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Authors: Philippe Vervliet, Siemon de Nys, Imke Boonen, Radu Corneliu Duca, Marc Elskens, Kirsten L. van Landuyt, Adrian Covaci

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Qualitative analysis of dental material ingredients, composite resins and sealants using liquid chromatography coupled to quadrupole time of flight mass spectrometry

Philippe VERVLIET[1,*], Siemon DE NYS[2], Imke BOONEN[3], Radu Corneliu DUCA[4], Marc ELSKENS[3], Kirsten L. VAN LANDUYT[2], Adrian COVACI[1,*]

[1] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[2]KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT & University Hospitals Leuven (UZ Leuven), Dentistry, Leuven, Belgium

[3]Department of Analytical, Environmental and Geo-Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Ixelles, Belgium

[4]Environment and Health, Department of Public Health and Primary Care, KU Leuven, Kapucijnenvoer 35, 3000 Leuven, Belgium

[*]Corresponding philippe.vervliet@uantwerpen.be; adrian.covaci@uantwerpen.be; fax: +32 3 265 27 22

Highlights

- A specific LC-QTOF-MS method for screening of dental materials was developed
- Ammonium fluoride improves LC-MS sensitivity for dental resin ingredients
- BPA ethoxylated methacrylates (BisEMA) standards contain a variety of oligomers
- Most identified compounds were not stated on the material safety data sheets

Abstract

Since 2011, the World Health Organization has encouraged a global phase-down of the use of dental amalgam and actively supported the use of alternative, resin-based dental materials. The resins consist of (meth)acrylate monomers derived from Bisphenol A (BPA), such as Bisphenol A glycidyl methacrylate (BisGMA) and Bisphenol A ethoxylate methacrylate (BisEMA) or triethylene glycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA) which lack the BPA backbone. Besides monomers, other compounds such as photoinitiators and stabilizing agents can be present in the dental resin matrices. The current study consists in the development of an analytical method for the separation and identification of dental material components using LC-QTOF-MS. The developed method was applied on several dental material ingredients, unpolymerized composite resins, and a common dental sealant. The acquired high resolution accurate-mass data was analyzed using suspect screening with an in-house developed library. Next to the main components, various isomers and impurities related to the production of the main component have been detected and identified in the dental material ingredients. In total, 39 chemicals have been identified in the analyzed dental materials. On average 15 chemicals have been identified. Major components, such as BisEMA, BisGMA and TEGDMA were identified although they were not always stated in the material safety data sheets. Minor components included photoinitiators, such as ethyl 4-dimethyl aminobenzoate (EDMAB) and (meth)acrylates impurities originating from production of main ingredients.

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Keywords: Dental Materials; Resin Composites; Resin Sealants; Suspect Screening; High-resolution mass spectrometry; Liquid Chromatography.

1. Introduction

In spite of its long-term use as the standard filling material for dental restorations, the use of amalgam has decreased (1, 2). Its good clinical performances did not outweigh the concerns for mercury pollution and its inferior aesthetics when compared to resin-based dental materials. In 2011, the World Health Organization (WHO) have encouraged a global phase-down of the use of dental amalgam and actively support the use of alternative dental materials (3).

Resin-based materials are widespread used alternative dental materials in e.g. endodontics and orthodontics, which have been in use for several decades (4, 5). Although these resin-based materials have proven to be satisfying alternatives to amalgam for teeth restorations, since recently, the safety of the usage of these materials has been under investigation (6, 7). Resin-based materials are a mixture of a polymer matrix with an inorganic filler, which is attached to the resin matrix by siloxane coupling (8). The resin-based materials can either be self-cured, light-cured or a combination of these two techniques. For both techniques, the polymerization is not complete. Studies have found that the degree of conversion of monomers to polymers varies from 35 – 77% and is in correlation with the leaching of unpolymerized monomers (9-12). Furthermore, the dental restoration can degrade when exposed to enzymes, bacteria, thermal or mechanical stress and thus may leach degradation products of the compounds present in the dental matrix (13-17). As unreacted monomers can leach out of the matrix, the dental restoration can be a source for systemic uptake of the incorporated compounds, either by uptake from the diffusion through the dentinal tubules, gastrointestinal tract after ingestion, or by uptake of the volatile compounds in the lungs (17-19).

The most commonly used monomers for dental resin systems are (meth)acrylates derived from Bisphenol A (BPA). Examples include Bisphenol A glycidyl methacrylate (BisGMA), Bisphenol A dimethacrylate (BisDMA), and Bisphenol A ethoxylate methacrylate (BisEMA). Some monomers, such as BisEMA, can vary in the length of the aliphatic side chains. Recent studies have also shown the presence of BPA and Bisphenol A diglycidyl ether (BADGE) in different dental resins and sealants (20, 21). Other frequently used monomers, such as triethylene glycol dimethacrylate (TEGDMA) or urethane dimethacrylate (UDMA), lack this BPA backbone.

Besides monomers, many other compounds such as photoinitiators and stabilizing agents can be present in the dental resin matrices. Often the complete composition is not communicated as this is regarded as a trade secret. As a result, material safety data sheets (MSDS) do not state exact compositions of the dental materials. This poses problems for research as studies on the composition of resins or leaching are based on the information stated on the MSDS (7, 22). On the other hand, many components, such as the monomers in the resin, are no longer present in their original form after the restoration as they get incorporated in the polymer matrix. Nevertheless, some compounds such as

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initiators are not chemically bound to the matrix and can leach over time. In addition, unpolymerized monomers, as well as degradation products of the polymer network, can also leach after the dental restoration. In order to correctly identify chemicals in leaching studies, it is necessary to know the original composition of the employed materials. An overview of the different compounds in this study can be found in Table 1.

When BPA-derived monomers are degraded, possibly even to BPA, and taken up into the human body, a range of toxicological effects can be expected similar to those resulting from exposure to BPA (23-28). As these effects are possibly compound-specific, an identification of all compounds present in the original unpolymerized material is required. Secondly, the presence of non-polymerized monomers might also lead to multiple effects, such as DNA-changes and induction of apoptosis and pro-inflammatory cytokines (29, 30). Due to their structural relation to BPA, endocrine disrupting effects can also occur due to degradation of the monomer to BPA, presence of BPA impurities from manufacturing or affinity of the monomer to the estrogen receptor (21, 31, 32).

In earlier studies, different methods have been developed to evaluate the release of substances from (polymerized) dental materials (20, 33-36). However, these methods focused on a quantitative targeted analysis of a number of selected chemicals. Yet no retrospective analysis could be performed to investigate the presence of other (non-targeted) compounds in the sample.

In order to elucidate extractables from dental resins, the first aim of this study was to develop a LC-QTOF-MS/MS method to identify compounds based on accurate mass and MS/MS spectra. Second, the developed LC-QTOF-MS/MS method was applied on several dental resins to characterize the composition of a number of commonly used dental materials. For the identification of compounds on the LC-QTOF-MS/MS system, an in-house library was developed containing known monomers, photoinitiators and other compounds used in dental resin-based materials.

