recommended polymerization time determined by the manufacturers for each fissure sealant. Polymerization time of 10 s with LED light is sufficient for Clinpro™ according to the manufacturer’s instruction. In this study, it was found that less BPA was released despite 40 s polymerization in all groups. The manufacturer’s instruction for Fissurit FX material is 20 s polymerization and it was found that less BPA was released from 20 s polymerization than 40 s polymerization in artificial saliva. The manufacturer’s instruction for Fuji Triage is 20 s of polymerization, but it was found to release less BPA at 40 s of polymerization in all groups.

Liquid Chromatography/Mass Spectrometry (LC/MS) is a technique that separates the components of a liquid mixture on their various interactions with different phases. LC is used in BPA release assessment because it can detect the amount of BPA. LC can also be coupled with MS to provide more accurate, sensitive identification, and measurement of BPA. Since LC works in the liquid phase, but MS is a gas-phase method, connecting the two requires specific expertise. An interface is needed to allow the separated components of a mixture to pass sequentially from LC to MS. LC/MS interfacing between the two is a very powerful technique (178). Most of the studies of BPA have been conducted with HPLC (178). LC/MS is used instead of HPLC when the samples requires both separation and identification, or when the sample is not compatible with high pressure. LC/MS can provide more accurate, sensitive, and selective detection of compounds than HPLC alone (205). The fact that most of the studies in this field have been conducted without LC/MS suggests the need for further research with this method. LC/MS is a superior device compared with HPLC and gives more detailed results. Therefore, LC/MS was used in this study to obtain more detailed results.

In vitro conditions were preferred in this study because it was difficult to standardize the steps of the procedures that will apply to the patient. At the same time, considering that BPA is present in everything plastic-based that is used in general life, it was taken into consideration that its background elimination would also be difficult.

Pulgar et al. (129) tested several resin containing fissure sealant materials and used HPLC and GC/MS devices to measure BPA release. After 24 hours, they concluded that there was BPA released from the fissure sealants in their study. The release of BPA was found to be statistically significantly different. Since the study was not a quantitative analysis, the amount of BPA release was not specified. In this study, BPA release was examined after 24 hours in all groups. Since quantitative analysis was performed, the amount of BPA released from the fissure sealants was recorded. Although, the fissure sealants used in this study were not the same as the ones tested by Pulgar et al. in this study statistically significantly different results were also obtained. However, as quantitative analysis was not performed in their study, the results could not be compared in terms of numerical values. In this study, LC/MS that takes more sensitive measurements and has more advanced technology, was used.

Geurtsen et al. (200) analyzed four different fissure sealants. They created 5 mm diameter fissure sealant discs. These discs were polymerized with an Elipar II (3M ESPE, St. Paul, MN, USA) light source. The polymerized discs were stored in 5 mL distilled water for 24 h. No BPA release was detected with GC/MS after 24 h from Fissurit FX. In this study, Fissurit FX fissure sealant disks with a diameter of 10x2 mm were created. Bluephase G4 (Ivoclar, Vivadent, Liechtenstein) was used for the polymerization of these discs and they were immersed in EtOH or artificial saliva for 24 h. In this study, BPA release after 24 h was found with Fissurit FX detected with LC/MS. The results between this study and Geurtsen et al. may have been varied due to the different methods employed during the experiments. However, this difference might also be due to the detection equipment used since it is a sensitive device that can detect even the lowest amounts of BPA and its isomers.

Ulu Güzel et al. (115) state that residual monomers were released from the fissure sealant materials after 24 h and showed the release of BPA from Fissurit FX, under in vitro conditions where discs were immersed in ethanol for 24 h and were polymerized for 20 s and 40 s. The discs were prepared as 10x1 in diameter, used LED lights (VALO Cordless, Ultradent, USA) for polymerization, and the analysis was performed with HPLC. After 24 h, an average of 20.47 ppm of BPA was released with 20 s of polymerization from Fissurit FX, while 19.80 ppm was released with 40 s of polymerization. A significant difference was found between the two polymerization times. In this study, it was detected that 41.83 ppm of BPA was released with 20 s of polymerization, while 40.18 ppm after 40 s of polymerization. The numerical differences might be due to the different devices used for measurements. The different LED devices used might also have caused numerical differences. Since the size of the discs might have different masses in mg, numerical differences could have occurred. However, both studies found statistically significant differences in BPA release.

