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RESEARCH ARTICLE

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Qualitative and quantitative analysis of leachables from dental composites under different extraction conditions using liquid chromatography coupled to mass spectrometry

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Abstract

Dental caries is the most widespread form of disease, affecting over 90% of the global population. Amalgam fillings, which have been in use for nearly two centuries, will face an European Union ban by 2025. Although photocomposite fillings are a suitable alternative, health concerns persist because of potential substance release into the oral cavity. This study aimed to evaluate the release of photoinitiator substances and monomers from dental materials into various solvents at different temperatures over 30 days. Cylindrical specimens of the composite resins were submerged in different extraction solutions and incubated at 37 and 50°C. The findings demonstrated that both the extraction solvent and extraction temperature significantly influenced the quantity of leachables ($p < 0.05$). Furthermore, most leachables were released within the initial days, although some monomers continued to elute for over 30 days. The estimated daily intake was calculated for the worst-case scenario, confirming the biocompatibility of the composite fillings. The weight loss of dental materials ranged up to 3.5% after 30 days, regardless of the extraction conditions and dental material ($p > 0.05$). In conclusion, this study contributes to filling several research gaps in the field by addressing the biocompatibility of various dental materials through quantitative and qualitative analyses supported by statistical evaluation.

KEYWORDS

cumulative extraction, dental composites, leachables, liquid chromatography, mass spectrometry, monomers

1 | INTRODUCTION

Dental caries is the most widespread form of disease, impacting over 90% of the global population. The harmful effects of dental caries, which lead to the erosion or decay of hard dental tissue, are progressive, with the inherent regenerative abilities of dental tissues being notably limited. Consequently, the application of

restorative materials becomes indispensable for the repair of damaged, esthetically compromised, or functionally impaired teeth.[1,2]

Amalgam, composed of 45–50 wt% mercury, has been a widely used restorative material for nearly 200 years because of its affordability, ease of application, and durability.[3] An amalgam phase-out is presently in progress, driven by the European Union's intention to enforce a total ban on the use of amalgam fillings by 2025. Photo-composite fillings emerge as a fitting and progressive alternative to amalgam, boasting numerous advantages: devoid of metal where it is not intended to be, absent of mercury, offering higher aesthetics, minimal invasiveness by virtue of not requiring undercut preparation, which removes significant healthy hard dental tissue, and possessing mechanical properties closely resembling those of original hard dental tissues.[4]

Composite dental materials are created by blending two different components: a continuous phase (polymer matrix) and a discontinuous phase (inorganic filler and reinforcement). The filler and reinforcement constitute up to 80% of the composite material, and their impact on the composite's properties is determined by factors such as material composition, morphology, size distribution, and concentration within the matrix.[5,6] In dental applications, polymer matrices are predominantly composed of acrylic resins, particularly methacrylate-based monomers such as 2-hydroxyethyl methacrylate (HEMA) monomer and 2-(dimethylamino)ethyl methacrylate (DMAEMA) co-initiator, or more commonly, dimethacrylate-based monomers including triethylene glycol dimethacrylate (TEGDMA), diurethane dimethacrylate (UDMA), bisphenol A-glycidyl methacrylate (BisGMA), and ethoxylated bisphenol A dimethacrylate (BisEMA). These monomers can create a highly crosslinked three-dimensional polymer network, resulting in enhanced properties.[7] Dental composites are typically created through the radical polymerization of monomers in conjunction with ceramic particles, a process commonly initiated by light. Presently, the predominant photoinitiator system employed consists of a camphorquinone (CQ) photoinitiator paired with the organic aliphatic tertiary amine co-initiator DMAEMA.[8,9]

Following polymerization, not all the double bonds of the monomers undergo reaction, because the flexibility of the polymer network diminishes during crosslinking.[8] As a result, concerns arise regarding the biocompatibility of these photo-composites, supported by several scientific studies,[10–25] which have demonstrated the release of potentially harmful substances (such as unreacted monomers, degradation products, and oligomers) through various mechanisms (including diffusion, osmotic effect, erosion, and degradation of the polymer matrix). The release of those substances may compromise the structural stability and biocompatibility of dental fillings.

Although, studies have utilized different extraction solvents in their in vitro evaluations of dental materials, including water,[11,12] artificial saliva,[11–13,24] or 70%–100% EtOH,[10–14,22] the collected data were not often subjected to statistical analysis to comprehensively assess the differences. Despite the varying temperatures experienced by dental fillings during their lifespan in the oral cavity, existing studies have typically conducted elution experiments at a single temperature, either room temperature or 37°C; thus, the influence of temperature has not been thoroughly investigated. Elution studies typically range from minutes[10] to several months,[11,12] covering the entire lifespan of their usage. While various analytical techniques, including LC-DAD,[10,22,26] LC-MS,[11,12,14–16,21,25,27–29] and GC-MS,[13,21,23] have been employed in available in vitro studies, it is important to note that these methods have limitations, and their reporting is often inadequate, with few exceptions. Additionally, studies have primarily focused on monomers listed in material safety data sheets (MSDS), neglecting substances from the photoinitiator system or other monomers not mentioned in MSDS. Therefore, these studies have quantified only a limited amount of compounds. Furthermore, dental composite fillings can release degradation products or oligomers of monomers, which have not been comprehensively investigated in the available literature.[10–25]

To assess dental materials that have not yet been explored in existing literature, four dental materials from two manufacturers were selected for evaluation. Both quantitative and qualitative analyses of leachables from dental composites were conducted using LC-ESI-TQ (liquid chromatography coupled to triple quadrupole with electrospray ionisation) and LC-ESI-IT (liquid chromatography coupled to ion trap with electrospray ionisation). The novelty of this research lies in the aim of analyzing both monomers specified and unspecified in MSDS, and oligomers and compounds of the photoinitiator system. Subsequently, the prepared specimens were immersed in various extraction media (H_2O, PBS, 100% EtOH) at different extraction temperatures (37 and 50°C) for up to 30 days. The obtained data were subjected to statistical evaluation to assess the influence of extraction solvent, temperature, and duration. Additionally, to address a gap in previous scientific studies, the weight loss of dental materials during extraction experiments was evaluated. Furthermore, the estimated daily intake (EDI) for acute and chronic exposure was calculated to assess biocompatibility and potential health risks. Finally, a literature review of potential degradation products and impurities from dental materials was conducted.

2 | MATERIALS AND METHODS

2.1 | Chemicals and standards

The following chemicals: Disodium hydrogen phosphate dodecahydrate (≥99%), potassium chloride (>99.5%), and hydrochloric acid (35%) were purchased from Lach:ner (Czech Republic). Sodium chloride (>99%) and formic acid (LC-MS grade) were purchased from Sigma Aldrich (Germany). Sodium hydroxide (>98%) was purchased from Penta (Czech Republic). Ethanol (LC-MS grade), acetonitrile (ACN) (LC-MS grade), and water (LC-MS grade) were purchased from VWR (Czech Republic). Nitrogen gas (4.7) and argon gas (5.0) were purchased from SIAD Czech spol. s.r.o. (Czech Republic). Nylon syringe filters (13 mm, 0.22 μm) were purchased from Chromservis (Czech Republic).

The following standards with physicochemical properties detailed in Table S1 were used. DMAEMA (>99%) was purchased from ABCR GmbH (Germany). HEMA (>99%), CQ (>99%), TEGDMA (>99%), UDMA (>97%), BisGMA (>98%), and BisEMA (>98%) were purchased from ESSCHEM Europe (United Kingdom).

Individual stock standard solutions were prepared in ACN at a concentration of 1 $mg \cdot mL^{-1}$ for the target compounds. A standard solution mixture (concentration of 10 $\mu g \cdot mL^{-1}$) containing all the target compounds was then prepared in Milli-Q water. Working standard solutions were obtained by further diluting the standard solution mixture. All standard solutions were stored in glass vials and kept at −20°C in a freezer.