2. Materials and methods

2.1. Chemicals and reagents

Bisphenol A dimethacrylate (BisDMA, CAS 3253-39-2), ethyl 4-(dimethylamino) benzoate (EDMAB, CAS 10287-53-3), triethylene glycol dimethacrylate (TEGDMA, CAS 109-16-0) and ammonium fluoride (>99.99% trace metals basis) were acquired from Sigma-Aldrich (Overijse, Belgium). Urethane dimethacrylate (UDMA, CAS 72869-86-4), Bisphenol A glycidyl methacrylate (BisGMA, CAS 1565-94-2), Bisphenol A ethoxylate methacrylate (BisEMA-10, CAS 41637-38-1), Bisphenol A ethoxylate methacrylate (BisEMA-6, CAS 41637-38-1) and Bisphenol A ethoxylate methacrylate (BisEMA-3, CAS 41637-38-1) were received from Esstech Inc (Essington, PA, United States). TCD-DI-HEA (CAS 861437-11-8) was kindly provided by Kulzer Benelux (Haarlem, The Netherlands). Deuterated urethane dimethacrylate (d-UDMA) was custom-made at the University of Leuven (Belgium) using the acquired UDMA from Esstech (34). The internal standard was dissolved in MeOH to obtain a solution with a concentration of 5 ng/µL.

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Methanol (LC-MS grade) was acquired from Fisher Scientific (Loughborough, United Kingdom). Ammonium acetate (pro analysi) and acetic acid (EMSURE® ACS,ISO,Reag. Ph Eur., CAS 64-19-7) were acquired from Merck (Darmstadt, Germany). Ultrapure water was prepared from demineralized water with an Elga Purelab Flex system (Veolia Water Solutions and Technologies, Tienen, Belgium).

The following dental materials were investigated. Filtek Supreme XTE Universal (Shade A2B) was acquired from 3M ESPE (Diegem, Belgium) and Quadrant Anterior (Shade A1) was acquired from Cavex (Haarlem, The Netherlands). AH Plus, Ceram.X Universal (Shade A3) and Dyract Extra (Shade A2) were acquired from Dentsply Benelux (Zoetermeer, The Netherlands). G-ænial Anterior (Shade A2), G-ænial Posterior (Shade A3), Gradia Direct Posterior (Shade A3) were acquired from GC Europe (Leuven, Belgium). Solitaire 2 (Shade A3) and Venus (Shade A3) were acquired from Kulzer (Haarlem, The Netherlands). N'Durance (Shade A3) was acquired from Septodont (Brussel, Belgium) and Fissurit FX was acquired from VOCO (Cuxhaven, Germany). An overview of these materials with the compounds listed on the MSDS can be found in the Supporting Information (Table SI-1).

2.2. LC-QTOF-MS/MS analytical method

For qualitative screening, the LC system consisted of an Agilent Infinity 1290 SL binary pump with an integrated two-channel solvent degasser, a thermostated Agilent 1200 HiP-ALS autosampler and an Agilent 1290 TCC SL column compartment coupled to an Agilent 6530 Accurate-Mass QTOF instrument (Agilent, Diegem, Belgium). Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18 (100 mm x 3.0 mm, 2.7 µm) column (Agilent, Diegem, Belgium). Injection volume was 5 µL and flow rate was set on 0.4 mL/min based on backpressure during the chromatographic run. Mobile phases consisted of ultrapure water (A) and methanol (B) with addition of selected additives. The chromatographic run started 0.6 min isocratic at 15 %B, followed by a first gradient to increase the percentage of B to 70 % at 1.5 min. This was held for 1.5 min before a second gradient increased the percentage of B to 95 % at 10 min. Next, the column was rinsed with 95 %B for 3 min and re-equilibrated at 15 %B for 6 min before the next injection. Column temperature was kept constant at 40°C. The mass spectrometer was operated in Extended Dynamic Range (2 GHz) mode providing a Full Width at Half Maximum (FWHM) resolution of approximately 5100 at *m/z* 118.0862 and 10000 at *m/z* 922.0098. The ions *m/z* 112.0508 and 922.0097 for positive mode and *m/z* 112.9855 and 940.0009 for negative mode were selected for a constant recalibration throughout the chromatographic run to ensure good mass accuracy. Eluting compounds were ionized with a jet stream electrospray ionisation source (AJS ESI source) operated both in positive and negative ionisation mode. Drying gas temperature and flow were 325 °C and 8 L/min, respectively. The sheath Gas temperature was 325 °C at a flow of 11 L/min. Nebulizer pressure was set at 40 psig. Capillary, Nozzle and Fragmentor Voltages were 3500 V, 0 V, and 125 V, respectively. The acquisition parameters set an m/z range from 80 to 1000 at a scan rate of 2.5 scans/s and 6.67 scans/s for the MS and MS/MS spectra, respectively.

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Collision energies were applied at 10 and 30 V. Signals were detected using a Data-Dependent Analysis method (Auto-MS/MS), selecting the three most abundant ions. An active exclusion of 0.2 min was applied to prevent repetitive acquisition of spectra for the same ion.

For optimization of the mobile phase composition, the LC-system consisted of an Agilent 1200 series binary pump and autosampler coupled to an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization source. In the first tests, the MS was operated in selected ion monitoring (SIM) mode in positive ionization to monitor different adducts for all selected monomers ($[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$). After a first selection of mobile phase additive, a multiple reaction monitoring (MRM) method was set up. An overview of the parameters for all selected compounds, including retention times can be found in Table 1. For both SIM and MRM methods, the chromatographic conditions were identical to those of the qualitative screening.

2.3. Workflow for identification of unknown substances on LC-QTOF-MS/MS

For suspect screening on LC-QTOF-MS/MS, an in-house library using the Personal Compound Database and Library (PCDL) Manager (Version Rev. B.07.01, Agilent Technologies, Santa Clara, USA) was prepared to facilitate the identification of chromatographic peaks. Based on literature, compounds were included when previously identified in leaching experiments (7, 19, 22, 36). Secondly, materials safety data sheets of dental products were screened and stated components were included in the database. The molecular formula and mono-isotopic mass were added to the database for all compounds. For compounds where an analytical standard was available, retention time and MS/MS spectra were also added to the database after analysis on the LC-QTOF-MS system.

2.4. Selection of used mobile phase additives

Mobile phases and additives were primarily selected in SIM mode, based on previous work by Putzeys *et al* (34). Next to mobile phases without additives, acetic acid (0.1% v/v) and ammonium acetate buffers pH 3.7 (2 mM and 10 mM) were also evaluated. For each mobile phase, a mixture containing selected compounds in a concentration of 1 ng/µL was injected and selection of the optimal mobile phase additive was based on peak height, peak width, and peak area. Secondly, after selecting appropriate MRM transitions, ammonium fluoride was also tested in a concentration of 1 mM, as this additive has showed previously promising improvements in sensitivity both in positive and negative ionisation in LC-MS (37). Results of the ammonium fluoride injections were compared to MRM results with the selected previous mobile phases.

2.5. Analysis of dental monomer standards

From the acquired standards, a stock solution of each compound with a concentration of 50 ng/µL in MeOH was prepared. Next, a solution for LC was prepared with a concentration of 1 ng/µL for analysis with the developed LC-QTOF-MS/MS method.

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2.6. Sample preparation of dental resins

For analysis of dental resin materials, approximately 50 mg of sample was dissolved in 50 mL of MeOH in a polypropylene falcon tube. While being protected from light, samples were vortexed and sonicated in two cycles for 30 seconds and 30 min, respectively. Next, samples were centrifuged at 2000 rpm for 5 min to precipitate filler particles. 160 µL of the supernatant was transferred to an amber LC-vial with insert and 40 µL of the internal standard solution was added. Appropriate procedural blanks containing only MeOH were also prepared with the same sample preparation. Before analysis with the developed LC-QTOF-MS/MS method, all samples were stored at -20 °C.

