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Quantification of organic eluates from polymerized resin-based dental restorative materials by use of GC/MS

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Abstract

Residual monomers, additives and degradation products from resin-based dental restorative materials eluted into the oral cavity may influence the biocompatibility of these materials. Emphasis has been placed on studies addressing cytotoxic, genotoxic and estrogenic potential of these substances. A prerequisite for analyzing the potential of exposure to eluted compounds from dental materials is reliable quantification methods, both real time and accelerated measurements. The purpose of the present study was to quantify nine eluates; 2-hydroxyethyl methacrylate (HEMA), hydroquinone monomethyl ether (MEHQ), camphorquinone (CQ), butylated hydroxytoluene (BHT), ethyl 4-(dimethylamino)benzoate (DMABEE), triethylene glycoldimethacrylate (TEGDMA), trimethylolpropane trimethacrylate (TMPTMA), oxybenzone (HMBP) and drometrizole (TIN P) leaching from specimens of four commonly used resin-based dental materials in ethanol and an aqueous solution. All analyses were performed by use of GC/MS, each component was quantified separately and the results presented in $\mu g\,mm^{-2}$. This study has shown that elution from various materials differs significantly, not only in the types of eluates, but also regarding amounts of total and of single components. A high amount of HMBP, a UV stabilizer with potential estrogenic activity, was detected from one material in both solutions.

Keywords: Quantification; Monomers; Gas chromatography/mass spectrometry; Resin-based dental composites; Eluates

1. Introduction

Dental resin-based restorative materials are complex polymers containing a variety of monomers, initiators, activators, stabilizers, plasticizers and other additives. The main organic ingredients are large monomers which during polymerization crosslink with smaller monomers to create a rigid polymer network. As the crosslinking propagates, diffusion inside the network is restricted, and complete cure is therefore not possible to achieve. The residual monomers and additives that are not chemically bond to the network are free to diffuse out from the cured materials. Several studies have shown that many of these compounds are leaching from the filling materials even after adequate polymerization [1–7]. It is known that some of the ingredients in the resin-based materials have cytotoxic [8–14], genotoxic [12,13] or allergenic effects [15–18] and/or exhibit estrogenic activity *in vitro* [19,20]. By use of chromatographic and mass spectrometric techniques, monomers and additives have been identified in aqueous and alcohol extracts of polymerized dental fillings [1–6,21]. In most studies, a limited number of compounds (mainly monomers) are quantified, few materials are investigated, and the results are sometimes contradictory. This might be explained by the fact that the quantitative results are obtained with different methods and presented in different ways. It is therefore difficult to compare various materials and the amount of single ingredients that can be extracted. The most extensive study was performed by Spahl et al. [2], who studied a large number of compounds eluted into water and methanol from four different resin-based materials. The various eluted compounds were not quantified separately, but their response in gas chromatograph/mass spectrometer (GC/MS) or liquid chromatograph/particle beam interface/mass spectrometer (LC/PB/MS) was compared to the response of caffeine.

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The adverse potential of leachables and degradation products, and the stability of the materials *in vivo*, is essential to consider the safety of a dental material. To evaluate the exposure of elutes to the human body, we need information about elution pattern and toxicokinetic factors, as well as reliable methods to measure the release of eluates. Both real time and accelerated measurements provides useful information about exposure in dose and time.

Recently we identified 32 eluates from four different resin-based dental filling materials [5]. The purpose of the present study was to quantify nine different leachables representing the various groups of ingredients; monomers, initiators, accelerators, inhibitors and stabilizers, from four different resin-based dental restorative materials (two composites, one compomer and one resin modified glass ionomer cement). The analytes were extracted into in ethanol and an aqueous solution and the quantitative results are presented as $\mu g/mm^2$ of specimen surface of the dental material.

2. Materials and methods

2.1. Standards and solvents

All standards listed in Table 1 were of analytical grade and obtained from Sigma–Aldrich, Oslo, Norway. Diethyl phthalate was purchased from Merck-Schuchardt, Hohenbrunn bei Munchen, Germany, and used as internal standard (I.S.). Ethanol was obtained from Arcus, Bergen, Norway. Ethyl acetate, NaOH and HCl were obtained from Merck, Darmstadt, Germany. All solvents and diethyl phthalate were checked to ensure they contained no compounds interfering with the analysis.