2.2 | Description of dental materials

In this study, we selected four dental materials: Fibrafill® DENTIN and Fibrafill® FLOW from ADM, a.s. and Ever X posterior and Ever X Flow from GC EUROPE N.V. These materials were chosen to address a current research gap because there are no existing studies evaluating the biocompatibility of these specific dental materials.

ADM, a.s. incorporates silane-treated silica as a filler, comprising a colloidal dispersion of SiO_2 particles. Additionally, the composite integrates glass-based bulk fillers with particle sizes ranging from hundreds of nanometers to units of micrometers. Inorganic microfibers serve as reinforcement, with both filler particles and reinforcement fibers coated with organosilane. The primary constituents in the resin matrix of Fibrafill® DENTIN, as per the safety data sheet (SDS), include BisEMA (<10%), UDMA (<10%), BisGMA (<5%), TEGDMA (<5%), HEMA (<1%), CQ (<1%), and DMAEMA (<1%). Similarly, Fibrafill® FLOW's resin matrix comprises BisGMA (<20%), UDMA (<10%), TEGDMA (<10%), BisEMA (<5%), HEMA (<5%), CQ (<1%), and DMAEMA (<1%), as indicated by the SDS.

GC EUROPE N.V. offers a diverse array of dental composites with varying compositions tailored to specific indications. However, both commercial composites analyzed by the company primarily utilize stained glass and SiO_2 particles as fillers. E-glass fibers serve as reinforcement, with both filler particles and reinforcement fibers coated with organosilane. According to the SDS, the major constituents of the resin matrix in EverX Posterior are BisGMA (10%–25%) and TEGDMA (5%–10%). Similarly, in EverX Flow, the resin matrix contains esterification products of BisEMA (10%–25%), UDMA (5%–10%), TEGDMA (2.5%–5%), and BHT (0.25%–0.5%), as specified in the SDS.

2.3 | Specimen fabrication

Cylindrical specimens were meticulously prepared by applying dental materials (Fibrafill® DENTIN, Fibrafill® FLOW, Ever X Flow, and Ever X Posterior) using appropriate dental instruments into lukopren molds to prevent the formation of air bubbles. Each cylindrical specimen hole in the mold measures approximately 6.60 mm in diameter and 1.80 mm in height. When the top sides of the specimen bodies were aligned with the top surface of the mold, the surface was covered with polyester foil to avoid the formation of an inhibited layer. Subsequently, the samples were cured using a dental curing light (Premium Plus™ C02 LED polymerization lamp; 700 $mW \cdot cm^{-2}$) for 30 s.

2.4 | Elution experiments

All extraction media and extraction temperatures were chosen to mimic conditions in the oral cavity, including water and aqueous buffer, along with ethanol, which exhibits comparable elution strength to the oral environment. Therefore, the fabricated specimens were placed into 12-mL glass vials and submerged in 2 mL of extraction medium (H_2O, phosphate-buffered saline [PBS], or 100% EtOH). Subsequently, these samples were placed in the dark on a shaker with vibratory motion (shaking frequency 600 rpm), and the shaker was then housed within an incubator set to a temperature of 37 ± 1°C or 50 ± 2°C. The extraction solutions were refreshed after 3, 10, and 30 days, spanning both acute and chronic exposure periods. Fresh solvent of the same volume was pipetted into the vessels without rinsing the samples beforehand. Before LC-MS analysis, the extraction

TABLE 1 MRM transitions of the selected analytes with their LoD and LoQ.

Abbreviation of substance	RT [min]	Quantitative transition			Qualitative transition			LoD [ng·g^{-1}]	LoQ [ng·g^{-1}]
		Precursor [m/z]	Product [m/z]	CE [eV]	Precursor [m/z]	Product [m/z]	CE [eV]		
DMAEMA	1.3	158.1	113.1	7.5	158.1	72.3	7.5	78.9	118.3
HEMA	2.3	131.1	113.1	2.5	131.1	69.3	2.5	58.1	87.2
CQ	3.6	167.0	167.0	0.1	167.0	139.0	5.0	59.9	89.8
TEGDMA	3.9	287.0	113.1	2.5	287.0	69.2	30.0	40.6	60.9
UDMA	5.2	471.4	113.1	10.0	471.4	341.4	2.5	308.4	462.6
BisGMA	6.0	530.0	142.9	20.0	530.0	277.2	10.0	355.3	532.9
BisEMA-5	9.9	602.3	113.1	15.0	602.3	113.1	15.0	238.7	358.1
BisEMA-4	10.2	558.2	113.1	20.0	558.2	291.2	20.0		
BisEMA-3	10.5	514.2	113.1	15.0	514.2	247.1	10.0		
BisEMA-2	10.9	470.1	113.1	15.0	470.1	247.1	10.0		

Abbreviations: BisEMA, ethoxylated bisphenol A dimethacrylate; BisGMA, bisphenol A-glycidyl methacrylate; CQ, camphorquinone; DMAEMA, 2-(dimethylamino)ethyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; LoD, limits of detection; LoQ, limits of quantification; MRM, multiple reaction monitoring; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate.

solution was filtered into a 1.8-mL glass vial through syringe nylon filters (0.22 μm, 13 mm diameter, Chromservis) and diluted, if necessary. Finally, the cumulative concentrations after 10 and 30 days were calculated by summing up the concentrations from the previous periods.

2.5 | Quantitative analysis of leachables

The quantification of leachables, with physicochemical properties detailed in Table S1, was conducted using ultra-performance liquid chromatography (UHPLC Agilent 1290 Infinity LC) coupled with triple quadruple mass spectrometry (Bruker EVOQ LC-TQ) employing ESI. An external gas generator (Peak Scientific—Genius 3045) was employed to supply nitrogen and air to the system.

Chromatographic separation was achieved using a Luna® Omega Polar C18 Phenomenex column (100 × 2.1 mm, 1.6 μm). The column temperature was optimized to 30°C, and the flow rate was maintained at 0.35 mL/min. The mobile phases comprised: (A) 0.1% formic acid in water and (B) ACN, following a gradient program of the A eluent (%): t(0 min) = 90, t(0.5 min) = 90, t(1.0 min) = 65, t(2.5 min) = 40, t(3.5 min) = 40, t(11.0 min) = 25, t(12.0 min) = 25, and t(12.9 min) = 90. The LC method's stop time was 13.0 min, with a re-equilibration time of 1.5 min. An injection volume of 7 μL was applied in all analyses.

The MS conditions were set as follows for electrospray ionization in positive mode: spray voltage: 4500 V; cone temperature: 300°C; cone gas flow: 50 arbitrary units (a.u.); heated probe temperature: 350°C; probe gas flow: 20 a.u.; nebulizer gas flow: 50 a.u.; and exhaust gas: ON. For the quantitative and qualitative analysis of leachables, the multiple reaction monitoring (MRM) mode was employed with specific MRM transitions detailed in Table 1. Argon was utilized as the collision gas at a pressure of 1.5 mTorr.

To quantify of leachables in the extraction media, an external calibration method was implemented. Despite the presence of both isomers in the standard for BisGMA monomer, quantification was conducted solely on one isomer (BisGMA-A) due to the significantly lower intensity of the other isomer, even at higher concentrations. BisEMA monomer quantification was performed as the sum of homologs BisEMA-2 to BisEMA-5, as the higher homologs exhibited low intensities even in the analytical standard. Leachables from dental composites were quantified using an external calibration method, with a coefficient of determination (R^2) exceeding 0.995 for each analyte. The calibration range spanned from 5 to 1000 ng·mL^{-1} for all analytes. The limits of detection (LoD) and limits of quantification (LoQ) for the method were established using signal-to-noise ratios of 3.3:1 and 10:1, respectively, as detailed in Table 1.