3. Results and discussion

3.1. Mobile Phase Selection Results

Based on previous work by Putzeys *et al,* ammonium adducts were expected for most of the compounds of interest, as they do not possess functional groups which get (de)protonated easily (34). In order to stimulate the formation of these adducts, mobile phases with ammonium were selected. As for some compounds (e.g. TCD-DI-HEA) a protonation was to be expected, acetic acid was also chosen as a possible additive.

For all compounds, the peak width in the SIM method was comparable between the different mobile phase additives. Consequently, peak height and peak area were correlated as shown in Figure SI-1 and the selection of the mobile phase additive was thus done based solely on peak area.

Figure 1 shows a comparison of the peak area of the selected dental monomers with the different mobile phase additives used in this study. Mobile phases with 0.1 % acetic acid and no additives led to the most intense signals. Ions present in MS were mostly sodium adducts and not $[M+H]^+$ or $[M+NH_4]^+$. As the formation of sodium adducts is difficult to control and led to poor fragmentation, both 0.1 % acetic acid and no additives were no longer considered as good options. For the remaining additives, results were comparable with a slightly more intense signal for 2 mM ammonium acetate acidified with acetic acid to pH 3.7. As this additive also showed good peak shape, it was selected for further analysis. Next, analysis with the selected additive were re-run with the MRM method and results were compared with results when adding 1 mM ammonium fluoride as additive. In a study by Pesek *et al*, NH_4F had been evaluated in LC-MS and led to an improvement in sensitivity up to 10 times for these compounds, and was therefore selected for evaluation in this study (37).

Figure 2 shows the combined chromatograms of the standard mixture used for evaluation. Using NH_4F as a mobile phase additive, a small forward shift in retention times (0.2 – 0.3 min) was observed for the monitored compounds which was in agreement with the results of Pesek *et al* (37). Intensity improved when using NH_4F instead of 2 mM ammonium acetate + acetic acid without any sacrifice in peak shape. For further analysis, NH_4F was selected as the additive for mobile phases A and B in a concentration of 1 mM.

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3.2. Suspect screening of dental monomer standards

Before analyzing the different materials, the ingredients for the production of dental resins and sealants have been analyzed in order to understand possible sources for chemicals identified in the materials. Next to the widely used monomers BisEMA, BisGMA, TEGDMA and UDMA we analyzed the older monomer BisDMA as well as the novel proprietary monomer TCD-DI-HEA.

Except for BisGMA and UDMA, all analyzed monomers and the photoinitiator EDMAB showed mainly a response in ESI+. In ESI+ mode TEGDMA, UDMA, BisDMA and EDMAB showed only 1 chromatographic peak, corresponding to the compound of interest. In ESI-, no other compounds have been identified.

3.2.1. BisGMA

BisGMA showed multiple chromatographic peaks (Figure 3). A main peak at 7.5 min and a smaller at 8 min corresponded to the molecular formula and isotopic pattern of BisGMA. Both features share the same fragment ions of ions in MS/MS (Figure SI-2 and Figure SI-3). Based on previous studies, the main peak can be identified as BisGMA, whereas the feature at 8 min is the isomer of BisGMA (iso-BisGMA) (38-40). Next to these isomers, two other compounds were identified in this standard as impurities resulting from the production of BisGMA. The peak at 5.4 min was identified as 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dihydroxy-1-propoxy)]diphenylpropane, a mono-methacrylate (BisGMA-H). 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dimethacryloyloxy-1-propoxy)]diphenylpropane (BisGMA-M) eluted after 9.5 min. These results are in agreement with earlier work (38-40) and show the capability of this method to separate and identify these compounds.

3.2.2. BisEMA standards

Analysis of the different standards of BisEMA showed a large number of co-eluting peaks in the total ion chromatogram. The MS spectra showed differences features with a difference of 44.02 amu, corresponding to an ethoxy group. This suggested a relation between these co-eluting peaks and suspect screening analyses identified different oligomers of BisEMA, of which an example can be seen in Figure 4. Table SI-2 gives an overview of the oligomers identified in the different BisEMA standards. The labelled BisEMA-3 standard contained oligomers ranging from BisEMA-2 to BisEMA-7, the BisEMA-6 standard contained oligomers from BisEMA-2 to BisEMA-12 and the BisEMA-10 standard contained oligomers BisEMA-4 up until BisEMA-13. As the differences in the epoxy chain spacer might have important effects on solubility of the monomer in water, hydrophilicity and viscosity (41), further studies are required to control if composition and thus properties remain identical between different batches of these products.

3.2.3. TCD-DI-HEA

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Multiple peaks corresponding to the molecular formula and isotopic pattern of TCD-DI-HEA were separated and identified when analyzing the reference standard. The isomers eluted at 4.1 – 5.6 minutes in the chromatographic run (Figure 5). However, as all isomers share the same fragment ions, there is no possibility for an unequivocal identification of these isomers on tandem mass spectrometry. Other techniques, such as NMR or Ion Mobility Mass Spectrometry, may be necessary to elucidate the structure of these isomers.

3.3. Screening of unpolymerized dental resins

Due to economic reasons, the inclusion of an internal standard for each compound of interest is not feasible, and thus the identified compounds cannot be quantified in suspect screening. However, a semi-quantitative approach using the respective relative area of identified compounds to the area of an appropriate internal standard can be used to compare levels of identified compounds between samples. As QC for this approach, the variation between samples introduced by autosampler injection was assessed using the relative standard deviation (RSD) of the area of the internal standard. The calculated RSD was 14.4%.

All samples were analyzed in both positive and negative ionization. However, in negative ionization no compounds have been identified that were not detectable in positive ionization. Therefore, only results of the positive ionization are discussed below.

In total, 39 compounds have been identified in the analyzed dental materials. The number of compounds, including isomers, detected per dental material ranged from 2 to 25 with an average of 15. Details on the detected compounds (including relevant MS/MS fragments) can be found in Table 2, a graphical representation of the relative areas per compound per sample can be found in Figure 6 and a detailed table on the relative area of the detected chemicals in the different materials can be found in the Supporting Information (Table SI-3). The most important ingredients in the dental materials, both in detection frequency and relative area were BisEMA (different oligomers), BisGMA, TEGDMA, and UDMA. Previous studies on the composition of dental resins based on their MSDS have also found these compounds to be used in the majority of the analyzed materials (19, 22).

3.3.1. BisEMA

One or more oligomers of BisEMA have been identified in 7 dental materials of which 6 were composites. In most cases, BisEMA was listed on the MSDS documents, except for Dyract Extra where only BisEMA-2 was listed and for Filtek Supreme XTE Universal where only BisEMA-6 was listed, but BisEMA-2 to BisEMA-12 were identified. In addition, Filtek Supreme XTE Universal was the composite with the highest relative areas of BisEMA-6 to BisEMA-12.

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All materials containing BisEMA had several oligomers present which can be related to the mixtures of isomers identified in the suspect screening of the BisEMA standards. As these standards contain a variety of oligomers materials with BisEMA will also contain a range of different oligomers.