Arenhovlt et al. (170) collected saliva samples from patients at 1 h and 24 h after the application of two different brands of fissure sealants, and examined BPA release. Their study was conducted under in vivo conditions. They used HPLC to analyze the saliva samples and BPA release after 24 h and, it was found that there was no BPA release from the two different fissure sealants. In this study, artificial saliva was used instead of human saliva because the study was conducted under in vitro conditions. BPA release after 1 h could not be examined because there was a travel distance of more than 1 h between two laboratories where the experiments and the analyses were performed. After 24 h, BPA release was detected. Furthermore, it is necessary to develop a methodology for the detection of BPA with LC/MS and to make calibration adjustments. Different results can be recorded due to variations in these methodologies. In addition, the use of artificial saliva in this study could have caused different results, although it can be argued that artificial saliva would be more consistent for experiments.

Joskow et al. (171) tested different sealant brands: Helioseal F (Ivoclar Vivadent, Liechtenstein) and Delton Light Cure Opaque (Dentsply, York, USA). Salivary samples were taken 24 h, 1 h, and immediately after treatment and measured with GC/MS. There was no BPA release in saliva 24 h after the procedure. In this study, BPA release in artificial saliva was also examined after 24 h. The reason for the different results in the studies might be due to the different materials used, the difference in the calibration of the analyzers, or the fact that the studies were performed under different conditions, such as in vivo versus in vitro. As before, the analyzer machine methodology and calibration adjustments might have given differing results.

Jo et al. (180) evaluated the BPA release from Clinpro™ fissure sealant in three different timepoints: 10 min, 1 h, and 24 h and at three different pH levels. The highest BPA release was found in acidic conditions at all timepoints, and the release after 24 h was found to be 5.5 times higher than the other timepoints. In the study, the BPA release from Clinpro™ fissure sealant in pH neutral conditions after 24 h was found to be 620 ng/mL. In this study, ethanol and artificial saliva were used and both were neutralized to pH 7. It was detected that BPA release occurred only in a neutral environment. Although acidic pHs were not investigated in this study, when compared with the neutral pH data by Jo et al, similar findings were observed for BPA release from Clinpro™ after 24 hours in this study. The Clinpro™ values found in artificial saliva were 7.4435 and 3.3163 ng/mL, and the values in ethanol were 17.8223 and 14.5723 ng/mL, respectively. No direct comparison with respect to polymerization time could be made between the studies since there was no information on polymerization time in Jo et al.'s study. BPA release was also found statistically significantly different between groups in this study, but the numerical differences found might be due to the differences in the device measurements or solutions.

Eun-Deok Jo (215), tested Fissurit FX in acidic, neutral, and basic environments and BPA release was analyzed with LC/MS. According to his study, BPA release was observed in all three different pH levels and there was a statistically significant difference among the groups. In this study, it was found that Fissurit FX released BPA in LC/MS analysis at pH 7 that was a statistically significantly difference in the two different solution media. These similarities might be due to similar pH conditions or the similar devices measuring BPA release.

Downs et al. (208) analyzed BPA release in their in vivo study using the Ecologiana supersensitive BPA ELISA kit. In order to quantify BPA in biological materials like serum and plasma, Downs et al., developed a competitive ELISA assay. In this study, however BPA release was investigated under in vitro conditions, but there were prolonged difficulties in experimental studies, such as placing the samples in the tube, taking the samples from the tube, or it could be difficult to find someone who with the appropriate qualifications to use the machine. At the same time, since LC/MS analysis is costly, therefore, developed kits are an alternative. Despite these disadvantages of LC/MC, the reliability of these kits is questionable so in this study it was preferred LC/MS analysis, which is the most reliable method. LC/MS analysis is the most reliable technique used to measure BPA release. Even though different analysis method were used in the above-mentioned study and this in vitro study similar results were obtained which can show the accuracy of the methodology used in this study.