2.6 | Qualitative analysis of leachables

Qualitative analysis of unknown substances was conducted using high-performance liquid chromatography

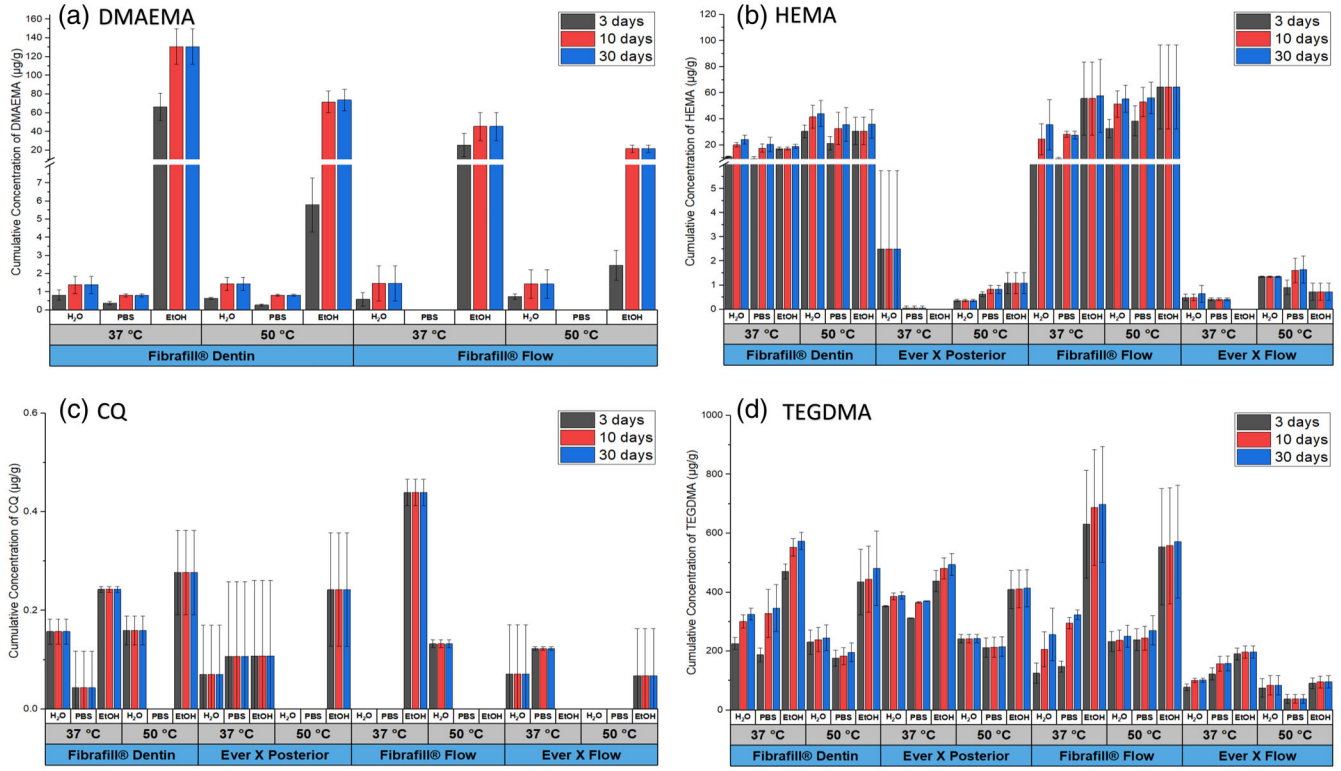

FIGURE 1 Cumulative concentrations of different extraction media at different temperatures: (a) DMAEMA; (b) HEMA; (c) CQ; and (d) TEGDMA. CQ, camphorquinone; DMAEMA, 2-(dimethylamino)ethyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate. [Color figure can be viewed at wileyonlinelibrary.com]

(HPLC Agilent 1100) coupled with a 3D ion trap mass spectrometer (Agilent 6320 Series Ion Trap LC/MS) equipped with ESI. An external gas generator (Peak Scientific—Genius 418LA) was employed to provide nitrogen.

Chromatographic separation was achieved using a Luna® Omega Polar C18 Phenomenex column (100 × 2.1 mm; 3.0 µm). The column temperature was optimized at 30°C, and the flow rate was set to 0.22 mL/min. Mobile phases consisted of (A) 0.1% formic acid in water and (B) ACN, following a gradient program of the A eluent (%) as follows: t(0 min) = 90, t(1.5 min) = 90, t(2.0 min) = 70, t(5.0 min) = 50, t(10.0 min) = 40, t(26.0 min) = 10, and t(26.5 min) = 90. The LC method's stop time was 27.0 min, with a re-equilibration time of 8.0 min. A consistent injection volume of 7 µL was utilized in all analyses.

The MS conditions were configured as follows: ESI operated in positive mode, with a cone temperature of 350°C. The cone gas flow was set at 12 L·min^{-1}, and the nebulizer gas flow was maintained at 25 psi. For qualitative analysis of the leachables, the full-scan mode was employed, covering an m/z range of 50–1000.

3 | RESULTS AND DISCUSSION

3.1 | Quantitative analysis of leachables

The cumulative elution profiles of various compounds (DMAEMA, HEMA, CQ, TEGDMA, UDMA, BisGMA, and BisEMA) from commercially available dental composite materials under different extraction conditions are illustrated in Figures 1 and 2. This study explores the impact of various extraction parameters on the quantity of leachables, including the extraction solvent, extraction temperature, and extraction time. In contrast to previous studies,[10–12,30,31] we quantified both the substances of the photoinitiator system and the monomers in the extraction medium. In the case of Ever X Flow and Ever X Posterior, no co-initiator DMAEMA was released (values under LoD). However, DMAEMA was eluted in concentrations ranging from 0.81 ± 0.09 µg·g^{-1} in PBS to 130 ± 20 µg·g^{-1} in 100% EtOH for Fibrafill® DENTIN and Fibrafill® FLOW after 30 days. Photoinitiator CQ was released in quantities from LoD (in both H_2O and PBS) in to 0.44 ± 0.03 µg·g^{-1} in 100% EtOH after 30 days. Furthermore, monomers were eluted in the following concentration range after 30 days of cumulative