3.3.2. BisGMA and related compounds

BisGMA was detected in 50% of the dental materials. The relative area of BisGMA in Filtek Supreme XTE Universal, Fissurit FX, Quadrant Anterior and Venus was greater than 10, whereas the 2 other materials (Ceram.X Universal and N'Durance) contained lower amounts of BisGMA. In only 2 out 6 materials (Filtek Supreme XTE Universal and Fissurit FX) where BisGMA was identified, it was listed as an ingredient on the MSDS. The production impurities BisGMA-H and BisGMA-M, which were identified in the BisGMA standard, were consequently also present in materials containing BisGMA. In all materials where BisGMA was identified, the monomethacrylate BisGMA-H was also detected. The presence of a monomethacrylate in resins containing BisGMA might actually be beneficial as a previous study by Labella *et al* has shown that the resins exhibited a lower shrinkage when mixed with monomethacrylates (42). BisGMA-M, the trimethacrylate, was identified in 5 out of 6 materials. Only in N'Durance BisGMA-M was not detected, possibly due to the relative low amount of BisGMA itself in N'Durance compared to other materials.

The composite Solitaire 2 contained the acrylate monomer 2,2-Propanediylbis(4,1-phenyleneoxy-2-hydroxy-3,1-propanediyl) bisacrylate (Bisphenol A glycidyl acrylate, BisGA) which is structurally closely related to BisGMA.

3.3.3. Ethylene glycol dimethacrylates and related compounds

The monomer triethyleneglycol dimethacrylate (TEGDMA) was detected in 2 out of 3 dental materials. In Ceram.X Universal and Quadrant Anterior, it was not listed on the MSDS, although the relative areas were comparable to other materials where it was stated. TEGDMA is often used as a copolymer for resins containing BisGMA or UDMA as it has a higher flexibility which compensates the rigidity of BisGMA (43). In this study, TEGDMA was an ingredient in 100% of the materials containing BisGMA and in 62.5% of the materials containing UDMA.

Next to TEGDMA, other dimethacrylates with an ethyleneglycol core were detected in lower relative concentrations: diethyleneglycol dimethacrylate (DEGDMA, Detection Frequency (DF) 75%), tetraethyleneglycol dimethacrylate (TEEGDMA, DF 75%), pentaethyleneglycol dimethacrylate (PEGDMA, DF 58.33%) and hexaethyleneglycol dimethacrylate (HEGDMA, DF 58.33%). Of these only PEGDMA was stated on the MSDS (Filtek Supreme XTE Universal).

Many materials where TEGDMA was detected also contained one or more impurities related to TEGDMA. In 6 out of 8 samples with TEGDMA, triethyleneglycol (TEG) was detected and 7 out of 8

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samples contained triethyleneglycol monomethacrylate (TEGMMA). Relative areas of TEG and TEGMMA were lower compared to TEGDMA, but the same trend could be observed within samples. Materials containing more TEGDMA contained also more TEG and TEGMMA.

Two samples where TEGDMA was identified also contained a monomer with a triethyleneglycol core with an acrylic and methacrylic functional group (TEGMAA). Relative areas were however low, when compared to other (meth)acrylates.

3.3.4. UDMA

Urethane dimethacrylate (UDMA) was detected in 8 out of 12 dental materials. In 2 of them (Quadrant Anterior and Solitaire 2), it was not stated on the MSDS although concentrations were in the same range as in materials where UDMA was stated on the MSDS (Filtek Supreme XTE Universal, Fissurit FX, G-ænial Posterior, Gradia Direct Posterior, N'Durance and G-ænial Anterior). Polydorou *et al* have previously reported the existence of different structures of urethane dimethacrylate, all abbreviated as UDMA (44). In this study, we have seen only the chemical with the molecular formula $C_{23}H_{38}N_2O_8$ according to Esstech (44), but there was only 1 chromatographic peak suggestion no separation between the different possible isomers or the presence of only 1 isomer.

3.3.5. Other methacrylates

Bisphenol A propoxy methacrylate (BisPMA) has been identified in 1 material (Filtek Supreme XTE Universal). It has a structural relationship to BisEMA as it also has a bisphenol A core with a linker to the methacrylate group, with BisPMA the length of this linker consists of 3 carbons. However, based on relative areas between BisEMA-oligomers and BisPMA, BisPMA is much less abundant.

Tricyclodecane dimethanol dimethacrylate (TCDDMDMA) was present in the dental composites, G-ænial posterior and Gradia Direct Posterior, both from GC Europe. Trimethylolpropane trimethacrylate (TMPTMA) was identified in several composites from different manufacturers. TCDDMDMA and TMPTMA both have a special core distinguishing them from other methacrylates.

Methyl methacrylate (MMA), the smallest detected methacrylate, was identified in Quadrant Anterior and Venus. Neopentylglycol dimethacrylate (DMPDMA) was only detected in G-ænial anterior and shares a structurally related core to TEGDMA. Isobutyl methacrylate (IBMA) was identified as an ingredient of Solitaire 2. However, the relative areas of MMA, DMPDMA and IBMA were small compared to other methacrylates.

3.3.6. Photo- / co-initiators

To initiate the polymerization reaction of the resins, blue visible light is often applied for a short-time period as defined by the manufacturer. This allows photo- and co-initiators present in the resin to start

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the polymerization reaction. Within our samples, we have identified several initiators. The photoinitiator ethyl 4-(dimethylamino)benzoate (EDMAB) had the highest detection frequency (42 %). 4-N,N-Dimethylaminobenzoic acid butyl ethoxy ester (DMABBE) is a co-initiator added to composites to accelerate the breakdown of initiators and thus also the polymerization (45). The photoinitiator 2,2-Dimethoxy-1,2-diphenylethan-1-one (DMPA) was identified in 2 materials. In one of the composites, diphenyliodonium (DPI) was detected. This compound is added to the formulation in order to increase the degree of conversion, as was proven in a previous study by Dressano et al (46).

Camphorquinone (CQ) is a photoinitiator with a widespread use, but with a difficult detection on LC-ESI-MS due to its structural properties. Most probably, this is the reason why it was not detected in the samples, although present in the in house PCDL database. In several samples, no photo- or co-initiator was detected; other techniques such as GC-MS could be applied to detect the presence of CQ in these (and other) materials.

3.3.7. Bisphenolic Diglycidylethers

In one material (root canal sealar AH Plus), Bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) have been identified. This root canal sealer does not undergo photo polymerization and has to set for 24 h with a complete polymerization after 7 days at 37°C allowing migration of BADGE and BFDGE. This raises concern given the known possible health effects of BADGE and BFDGE (47). Earlier studies by Xue *et al* have already investigated the presence of BADGE and its derivatives in sealants from the US and Korean market and found the different BADGEs in 87% of the analyzed sealants, showing their widespread occurrence in different sealants.

3.3.8. Other compounds

Other identified compounds have different functions in the studied materials. Relative areas of these compounds are generally quite low and detection frequencies do not exceed 33 %. These compounds include 3,5-Di-t-butyl-4-hydroxybenzoic acid methyl ester (BHM). BHM might originate from butylated hydroxytoluene (BHT), either as an impurity or reaction product of BHT with MMA. BHT and other synthetic phenolic antioxidants are known to be a part of dental materials, as was previously investigated by Wang *et al* (35). The photostabilizer 2-hydroxy-4-methoxy benzophenone (HMBP) and 2-methyl-5-methylene-hexadecanoic acid methyl ester (MMHM) were also identified. MMHM might be an impurity resulting from the reaction between 2 MMA molecules during the production of the monomers or materials.