Polydorou et al. (178) investigated two resin composite materials: a nanohybrid Filtek Supreme XT and a chemically cured Clearfil Core resin composite. Halogen unit Elipar Highlight was used and the samples were polymerized for 20 s and 40 s. Specimens were 4.5 mm in diameter and 2 mm thick. Each sample was immediately immersed in 1 ml of 75% ethanol and LC/MS was used for analysis. There was no significant difference between samples polymerized for 20 s compared with 40 s. In this study, Fuji Triage, Fissurit FX, and Clinpro™ dental sealants were used, and Bluephase G4 the light source to polymerize at 20 s and 40 s. Specimens were 10 mm diameter and 2 mm thick. Each sample was immediately immersed in 5 mL 96% ethanol or artificial saliva. In this study, there were statistically significant differences between the polymerization times. This difference might be due to differences in the materials used, differences in the polymerization sources, or differences in the solutions.

Berge et al. (209) tested BPA release in dental sealants after Satelec Mini LED light emission rate of 600–700 mW/cm^2, polymerized for 20 s. BPA was analyzed with LC/MS after 24 h and there was BPA release found. In this study, Bluephase G4 was used for polymerization with emission rate of 600–700 mWcm^2. The samples were polymerized for 20 s and immersed in artificial saliva, then analyzed with LC/MS 24 h later where it was observed that BPA was still released. Although the polymerization devices and dental sealant brands used were different, BPA was released after 24 h in both studies. The similarities in the results of both studies might be due to the comparable devices used.

Becher et al. (203) tested Clinpro™ sealant on discs that were 5 mm in diameter and 0.5 mm in width, and 0.5 ml of sealant was placed inside each disc. Ivoclar Vivadent 20i was used as the polymerization device for 20 s. In the study, only methanol was used in the samples and they stored the samples at -18°C until the day of analysis with UPLC-MS-MS. They detected BPA release from the Clinpro™ sealant after 24 h, but no statistically significant difference was found among groups. In this study, discs were 10 mm wide and 2 mm thick, 0.12 mL of sealant was placed on the discs, and Bluephase G4 used as the light source. In this study, two different solutions were used: ethanol and artificial saliva and samples were stored at -20°C until the day of analysis with LC/MS. The observed BPA release from Clinpro™ after 24 h was statistically significantly different in all groups. Similar results were seen in both of these studies that can be attributed to methodology of the experiments.

Tabatabaee et al. (213) reported that the amount of residual monomer released from nanohybrid composite, flowable composite, and the flowable composite with low filler content caused more residual monomer release. In this study, only BPA release only from the residual monomers was investigated. In this study, in accordance with Tabatabaee et al., low filler containing Clinpro™ showed more residual monomer release than Fissurit FX with its high filler content. Even if the materials used were not the same, they all contain resin and similar results were obtained. When materials with different filler rates were compared, among the samples immersed in artificial saliva with 20 s polymerization (S1), Clinpro™ released the highest amount of BPA than other experiment groups. Although different materials were assessed in both these studies, the amount of release decreased in both studies as the filler content of the material increased.

Batiano et al. (207) used GC/MS analysis for analyzing BPA release. The calibration curve and response factor were computed with reference BPA at different concentrations from 0.01 to 50 μg/mL. Capillary column is a type of chromatographic column that was 300 mm in length with an internal diameter of 320 μm, and a flow rate of 1.2 mL per min. There was BPA release found. In this study, BPA was measured at different concentrations ranging from 50 and 250 μg/L. The capillary column was length of 150 mm, internal diameter of 350 μm, and flow rate of 0.6 mL/min. There was also BPA release found.