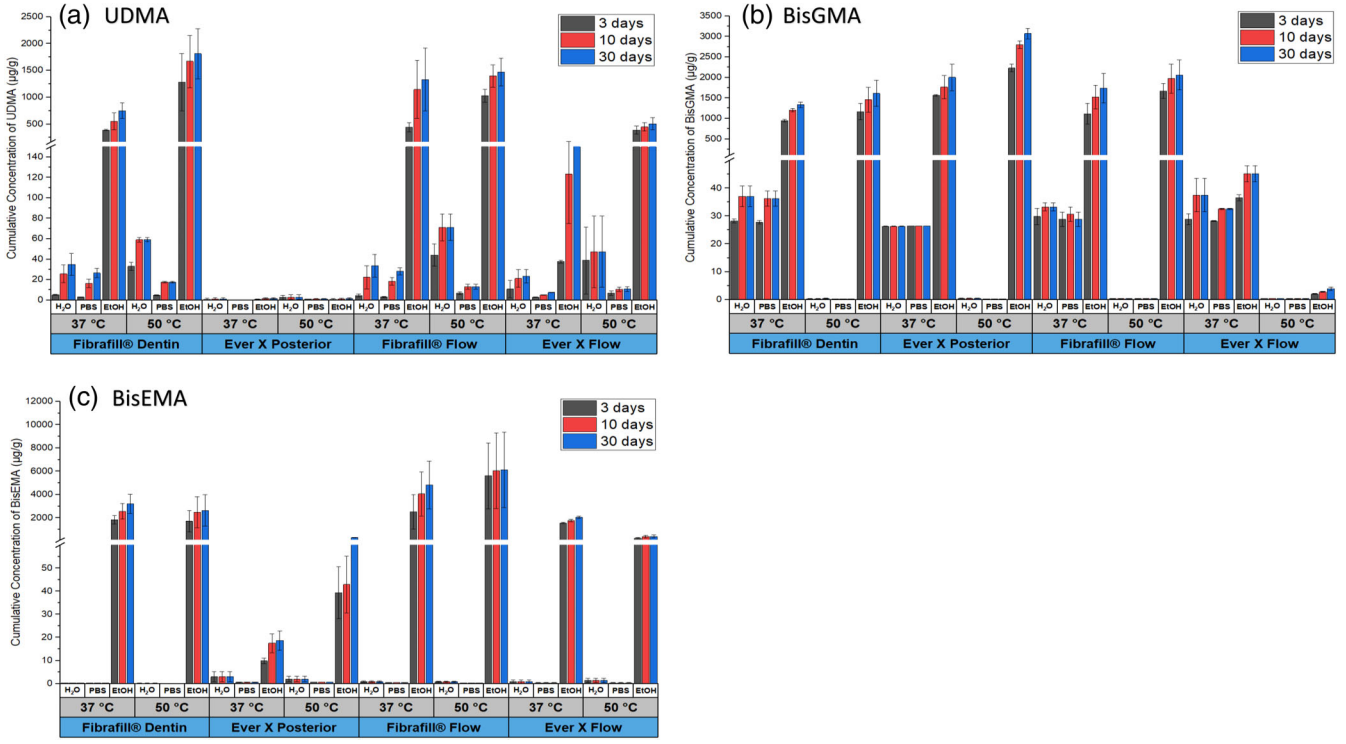

FIGURE 2 Cumulative concentrations of different extraction media at different temperatures: (a) UDMA; (b) BisGMA; and (c) BisEMA. BisEMA, ethoxylated bisphenol A dimethacrylate; BisGMA, bisphenol A-glycidyl methacrylate; UDMA, diurethane dimethacrylate. [Color figure can be viewed at wileyonlinelibrary.com]

extraction: HEMA concentrations ranged from LoD to $65 \pm 32\ \mu g \cdot g^{-1}$ in 100% EtOH; TEGDMA from $38 \pm 15\ \mu g \cdot g^{-1}$ in PBS to $699 \pm 196\ \mu g \cdot g^{-1}$ in 100% EtOH; UDMA from $0.11 \pm 0.07\ \mu g \cdot g^{-1}$ in PBS to $1811 \pm 465\ \mu g \cdot g^{-1}$ in 100% EtOH; BisGMA from $0.30 \pm 0.01\ \mu g \cdot g^{-1}$ in PBS to $3070 \pm 132\ \mu g \cdot g^{-1}$ in 100% EtOH, and BisEMA from $0.006 \pm 0.004\ \mu g \cdot g^{-1}$ in PBS to $6135 \pm 3241\ \mu g \cdot g^{-1}$ in 100% EtOH.

In contrast to previous studies,[10–12,30,31] which primarily focused on quantifying monomers in numerous dental materials, such as Tetric Evo Ceram Bulk Fill, X-tra Fill, Sonic Fill, Filtek Bulk Fill, SDR, EQUIA, G-aenial Anterior, G-aenial Posterior, Venus, Venus pearl, Venus Diamond, Ceram X Mono, Dyract, Filtek Supreme XTE, Surefil SDR, Venus Bulk Fill, X-tra base, Filtek Bulk Fill flowable, Tetric Evoceram Bulk Fill, SonicFill™, Grandioso Flow, Venus® Diamond Flow, X-Flow, Filtek™ Supreme XTE flowable, Grandioso, Venus Diamond, TPH® 3 Spectrum, Filtek™ Z250, Filtek Ultimate, Signum, Gradia, and Solidex from various manufacturers (such as Ivoclar Vivadent, VOCO GmbH, Kerr Corporation, 3M ESPE Dental Products, Dentsply DeTrey GmbH, GC Europe, Heraeus Kulzer, Shofu Inc., Coltène/Whaledent AG), our study provides a comprehensive analysis of both released monomers and compounds of the photoinitiator system. Notably, none of these previous studies analyzed any of the dental materials evaluated in our study, namely Fibrafill® DENTIN, Fibrafill® FLOW, Ever X Flow, and Ever X Posterior.

Although these studies have evaluated several dental materials and quantified eluting monomers, the majority of them have reported results in different units such as molarity (M),[10,31] nmol,[11] or in $\mu g \cdot mL^{-1}$, making it challenging to directly compare our results as the weight of the dental material is not reported in those studies. Nonetheless, the quantity of eluted substances depended on the extraction solvent and extraction time, as discussed below. Moreover, these studies used different volumes of extraction solvents ranging from 0.5 to 1.5 mL and different sizes of dental specimens (2–4 mm thickness × 4–6 mm diameter), typically with a single dental specimen per vial, unlike our experiment with two specimens per vial.[10–12,30,31] All of these factors can affect the elution kinetics and quantity of eluted substances.

Although study[30] quantified monomers HEMA (up to $3.34\ \mu g \cdot g^{-1}$), TEGDMA (up to $34.16\ \mu g \cdot g^{-1}$), UDMA (up to $212.81\ \mu g \cdot g^{-1}$), and BisGMA (up to $178.06\ \mu g \cdot g^{-1}$) released from dental materials (Filtek Z550, X-tra fill, Beautifil Bulk Restorative, and Fill Up) into 75% EtOH after 1 month. Meanwhile, a study[11] reported their results as EDI, enabling comparison with our results

(Section 3.1.4). Compared with these studies, our results show either similar or significantly higher quantities of substances released from dental materials. This could be attributed to the dental materials themselves, the selected spectrum of quantified substances, experimental design (volume of extraction solvent, size of dental specimen), or polymerization conditions (polymerization lamp and curing time).[30] Nonetheless, the materials' biocompatibility was assessed through EDI in Section 3.1.4. In addition, Figures 1 and 2 could suggest that a higher overall amount of leachables is eluted from dental materials manufactured by ADM, a.s. (Fibrafill® DENTIN and Fibrafill® FLOW). However, the standards used for the recognition of the substances in the measurement were direct ingredients of these two materials, indicating that differences in ingredients (both type- and manufacturer-wise) used by GC EUROPE N.V. (Ever X posterior and Ever X Flow) may play an important role in measurement specificity. In general, the extent and rate of elution of leachables depend on several factors: dental material (both monomer and filler/reinforcement composition-wise), degree of double bond conversion, physicochemical properties of leachable species (Mw, polarity, molecular structure due to steric factors), extraction solvent, elution temperature, and extraction time.