3.3.9. Detection vs listed on MSDS

The number of peaks detected in all samples summed to 180. Of these, only 61 (34%) were stated on the MSDS. Since important monomers such as BisEMA, BisGMA, TEGDMA and UDMA were not always

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stated on the MSDS, this gives an additional perspective on earlier studies on the composition of dental resins. The number of materials containing either one of these monomers could actually be higher than previously reported (22). The results in this study underline once more the need for a correct and complete ingredient list for dental materials, in spite of trade secrets.

3.3.10. False negatives

Several compounds were stated on the MSDS, but have not been detected in the materials. In Filtek Supreme XTE Universal, 2-(2-Hydroxy-5-methacryloyloxyethylphenyl)-2H-benzotriazole (BenzotriazolMA) and EDMAB were listed, but have not been detected. 2(2-Hydroxy-5-methylphenyl) benzotriazole and 2,2-Dimethylpropane-1,3-diyl bis(2-methylprop-2-enoate) (DMPDMA) were stated on the MSDS of respectively in G-ænial posterior and Gradia Direct Posterior, but could not be identified. Dimer Dicarbamate Dimethacrylate (DDCDMA) is a proprietary monomer of Septodont and is listed as an ingredient of N'Durance, but was also not identified with suspect screening.

3.3.11. Limitations

As monomers in dental resins are often derived from BPA, residual BPA can be expected to be present in the dental resin as an impurity from production, as was shown by Xue *et al* (20). As these amounts are below the limit of detection of the instrument on hand, specific targeted methods for bisphenols should be applied to samples as this type of analysis are optimized to detect the low concentrations using specific cleanup steps (33). Also, as shown with the photoinitiator camphorquinone, LC-ESI-MS is not capable to detect all possible compounds present in samples if ionisation efficiency is poor. Application of other (complementary) techniques, such as GC-MS, could unravel the presence of several other compounds in various dental materials. Wang *et al* have identified different synthetic phenolic antioxidants in dental sealants from the US market and these compounds could thus be expected in different materials (35).

In addition, the number of compounds that can be detected and identified depends on the number of chemicals included in the database. In this study, we used available literature and MSDS sheets of materials from different manufacturers to compile a list of 135 chemicals. However, not all chromatographic peaks could be identified, showing the need for additional non-target screening workflows.

4. Conclusions

In the present study, a LC-QTOF-MS/MS method was developed for qualitative screening of dental samples. The use of ammonium fluoride as a mobile phase proved to be an improvement for the sensitivity for detection of monomers and photoinitiators. Application of the developed method on different standards of dental ingredients discovered the presence of a range of oligomers in BisEMA standards, production impurities in BisGMA and isomers of TCD-DI-HEA. The method was applied on

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different dental composite resins and sealants to explore the capability to detect compounds not stated on MSDS. BisGMA, TEGDMA, UDMA and different oligomers of BisEMA were in terms of abundance and detection frequency the most important monomers present in the different materials, but were not always stated on material safety data sheets. Degradation products and impurities of BisGMA and TEGDMA were detected in several samples and one dental root canal sealer contained BADGE and BFDGE.

Conflicts of interest

The authors declare to have no competing financial interests.

5. ACKNOWLEDGEMENTS

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6. ADDITIONAL INFORMATION

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Figures and tables

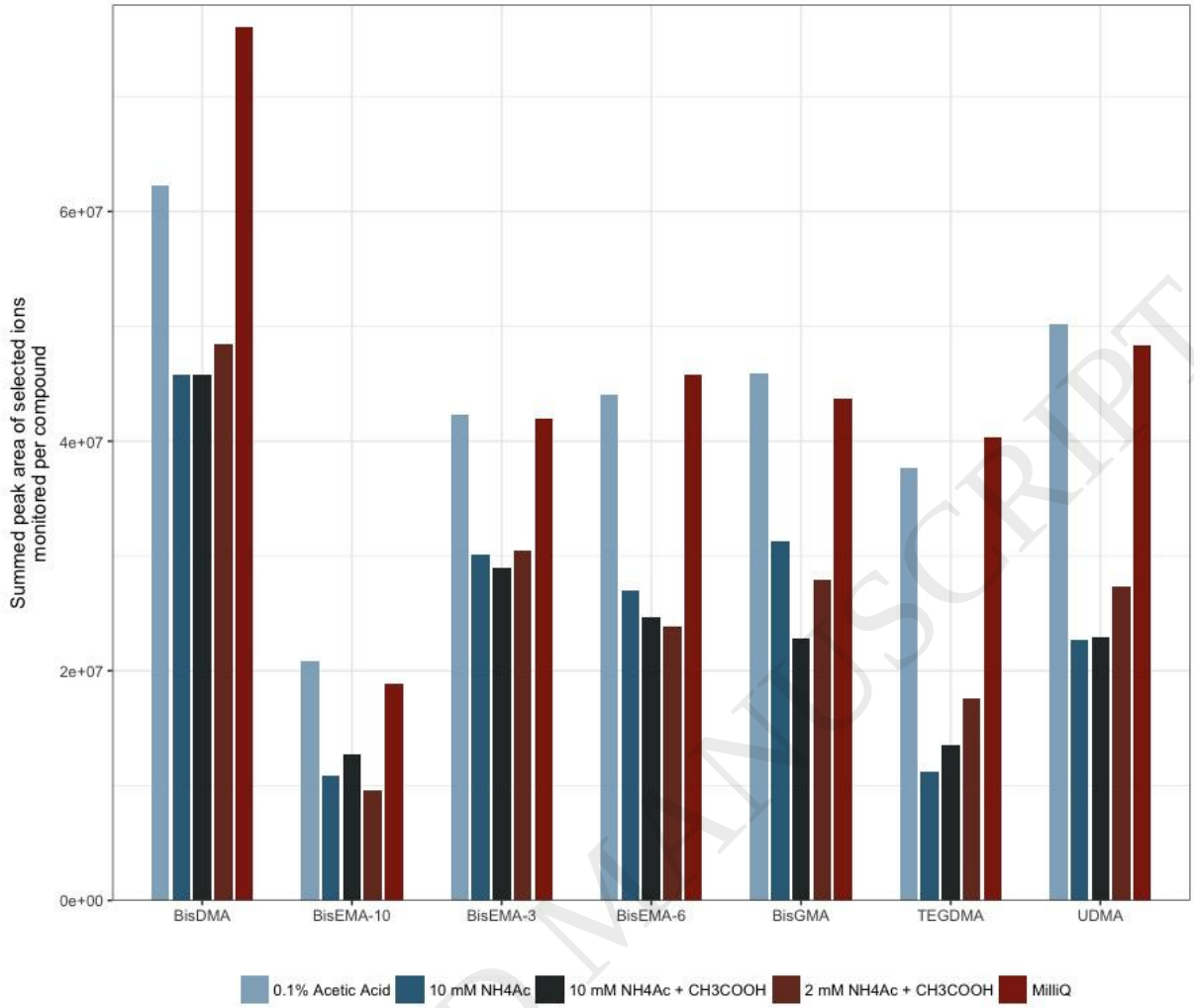

Figure 1: Comparison of summed peak area of $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$ ions monitored of selected dental monomers with different mobile phase additives