Kang et al. (210) used LC/MS to determine the release of BPA and a Zorbax Eclipse XDB-C18 column (particle size, 5 mm; 3x150 mm, Agilent) was used. The mobile phase was at a 60:40 volume/volume mixture of 0.1% acetic acid and acetonitrile. The flow rate was 0.7 mL per millimeter. The peak was detected in the selected ion monitoring mode, and the electrospray positive mode was used for ionization. Nitrogen gas was used as the nebulizing gas at a nebulizing pressure of 40 psi with a nebulizing gas flow rate of 12 L per min, a gas temperature of 300°C, and capillary voltage of 3500 V. In their results, BPA release was observed after 24 h, but was not statistically significant. In this study, LC/MS was used to determine the release of BPA. The mobile phase was at 70:30 volume/volume mixture of 0.1% acetic acid and acetonitrile 0.3 mL per millimeter, 45 psi with nebulizing gas flow of 10 L per minute, gas temperature of 300°C, and capillary voltage of 3500 V. In comparison, in this study, BPA release was found to be statistically significant. Although the same instruments were used for the analysis, the results could have been different due to the machine settings used.

Limitations:

- The pre-existing BPA in the environment or on the surfaces it came into contact with during the creation of fissure sealant discs may have influenced the recorded BPA values.
- The experiment and analysis were conducted at different locations, with a significant distance between them.
- The samples were stored frozen, and dissolution may have occurred during the transportation of the discs.
- There is no standardized protocol for calibrating the LC/MS device, leading each researcher to develop their own methodology and calibration.
- LC/MS results may vary based on the expertise of the individual performing the analysis.
- Although various fissure sealants are available on the market, only three could be utilized in this study.

6. CONCLUSION

Three different fissure sealants were polymerized at 20 s and 40 s and the release of BPA after 24 h from discs immersed in artificial saliva and in EtOH were examined. In this study, it was observed that:

- Fuji Triage is a GI-based fissure sealant that does not contain resin, and was the least BPA releasing fissure sealant.
- BPA was still released after 24 h in all groups.
- In all groups, BPA release was recorded at the sixth minute after the measurement was started.
- The difference between the groups in terms of total BPA release was found to be highly statistically significant ($p=0.000$).
- The highest BPA release was determined to be from Fissurit FX fissure sealant (S3) ($p=0.000$) (41.8348 ng/mL).
- Clinpro™ fissure sealants were found to release the most BPA in artificial saliva.
- Fissurit FX fissure sealant, which has the highest filler rate, released less BPA than Clinpro™ fissure sealant, which has a lower filler rate, after 20 s polymerizations and immersion in artificial saliva.
- BPA from Fuji Triage and Clinpro™ in artificial saliva with a polymerization time of 40 s (S2,S4) was found to be less than that from the 20 s (S1,S3) group.
- BPA from Fissurit FX was found to be released less with 20 s polymerization when immersed in artificial saliva.
- Resin-free Fuji Triage fissure sealant material was the most reliable material because it released the least BPA in all groups immersed in artificial saliva.
- The order of preference for clinical conditions in terms of BPA release are Fuji Triage, Fissurit FX and Clinpro™.
- In this study, the amount of BPA release after 24 h was investigated and future studies could be conducted at different timepoints.

In conclusion, BPA release from contemporary dental materials, including those used in fissure sealant application, poses systemic risks. While BPA's adverse effects have garnered attention, further research is imperative. Exploring alternative sealant materials with safer compositions is recommended for mitigating these risks. Continued investigation into commercially available options will benefit both patients and practitioners. With advancing technology, the development of BPA-reducing materials is anticipated. Additionally, *in vivo* studies should complement *in vitro* research for BPA release from dental materials.

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8. CURRICULUM VITAE

Personal Informations

Name	Gülfem	**Surname**	Çavuşoğlu Çarpar

Education

Degree	**Department**	**Graduated Institution**	**Graduation year**
University	Dentistry	**Marmara University**	2019
High school	-	**Bartın Anadolu Öğretmen Lisesi**	2013

Languages	**Grades (#)**
Tıp/Dil	85

Computer Skills

Program	**Level**
Microsoft Word	Good
Excel	Good

***Excellent , good, average or basic**

Meetings Attended

Association of Turkish Pediatric Dentistry Congress, 2021
International Association of Pediatric Dentistry Congress, 2023