3.1.1 | Influence of the extraction solvent

The influence of the extraction solvent on the amount of leachables in H_2O, PBS buffer, or 100% EtOH was statistically evaluated using the ANOVA post hoc test (Tukey HSD). Consistent with previous studies,[11,12,20,23,32] the extraction solvent significantly affected the amount of leachables ($p < 0.05$). Specifically, a higher amount of leachables, including DMAEMA, TEGDMA, UDMA, BisGMA, and BisEMA, was eluted into 100% ethanol compared with H_2O or PBS. Meanwhile, no significant differences were observed when comparing H_2O with PBS ($p > 0.05$). There was no clear solvent effect observed in the case of CQ due to its overall low concentration in dental material. Moreover, the solvent choice did not influence the amount of eluted HEMA due to its high polarity (log $p = 0.47$) compared with other monomers (log $p > 1.5$ up to 6.4). In addition, solvent effect was evaluated using principal component analysis (PCA) in Figures 3 and 4 (elution up to 3 days) or Figures S1–S4 (elution up to 30 days), where H_2O and PBS extract samples were closely clustered together, while EtOH extracts were distinctively separated from these, regardless of the dental material. In agreement with previous studies,[10,11,31] a higher amount of leachables was eluted with 100% EtOH because of its greater elution strength

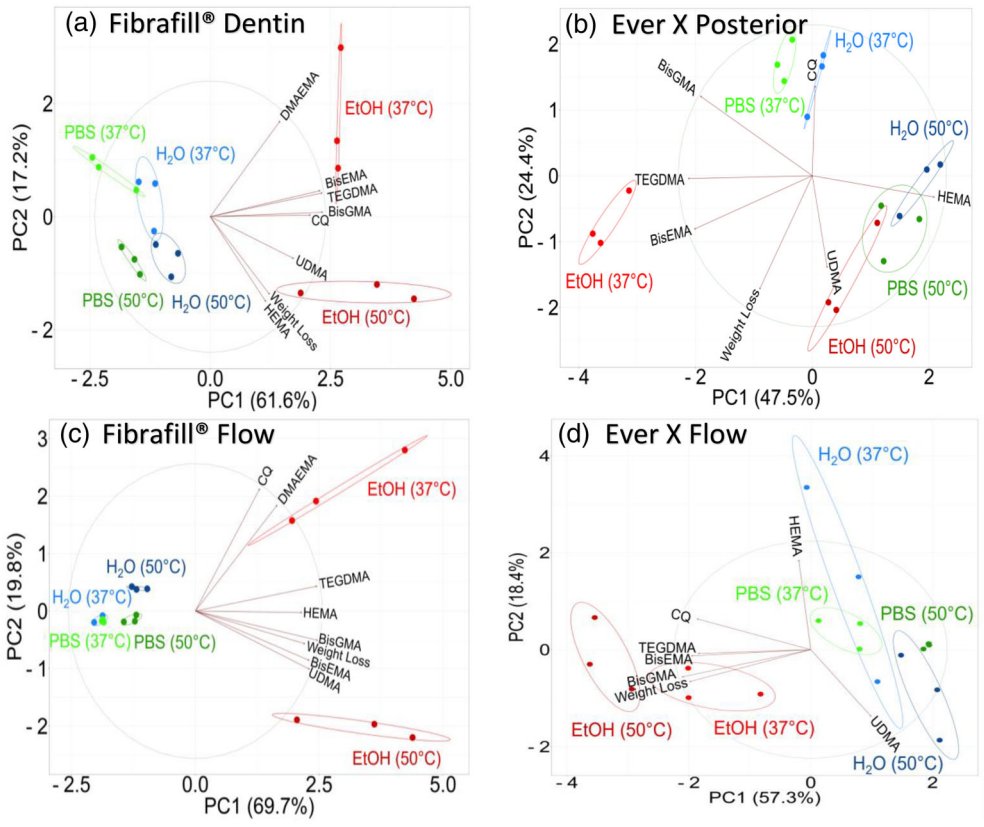

FIGURE 3 PCA of dental materials under varied extraction conditions after 3 Days: (a) Fibrafill® Dentin, (b) Ever X Posterior, (c) Fibrafill® Flow; and (d) Ever X Flow (created using SRplot[35]). BisEMA, ethoxylated bisphenol A dimethacrylate; BisGMA, bisphenol A-glycidyl methacrylate; CQ, camphorquinone; DMAEMA, 2-(dimethylamino)ethyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate. [Color figure can be viewed at wileyonlinelibrary.com]

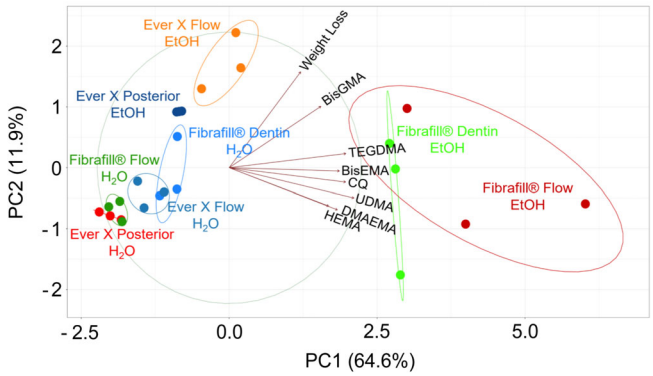

FIGURE 4 PCA of various dental materials extracted into H_2O and EtOH at 37°C after 3 days: comparative analysis of Fibrafill® Dentin, Ever X Posterior, Fibrafill® Flow, and Ever X Flow (created using SRplot[35]). BisEMA, ethoxylated bisphenol A dimethacrylate; BisGMA, bisphenol A-glycidyl methacrylate; CQ, camphorquinone; DMAEMA, 2-(dimethylamino)ethyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate. [Color figure can be viewed at wileyonlinelibrary.com]

and enhanced ability to swell the polymer network. This property allows ethanol to penetrate deeply into the polymeric chains, facilitating the diffusion of monomers and other substances.

The oral environment is commonly compared in terms of elution power with 75% EtOH by the United States Food and Drug Administration (US FDA). A higher amount of leachables is released into 100% EtOH because of its stronger elution strength. Although the three-dimensional polymer network is practically insoluble, it can swell in a suitable solvent, allowing more efficient diffusion of unreacted monomers. On the basis of the solubility parameters of the solvent, it is possible to predict which solvent would be the most efficient for the extraction of a given substance. Higher concentrations (in some cases approximately 10× to almost 10,000×) were measured when elutions were performed using 100% EtOH compared with H_2O or PBS buffer. This was observed especially in the case of less polar substances (e.g., UDMA, BisGMA, and BisEMA).

3.1.2 | Influence of the extraction temperature

To the best of our knowledge, no study has yet evaluated the effect of temperature on the quantity of eluted compounds from dental composite fillings. Therefore, the influence of extraction temperature on the amount of leachables was statistically evaluated using ANOVA post hoc test (Tukey HSD). Overall, statistically significant effects of temperature were only observed in the case of DMAEMA and TEGDMA ($p < 0.05$), where lower temperature (37°C) resulted in higher eluted concentrations. This is likely due to degradation, such as hydrolysis or alcoholysis, occurring at 50°C. Meanwhile, the elution of HEMA and UDMA was more efficient at 50°C ($p < 0.05$), which can be explained either by more effective swelling of the polymer network and faster diffusion or by slight degradation of the polymer network (via thermolysis). Consequently, a multicomponent statistical technique such as PCA was used to evaluate the overall elution trends, as shown in Figure 3 (elution up to 3 days) or Figures S1–S4 (elution up to 30 days). PCA suggests that temperature is less accountable for the differences between the samples, as there are shifts on the *y*-axis (PC2), compared with the solvent effect accountable for the shift on the *x*-axis (PC1). However, in the PCA plot, the majority of samples are distinguished due to different extraction temperatures.

3.1.3 | Influence of the extraction time

In agreement with previous studies,[10,11,14,15] most substances are eluted within the first few hours to days (Figures 1 and 2). However, leachables can continue to elute from dental fillings for an extended period, as demonstrated by studies,[10,14,15] which have investigated elution for up to 30 days, and a study,[11] which investigated elution for up to 52 weeks. Our results confirm that the release of some substances from dental composites may indeed persist beyond 30 days, although only in fractions of the initial amounts. This is also supported by Figures S1–S4, where samples from days 3, 10, and 30 are located nearby in PCA plots, indicating a gradual increase in cumulative concentration over time, although not as rapid as initially observed. The continuous release over this period can be attributed to residual unreacted monomers, degradation of the polymer matrix, and degradation of cleaved monomers. Nevertheless, the significant decrease in compounds over time from dental fillings is a positive finding for biocompatibility.