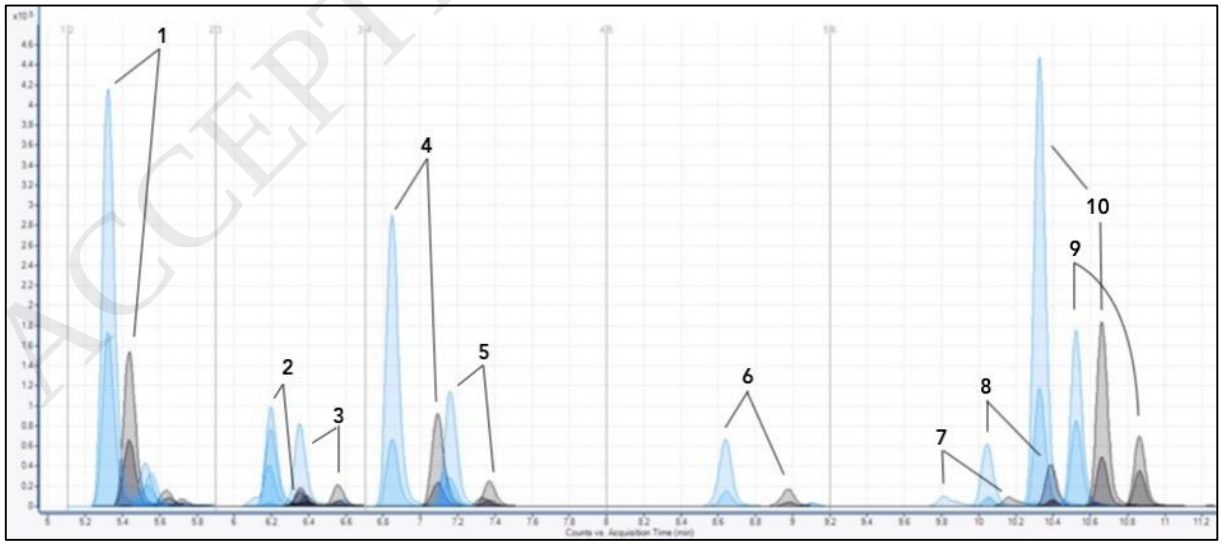

Figure 2: Comparison of chromatograms of the standard mixture using 1 mM NH_4F (blue) or 2 mM NH_4Ac + CH_3COOH (black) as mobile phase additive. For each compound, chromatographic peaks of quantifier and qualifier transitions are shown. 1: TEGDMA; 2: EDMAB; 3: TCD-DI-HEA; 4: UDMA; 5: dUDMA; 6: BisGMA; 7: BisEMA-10; 8: BisEMA-6; 9: BisEMA-3; 10: BisDMA.

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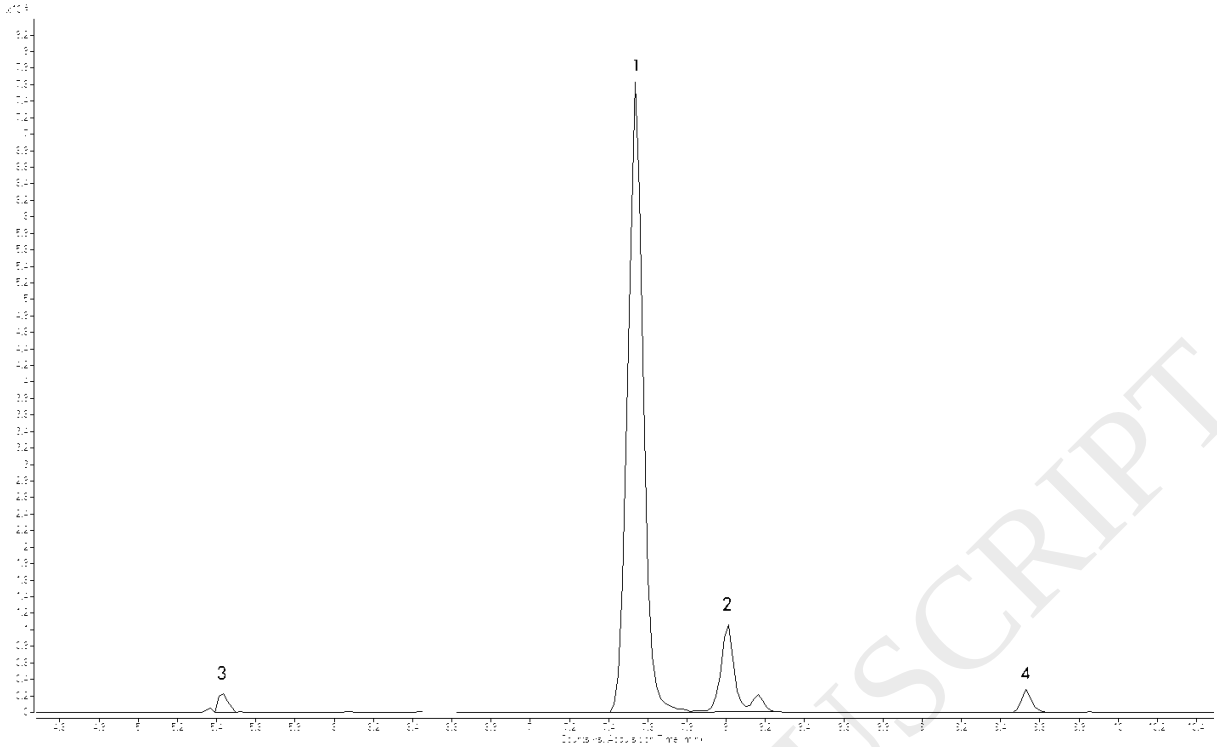

Figure 3: Total ion chromatogram of compounds identified in the reference standard of BisGMA. 1: BisGMA ; 2: iso-BisGMA ; 3: monomethacrylate BisGMA-H ; 4: trimethacrylate BisGMA-M