Specifically, CQ was detected only in the first extraction medium (extraction within the first 3 days). DMAEMA elution was completed after 10 days, except for Fibrafill® FLOW to EtOH at 50°C, where significantly low elution continued up to 30 days. In the case of HEMA, TEGDMA, and UDMA, elution likely continued for over 30 days in most samples, regardless of the

extraction media. The release of BisGMA and BisEMA continued for over 30 days only when the leachables were extracted with 100% EtOH. This continuous release of organic substances beyond 30 days could be attributed to several factors, including slow diffusion, degradation of the polymer network, formation of microcracks and porosity, and surface erosion.[11,33]

3.1.4 | Estimation of the potential exposure to leachables

Dental composite fillings, which are commonly composed of acrylic resins and methacrylates, are widely used. However, their leachables can trigger various adverse effects.[6,34] These substances have been linked to cytotoxicity, cell cycle arrest, ROS accumulation, GSH depletion, inflammation, diminished cell adhesion, and cell death, along with notable changes in cell morphology. Concerns extend to other tissues, because these materials may release a spectrum of toxic compounds capable of permeating biological membranes, potentially causing local or systemic harm.[6,34] Moreover, a study[34] mentioned that methacrylate degradation products exhibit mutagenic properties, while impurities could induce cell proliferation via ubiquitination rather than apoptosis.

Therefore, the EDI of leachables released from total crown restorations was calculated for the worst-case scenario based on the highest measured result across all experiments for each substance extracted in 100% EtOH. Potential estimated exposures were calculated using the average total crown surface area and assuming body weights of 70 kg for adults and 20 kg for children. Acute exposure (from cumulative extraction over 3 days) and chronic exposure (from cumulative extraction over 30 days) were calculated (Table 2). Estimated EDIs are higher for children in both acute and chronic exposures due to their lower weight. However, these values (in μg/kg·bw/day) are still significantly lower than the available toxicological data (Table S1), where LD_{50} (oral) for all quantified leachables are in the range of (1600–10,837) $mg{\cdot}kg^{-1}$, indicating that these dental materials are safe, as LD_{50} is more than approximately 1000 times higher. Nevertheless, the potential synergistic toxicological effects of different leachables were not considered. Furthermore, our EDI values were compared with those of a study,[11] where EDIs were reported in ng/kg·bw/day, indicating significantly higher amounts of leachables eluted within our study. However, this difference could be due to variations in dental materials or experimental conditions in vitro.

3.2 | Qualitative analysis of the leachables

3.2.1 | Analysis of the standard mixture

A qualitative analysis of a mixture of standards (DMAEMA, HEMA, CQ, TEGDMA, UDMA, BisGMA, and BisEMA) in full-scan ion mode was conducted. In this mixture, individual substances and their isomers were identified (Table S2), and a chromatogram of the standard mixture was generated (Figure S5). During electrospray ionization, the common adducts $[M + H]^+$, $[M + NH_4]^+$, $[M + Na]^+$, and $[M + H\text{-}H_2O]^+$ were observed. Structural isomers were detected for CQ, UDMA, BisGMA, and BisEMA. However, we were able to separate two structural isomers of BisGMA, distinguishable only by separation rather than by MS or MS/MS spectra. BisEMA consists of a polymerizable methacrylate group linked to bisphenol by oligo- or polyethoxy groups of varying lengths (degrees of ethoxylation). We were able to separate and identify monomers up to BisEMA-13 (Figure S6), which is in agreement with a previous study[36] that analyzed BisEMA homologs up to BisEMA-10 using GC-MS. Although our LC-MS data did not confirm this due to insufficient LC separation, different structural isomers of each BisEMA homolog could also be present, both in standard and composite fillings. Typical product ions for methacrylates (e.g., HEMA or DMAEMA) and dimethacrylates (e.g., TEGDMA, UDMA, BisGMA, and BisEMA monomers) were observed in MS/MS at m/z 113.1 ($CH_2{=}C(CH_3)CO_2CH_2CH_2^+$) and m/z 69.4.

3.2.2 | BisEMA homologs in dental materials

Figure 5d displays the relative abundance of individual BisEMA homologs in a standard solution of 1.0 $\mu g{\cdot}mL^{-1}$. The highest intensity among the BisEMA homologs was observed for BisEMA-3, with a decrease in intensity as the degree of ethoxylation (m + n) increases. Subsequently, Figure 5a,b illustrates that the relative abundances of different BisEMA monomer homologs are similar when the compounds from the tested dental composites are extracted into H_2O and PBS, respectively. However, there is a notable difference in relative abundances when BisEMA was extracted into 100% EtOH (Figure 5c).

Short chains of BisEMA, particularly BisEMA-2 and BisEMA-3, exhibit the highest release into H_2O and PBS (Figure 5a,b, respectively), with decreasing intensity observed for longer chains (BisEMA-7 is no longer

TABLE 2 Estimated daily intake (EDI) of different leachables (μg/kg·bw/day) for adults and children after acute and chronic exposure due to dental filling.

Typical restorations		Surface area [mm^2]	Acute exposure (3 days) [μg/kg·bw/day]							Chronic exposure (30 days) [μg/kg·bw/day]						
Adults (70 kg)			**DMAEMA**	**HEMA**	**CQ**	**TEGDMA**	**UDMA**	**BisGMA**	**BisEMA**	**DMAEMA**	**HEMA**	**CQ**	**TEGDMA**	**UDMA**	**BisGMA**	**BisEMA**
Front teeth	Central incisor	223	1.33	1.29	0.009	12.6	25.7	44.7	112.3	0.26	0.13	0.001	1.4	3.6	6.2	12.3
	Lateral incisor	178	1.06	1.03	0.007	10.1	20.5	35.7	89.6	0.21	0.10	0.001	1.1	2.9	4.9	9.8
	Canine	210	1.25	1.22	0.008	11.9	24.2	42.1	105.7	0.25	0.12	0.001	1.3	3.4	5.8	11.6
Premolars	First premolar	203	1.21	1.18	0.008	11.5	23.4	40.7	102.2	0.24	0.12	0.001	1.3	3.3	5.6	11.2
	Second premolar	191	1.14	1.11	0.008	10.8	22.0	38.3	96.2	0.22	0.11	0.001	1.2	3.1	5.3	10.5
Molars	First molar	315	1.88	1.83	0.012	17.9	36.3	63.2	158.6	0.37	0.18	0.001	2.0	5.1	8.7	17.4
	Second molar	276	1.65	1.60	0.011	15.7	31.8	55.3	138.9	0.32	0.16	0.001	1.7	4.5	7.6	15.2
	Third molar	247	1.47	1.43	0.010	14.0	28.4	49.5	124.3	0.29	0.14	0.001	1.6	4.0	6.8	13.6
4 quadrants		7372	43.97	42.75	0.291	418.1	848.9	1477.9	3711.3	8.65	4.28	0.029	46.3	120.0	203.4	406.4
Children (20 kg)		**Surface Area [mm^2]**	**DMAEMA**	**HEMA**	**CQ**	**TEGDMA**	**UDMA**	**BisGMA**	**BisEMA**	**DMAEMA**	**HEMA**	**CQ**	**TEGDMA**	**UDMA**	**BisGMA**	**BisEMA**
Front teeth	Central incisor	223	4.66	4.53	0.031	44.3	89.9	156.5	392.9	0.92	0.45	0.003	4.9	12.7	21.5	43.0
	Lateral incisor	178	3.72	3.61	0.025	35.3	71.7	124.9	313.6	0.73	0.36	0.002	3.9	10.1	17.2	34.3
	Canine	210	4.38	4.26	0.029	41.7	84.6	147.4	370.0	0.86	0.43	0.003	4.6	12.0	20.3	40.5
Premolars	First premolar	203	4.24	4.12	0.028	40.3	81.8	142.4	357.7	0.83	0.41	0.003	4.5	11.6	19.6	39.2
	Second premolar	191	3.99	3.88	0.026	37.9	77.0	134.0	336.5	0.78	0.39	0.003	4.2	10.9	18.4	36.8
Molars	First molar	315	6.58	6.39	0.044	62.5	126.9	221.0	555.0	1.29	0.64	0.004	6.9	17.9	30.4	60.8
	Second molar	276	5.76	5.60	0.038	54.8	111.2	193.7	486.3	1.13	0.56	0.004	6.1	15.7	26.7	53.2
	Third molar	247	5.16	5.01	0.034	49.0	99.5	173.3	435.2	1.01	0.50	0.003	5.4	14.1	23.9	47.7
4 quadrants		7372	162.24	157.75	1.074	1542.7	3132.2	5453.4	13,694.2	31.93	15.78	0.107	170.7	442.8	750.5	1499.4

Abbreviations: BisEMA, ethoxylated bisphenol A dimethacrylate; BisGMA, bisphenol A-glycidyl methacrylate; CQ, camphorquinone; DMAEMA, 2-(dimethylamino)ethyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, diurethane dimethacrylate.

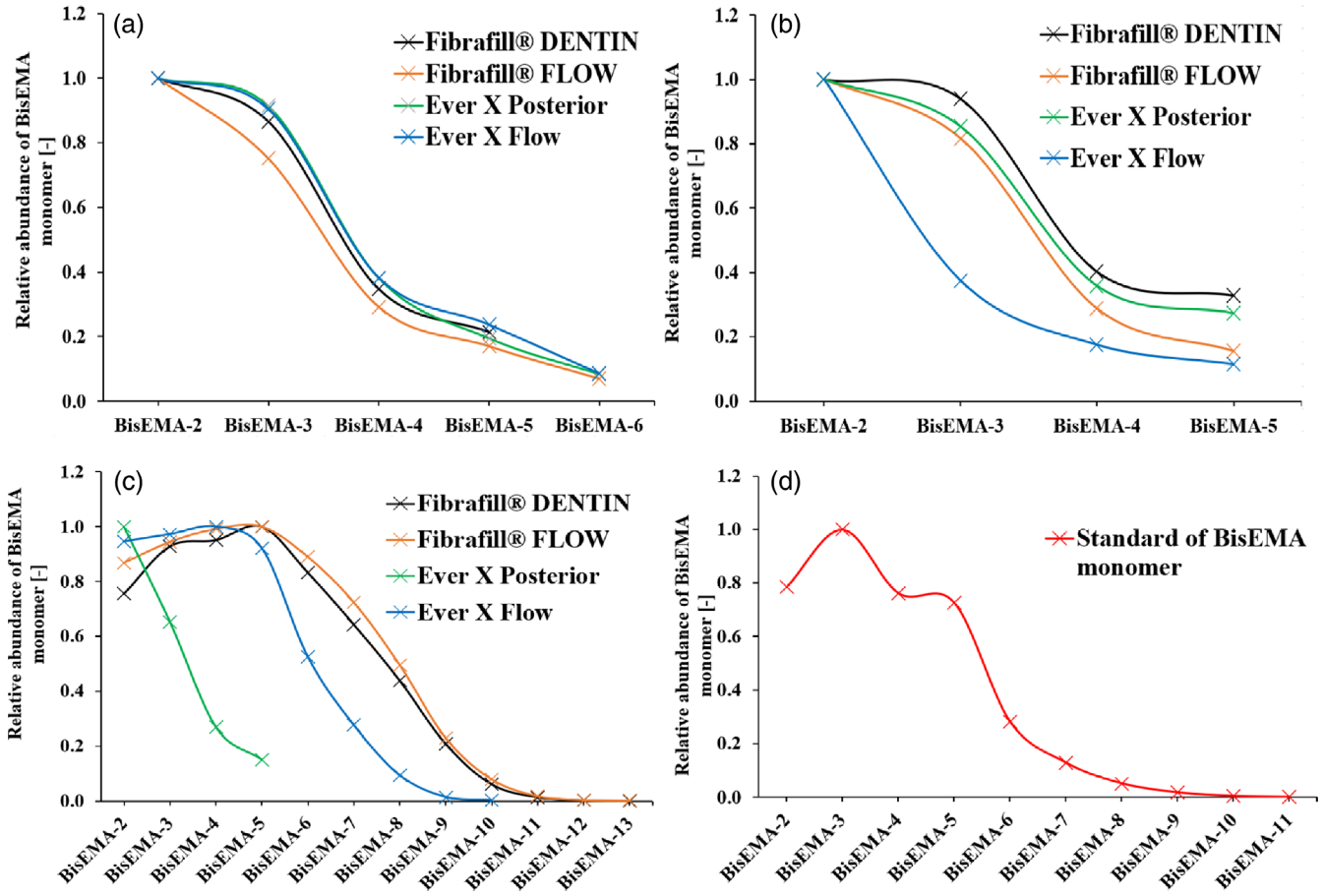

FIGURE 5 Relative abundance of individual BisEMA monomers at extraction temperature 37°C: (a) extraction medium H_2O, (b) extraction medium PBS, (c) extraction medium 100% EtOH, and (d) BisEMA standard. BisEMA, ethoxylated bisphenol A dimethacrylate. [Color figure can be viewed at wileyonlinelibrary.com]

detected in H_2O or PBS). This trend may be attributed to the inability of H_2O and PBS to sufficiently swell the polymer network, hindering the efficient elution of longer BisEMA homologs due to steric effects. Conversely, during elution into 100% EtOH (Figure 5c), BisEMA-5 exhibited the highest relative abundance, consistent with the BisEMA standard (Figure 5d), whereas the intensities of BisEMA-2 to BisEMA-6 remained relatively high. However, other homologs exhibit decreasing intensity with increasing $m + n > 7$, as 100% EtOH effectively swells the polymer network, allowing efficient elution up to the BisEMA-6 monomer, beyond which steric effects become more pronounced.

3.2.3 | Untargeted analysis

Nondiluted dental material extracts were subjected to full scan mode analysis using LC-ESI-IT. The choice of solvent significantly influenced the quantity of released substances, as depicted in Figures S7–S12 (and corroborated by statistical analyses in the quantitative analysis, Section 3.1.1), where peaks are labeled according to Table S2. These results demonstrated that a higher amount of substances is indeed released from all tested dental composites into 100% EtOH, because of its superior elution strength and enhanced ability to swell the polymer network. Furthermore, the influence of temperature (Section 3.1.2), as confirmed by the quantitative results and subsequent PCA plot (Figure 3), is also evident when comparing the total ion chromatograms (TIC) in Figures S7–S12. These differences may arise from the potential degradation of dental materials or previously eluted compounds.