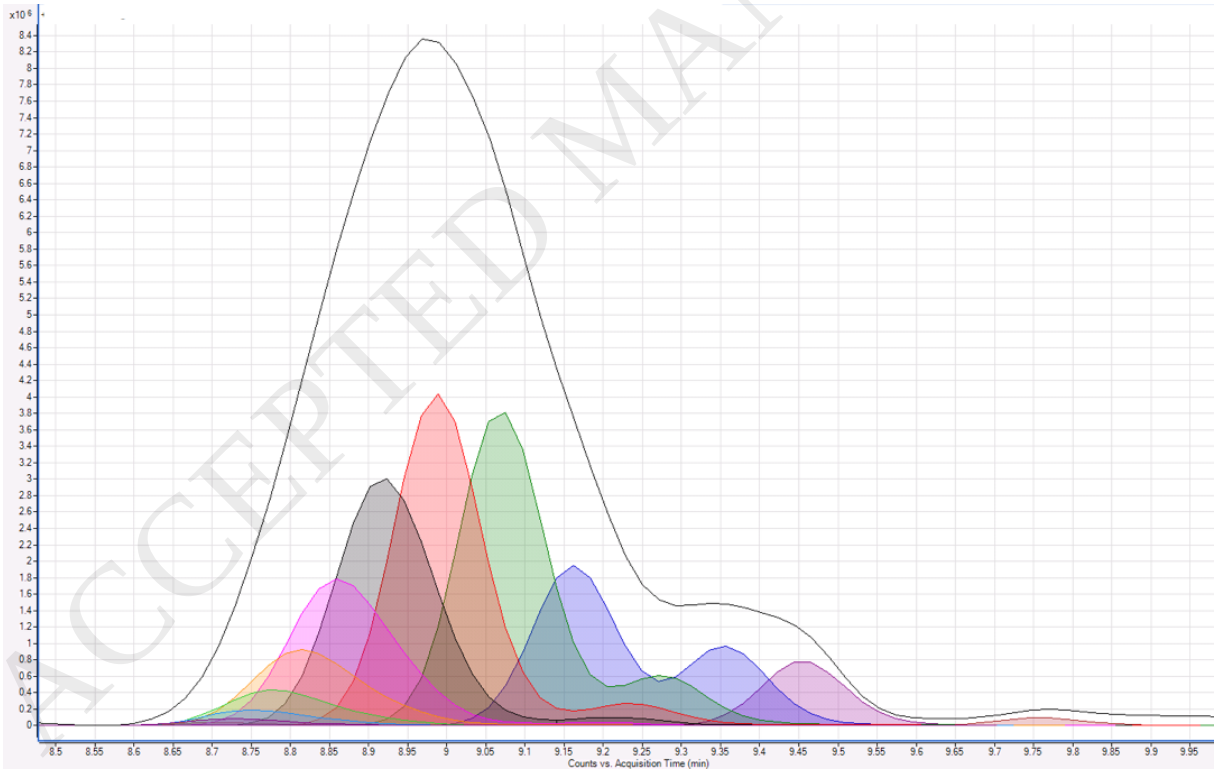

Figure 4: Example of a chromatogram of an injection of the BisEMA-6 standard. In black the TIC, in color the different EICs of BisEMA-2 to BisEMA-13.

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Figure 5: Tentative structure (A) and Total Ion Chromatogram (B) of the monomer TCD-DI-HEA

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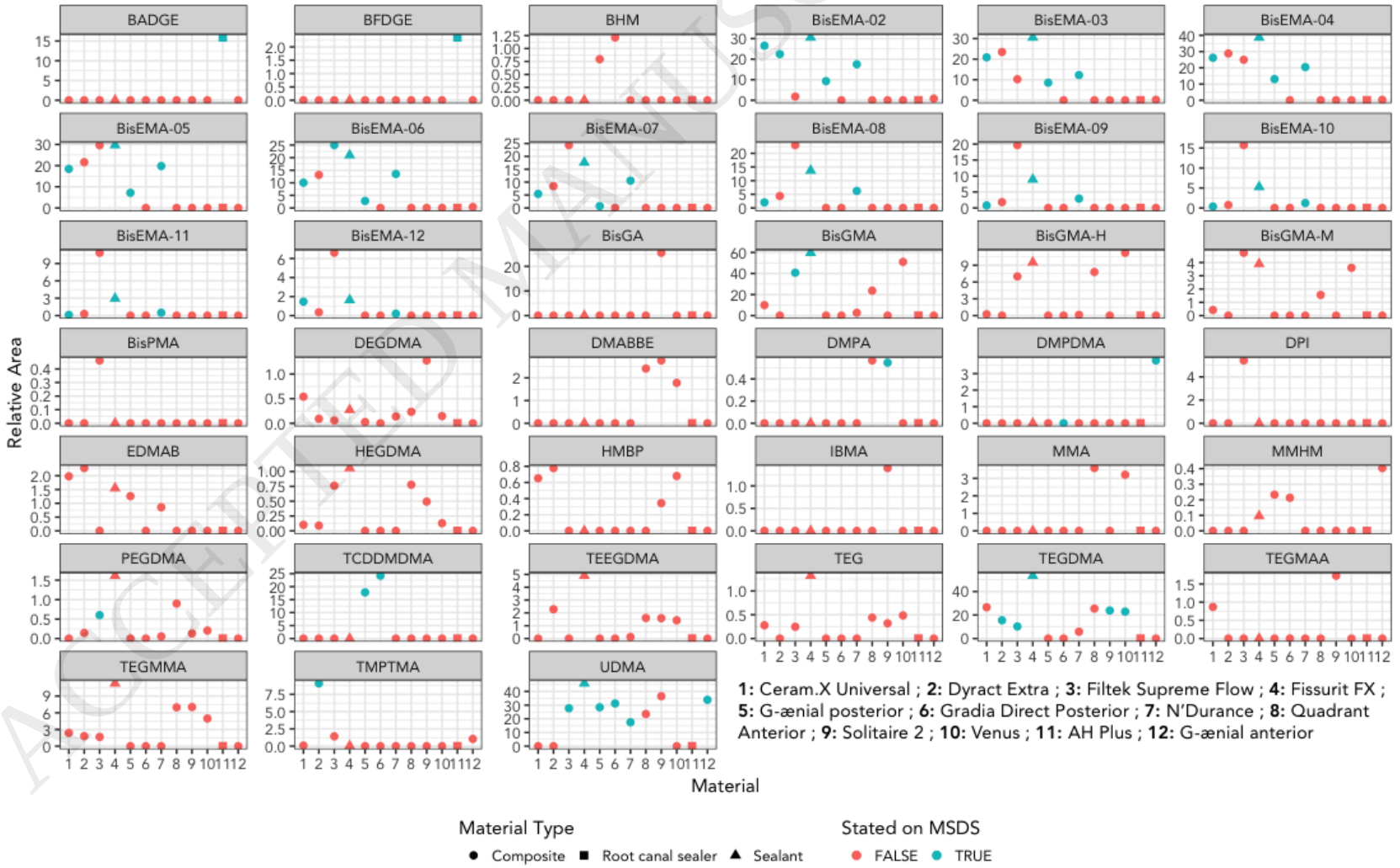

Figure 6: Graphical representation plotting the relative area of the compounds identified in the suspect screening of different dental composites (●), root canal sealer (■) and sealant (▲). Points colored in red are not stated on safety data sheets of the materials, points colored in blue were stated.

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Table 1: Overview of selected monomers and photoinitiators used for optimisation of the mobile phase additive. For each compound the retention time with NH_4F in MRM, monitored SIM ions ($[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$) where applicable and MRM parameters (precursor and qualifier/quantifier m/z values with collision energy (CE) applied of the LC-QqQ analysis are shown.

Compound	RT (min)	SIM ions (m/z)	MRM transition 1 (m/z)	Collision energy 1 (V)	MRM Transition 2 (m/z)	Collision energy 2 (V)
TEGDMA	5.32	287 / 304 / 309	304 → 113	10	287 → 113	20
IS-dT	5.52	NA	314 → 119	10	295 → 118	10
EDMAB	6.20	NA	194 → 151	20	194 → 134	30
TCD-DI-HEA	6.35	NA	479 → 99	15	501 → 429	25
UDMA	6.85	471 / 488 / 493	471 → 113	20	488 → 113	20
d-UDMA	7.13	NA	481 → 119	10	/	/
BisGMA	8.64	513 / 530 / 535	530 → 143	25	530 → 277	10
BisEMA-10	9.81	805 / 822 / 827	822 → 113	35	822 → 69	40
BisEMA-6	10.04	629 / 646 / 651	646 → 113	30	646 → 291	20
BisDMA	10.33	365 / 382 / 387	382 → 203	10	382 → 365	10
BisEMA-3	10.52	497 / 514 / 519	514 → 113	20	514 → 247	10

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Table 2: Overview of identified compounds in dental composites and sealants.