A scientific study[16] identified up to 180 compounds in the extraction medium, many of which remain unidentified. This underscores the necessity for MSDS to comprehensively list all substances used, not just the primary ingredients of the composite above a threshold of ≥1%. Despite the analysis of various dental materials from different manufacturers, their total ion chromatograms exhibited similar substance profiles and intensities in this

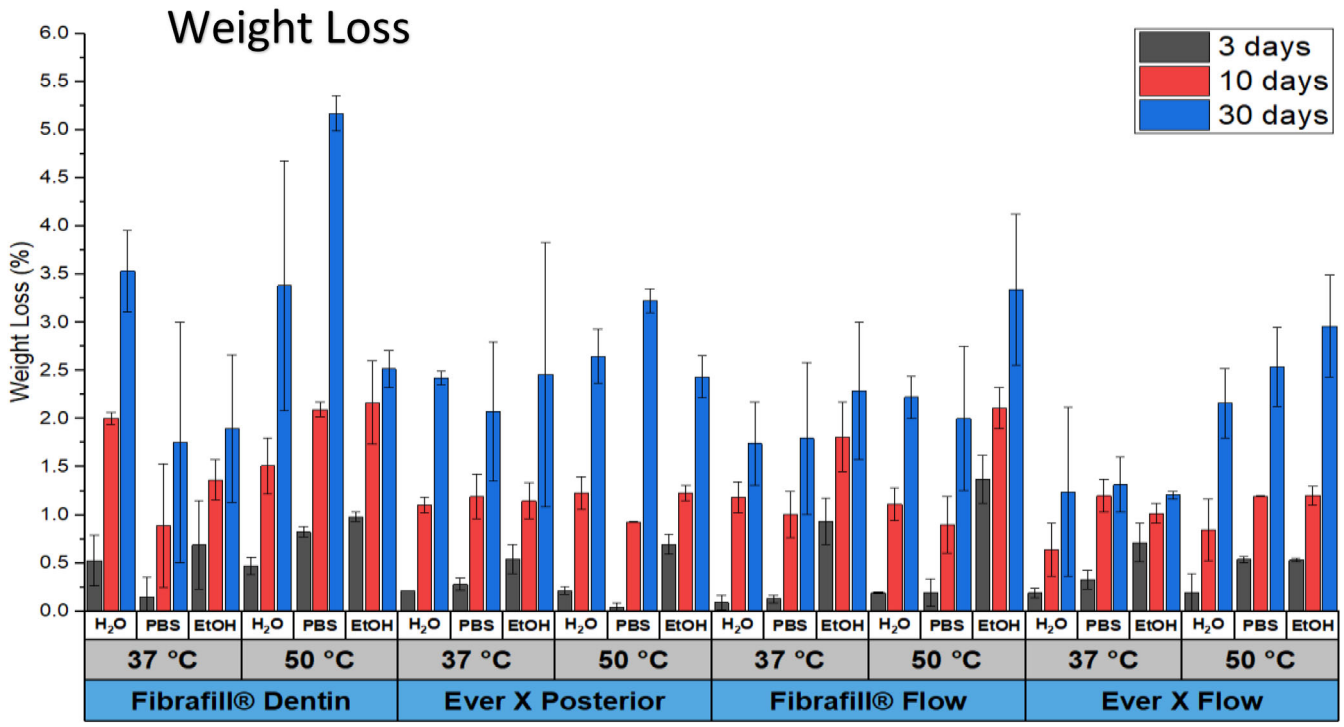

FIGURE 6 Weight loss of dental materials during the extraction experiments. PBS, phosphate-buffered saline. [Color figure can be viewed at wileyonlinelibrary.com]

study, as confirmed by PCA in Figure 4 (quantitative results). Unlabeled peaks observed in the chromatograms (Figures S7–S12) may represent degradation products or potential oligomers,[37] for which commercial standards are not available. Although studies suggest the diffusion of these substances from dental composites, specific oligomers have yet to be identified.

The peaks observed in retention times ranging from 9.0 to 12.0 min indicate oligomers derived from the hydrophilic monomer HEMA. These peaks exhibit particularly high intensity when extracted into H_2O and PBS (Figures S7–S12). These polar oligomers manifest as $[M + H]^+$ ions with m/z values of 453.2, 566.4, 679.5, 792.6, and 905.7. Notably, these m/z values are multiples of 113, which is a characteristic fragment of methacrylate-based monomers. Conversely, the peaks observed in retention times of 25.0–27.0 min correspond to less polar oligomers derived from the hydrophobic monomer. These oligomers are predominantly intense when extracted with 100% EtOH. The hydrophobic oligomers exhibit m/z values of 719.3, 675.3, and 631.3, respectively, showcasing a typical m/z difference of 44, which is consistent with the different ethoxylation states of BisEMA.

In addition, the identification of degradation products necessitates the use of LC-HRMS instrumentation due to its high resolution, mass accuracy, and scan speed. Although only a few studies[28,29] have successfully focused on the identification of these degradation products, they have been limited to certain monomers. Consequently, we have compiled a comprehensive overview in Table S3, which encompasses degradation products, standard impurities, and monomers formed by cleavage from other monomers (e.g., HEMA from TEGDMA monomer).

3.3 | Weight loss during the extraction experiments

During the cumulative extraction process, we monitored the weight loss of the dental materials under various extraction conditions, as illustrated in Figure 6. Over the course of the in vitro experiments, we observed a gradual increase in weight loss, which was primarily attributed to the depletion of filler or reinforcement particles, which can constitute up to 80% of dental materials. This progressive weight loss raises concerns about the structural integrity of the material and its potential impact on biocompatibility. On average, the weight loss of dental materials ranged from 1.2% to 3.5% after 30 days. Notably, there was an outlier in the weight loss of the Fibrafill® DENTIN material when exposed to PBS at 50°C, showing a weight loss of (5.2% ± 0.2%). However, we found no statistically significant or discernible trends in weight loss

among different dental materials based on extraction media ($p > 0.05$), extraction temperatures ($p > 0.05$), or dental material ($p > 0.05$). Nevertheless, weight loss can persist beyond 30 days and throughout the lifetime of a dental filling due to mechanical wear, the formation of microcracks, and surface erosion.

4 | CONCLUSION

This study provides valuable insights into the elution profiles of both disclosed and undisclosed compounds in the MSDS from four dental materials. The present study evaluated the biocompatibility of Fibrafill® DENTIN, Fibrafill® FLOW, Ever X posterior, and Ever X Flow, which have not been previously assessed in the literature, thereby filling another research gap. Both quantitative and qualitative analyses of various compounds were conducted using liquid chromatography with electrospray ionization and triple quadrupole or ion trap mass spectrometry.

The results revealed that both the extraction solvent and extraction temperature significantly influenced the quantity and elution profile of numerous leachables ($p < 0.05$). Consistent with prior research, the majority of leachables were released within the initial days, whereas certain monomers continued to elute for over 30 days. Consequently, the estimated daily intake was calculated for the worst-case scenario, confirming the biocompatibility of the composite fillings. Previously unexplored, the weight loss of dental materials ranged from 1.2% to 3.5% after 30 days, irrespective of extraction conditions and dental material ($p > 0.05$). Additionally, several hydrophilic and hydrophobic oligomers were identified, and a literature review on the degradation products of monomers was conducted, addressing another research gap. In conclusion, this study comprehensively addressed the biocompatibility of various dental materials through rigorous statistical analyses, thereby contributing to the resolution of several research gaps in the field.

Despite the demonstrated biocompatibility of all dental materials, caution regarding human health should be exercised. Existing studies overlook potential toxicological synergistic effects, and the majority of degradation products and released monomers remain unidentified.

AUTHOR CONTRIBUTIONS

Jan Fučík: Conceptualization (lead); data curation (lead); investigation (lead); methodology (lead); validation (lead); writing – original draft (lead). **Pavel Kejík:** Conceptualization (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting). **Zdeněk Bystřický:** Conceptualization (supporting); data curation (supporting); investigation (supporting); methodology (supporting). **Anna Amrichová:** Investigation (supporting); methodology (supporting). **Marie Hamplová:** Investigation (supporting); methodology (supporting). **Ludmila Mravcová:** Conceptualization (supporting); investigation (supporting); methodology (lead); project administration (lead); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors from BUT FCH have no competing interests. The authors employed at ADM, a.s., work in the R&D and manufacture of dental composite materials.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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