Compound	Formula	RT (min)	Precursor ion (MS-TOF) [m/z]	Adduct Type	Δm (ppm)	Product ions (MS/MS-TOF) [m/z]	Confidence level▲	Detection Frequency (%)
BADGE	C21H24O4	6.15	358.2005	NH4	-2.30	341.1737 ; 191.1068 ; 161.0959 ; 135.0801 ; 107.0488 ; 57.0332	L1	8
BFDGE	C19H20O4	5.15	330.1722	NH4	6.59	189.0902 ; 163.0707 ; 133.0642 ; 91.0507	L2b	8
BHM	C16H24O3	6.48	282.2065	NH4	0.63	265.1778 ; 247.1697 ; 179.1424 ; 161.1311	L2b	17
BisEMA-2	C27H32O6	9.92	470.2522	NH4	-3.23	453.2269 ; 247.1317 ; 113.0588 ; 69.0328	L2b	58
BisEMA-3	C29H36O7	9.61	514.2801	NH4	0.34	497.2501 ; 291.1576 ; 247.1316 ; 113.0597 ; 69.0338	L1	58
BisEMA-4	C31H40O8	9.32	558.3093	NH4	5.69	541.2799 ; 335.1849 ; 291.1597 ; 247.1324 ; 113.0595 ; 69.0334	L2b	58
BisEMA-5	C33H44O9	9.21	602.3353	NH4	4.94	379.2136 ; 335.1858 ; 291.1593 ; 247.1337 ; 113.0592 ; 69.0331	L2b	50
BisEMA-6	C35H48O10	9.14	646.3606	NH4	3.21	379.2125 ; 335.1866 ; 291.1595 ; 113.0605 ; 69.0343	L1	58
BisEMA-7	C37H52O11	9.11	690.3846	NH4	-0.32	423.2381 ; 379.2113 ; 335.1857 ; 291.1601 ; 113.0604 ; 69.0344	L2b	50
BisEMA-8	C39H56O12	9.08	734.4133	NH4	3.10	467.2663 ; 423.2402 ; 379.2159 ; 335.1880 ; 291.1609 ; 113.0615	L2b	42
BisEMA-9	C41H60O13	9.02	778.4391	NH4	2.41	467.2680 ; 423.2432 ; 379.2169 ; 335.1888 ; 291.1626 ; 113.0618	L2b	42
BisEMA-10	C43H64O14	8.94	822.4699	NH4	7.88	467.2633 ; 423.2377 ; 379.2110 ; 335.1859 ; 291.1595 ; 113.0603	L1	42
BisEMA-11	C45H68O15	8.86	846.492	NH4	2.74	511.2906 ; 467.2640 ; 423.2387 ; 379.2119 ; 335.1866 ; 113.0605	L2b	42
BisEMA-12	C47H72O16	8.75	910.5183	NH4	2.72	511.2904 ; 467.2639 ; 423.2373 ; 379.2102 ; 335.1852 ; 113.0599	L2b	42
BisGA	C27H32O8	6.14	502.2447	NH4	2.34	467.2102 ; 263.1290 ; 173.0972 ; 129.0558 ; 55.0191	L2b	8
BisGMA	C29H36O8	7.76	530.2801	NH4	9.95	495.2386 ; 427.2128 ; 277.1437 ; 173.0966 ; 143.0707 ; 135.0810 ; 87.0448 ; 69.0341	L1	50
BisGMA-H	C25H32O7	5.48	462.2488	NH4	0.38	427.2106 ; 277.1420 ; 209.1161 ; 143.0695 ; 135.0796 ; 69.0334	L2b	50
BisGMA-M	C33H40O9	9.72	598.3042	NH4	5.31	563.2674 ; 495.2417 ; 277.1452 ; 173.0964 ; 143.0707 ; 69.0343	L2b	42
BisPMA	C29H36O6	9.75	498.2866	NH4	3.16	N.A.	L1	8
DEGDMA	C12H18O5	4.16	243.1226	H	-0.31	113.0571 ; 55.0171	L2b	75
DMABBE	C15H23NO3	6.77	266.1772	H	7.98	192.1009 ; 166.0857 ; 148.0756 ; 77.0391	L2b	25
DMPA	C16H16O3	5.31	264.0679	NH4	9.00	N.A.	L4	17
DMPDMA	C13H20O4	6.06	241.1456	H	8.81	155.1084 ; 87.0447 ; 69.0349	L2b	8
DPI	C12H10I	2.73	280.9824	-electron	-1.07	154.0768 ; 77.0386 ; 51.0232	L2b	8
EDMAB	C11H15NO2	5.19	194.1172	H	-1.94	179.0920 ; 166.0843; 151.0603 ; 134.0577	L1	42
HEGDMA	C20H34O9	4.32	436.2561	NH4	4.53	419.2297 ; 289.1625 ; 245.1403 ; 201.1149 ; 157.0862 ; 113.0600 ; 69.0337	L2b	58
HMBP	C14H12O3	6.16	229.0858	H	-0.33	151.0353 ; 105.0309 ; 95.0470 ; 77.0370	L2a*	33
IBMA	C8H14O2	1.45	160.1331	NH4	-0.77	86.0964 ; 58.0413	L2b	8
MMA	C5H8O2	1.09	118.0873	NH4	9.12	59.0729 ; 58.0655	L2b	17
MMHM	C10H16O4	4.46	218.1388	NH4	0.35	106.0123 ; 88.0020 ; 57.0699	L2b	33
PEGDMA	C18H30O8	4.23	392.2289	NH4	2.49	375.2038 ; 245.1428 ; 201.1092 ; 157.0864 ; 113.0615 ; 69.0352	L2b	58

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TCDDMDMA	C20H28O4	9.84	333.2086	H	7.57	247.1675 ; 161.1320 ; 133.1007 ; 67.0543	L2b	17
TEEGDMA	C16H26O7	4.20	348.2008	NH4	-2.65	331.1757 ; 245.1404 ; 157.0869 ; 113.0610 ; 81.0699 ; 69.0350	L2b	50
TEG	C6H14O4	1.82	151.0966	H	0.82	89.0594	L2b	50
TEGDMA	C14H22O6	4.21	304.1776	NH4	7.16	287.1469 ; 113.0589 ; 69.0332	L1	67
TEGMAA	C13H20O6	3.71	290.1599	NH4	0.27	273.1325 ; 113.0595 ; 99.0439 ; 69.0335 ; 55.0179	L2b	17
TEGMMA	C10H18O5	3.15	236.1485	NH4	-3.06	219.1236 ; 133.0851; 113.0595 ; 89. 0596 ; 69.0330	L2b	58
TMPTMA	C18H26O6	7.25	356.2097	NH4	8.36	253.1461 ; 99.0814 ; 81.0710 ; 69.0347	L2a*	42
UDMA	C23H38N2O8	5.93	471.2709	H	1.75	385.2323; 255.1692 ; 113.0599 ; 69.0339	L1	67

▲*: Confidence levels according to Schymanski et al(48). Level 1 (L1): confirmed using a reference standard; Level 2a (L2a): Probable structure by library spectrum match; level 2b (L2b): Probable structure by diagnostic evidence (MS/MS), level 4 (L4): Unequivocal molecular formula using MS isotopes/adducts ;* *******: confirmed using mzCloud (https://www.mzcloud.org)*

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