2.2. Preparation of specimens

Four different resin-based dental restorative materials were investigated (Table 2). The applied leaching model has previously been described in detail by Michelsen et al. [5].

Cylindrical stainless steel moulds were filled with uncured material to produce specimens with a diameter of 6 mm and a thickness of 2 mm. Care was taken to avoid air bubbles. Fourteen specimens of each material were prepared. The uncured materials were covered with a polyester film (Odus universal-strips, Odus Dental AG) and a glass plate to exclude the oxygen-inhibiting layer, and were polymerized by visible light with an Optilux 400 curing lamp (Demetron Research Corp., Danbury, CT, USA). The 14 specimens of each material were cured for 40 s. The light intensity was measured to be above 350 mW/cm^2 by a Curing Radiometer Model 100 (Demetron Research Corp.). Polymerization time of 40 s was in agreement with specification from the manufacturer for TC, DY and FU. For FZ, the manufacturer recommended a polymerization time of 20 s. However, after pilot studies we decided to apply the same curing time for all the materials.

Specimens were immersed in ethanol or Ringer's solution (9.0 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2 \cdot 2H_2O$, in distilled water, total volume 1 l, pH adjusted to 7 with NaOH or HCl). Two series of seven glass tubes were prepared, one set with each glass tube containing 3 ml of Ringer's solution, and one parallel set with each glass tube containing 5 ml ethanol. The cured specimens were detached from the stainless steel moulds, and seven parallel specimens from each resin-based material were immediately immersed in Ringer's solution in the separate glass tubes. An identical series of seven parallel specimens was immersed likewise separately in the glass tubes containing ethanol. The glass tubes were secured with a ground glass stopper to prevent evaporation, and kept at 37 °C with constant agitation (200 rpm).

2.3. Specimens in ethanol

After 24 h, 1 ml of each ethanol solution was transferred to separate 10.5 ml glass vials (Karl Hecht, Germany), each vial

Table 1
The following authentic reference substances were used

Eluate	Cas nr	Mol. formula	Trivial name	Monoisotopic MW
HEMA	868-77-9	$C_6H_{10}O_3$	2-Hydroxyethyl methacrylate	130.1
MEHQ	150-76-5	$C_7H_8O_2$	4-Methoxyphenol (mequinol)	124.1
CQ	10373-78-1	$C_{10}H_{14}O_2$	(±)-Camphorquinone	166.1
BHT	128-37-0	$C_{15}H_{24}O$	Butylated hydroxytoluene	220.2
DMABEE	10287-53-3	$C_{11}H_{15}NO_2$	Ethyl 4-(dimethylamino)benzoate	193.1
TEGDMA	109-16-0	$C_{14}H_{22}O_6$	Triethyleneglycol dimethacrylate	286.1
TMPTMA	3290-92-4	$C_{18}H_{26}O_6$	Trimethylolpropane trimethacrylate	338.2
HMBP	131-57-7	$C_{14}H_{12}O_3$	2-Hydroxy-4-methoxybenzophenone (oxybenzone)	228.1
TIN P	2440-22-4	$C_{13}H_{11}N_3O$	2-(2-Hydroxy-5-methylphenyl) benzotriazole (drometrizole)	225.1

Table 2
The four different resin-based dental restorative materials investigated

Abbreviation	Type of material	Product name	Specifications	Manufacturer
TC	Composite	Tetric Ceram	Color A3, lot B42131	Vivadent Ets. Schaan, Lichtenstein
FZ	Composite	3M™ Filtek™ Z250	Color A3, lot 19991122	3M Svenska AB, Sollentuna, Sweden
DY	Compomer	Dyract AP	Color A3, lot 9909000451	Dentsply DeTrey GmbH, Konstanz, Germany
FU	Resin modified glass ionomer cement	GC Fuji II LC	Color A3, lot 080291	GC Corporation, Tokyo, Japan

containing 1 ml of ethyl acetate with an internal standard of diethyl phthalate (2 μg/ml). The solutions were evaporated to approximately 200 μl at 60 °C, and transferred to sample vials (Cromacol, London, UK).

2.4. Specimens in Ringer's solution

The specimens in Ringer's solution were removed from the glass tubes after 7 days. One millilitre of freshly distilled ethyl acetate with an internal standard (diethyl phthalate 2 μg/ml) was added to each of the seven parallel solutions, agitated for 1 min and rested. The solution from each glass tube was extracted three times with 2 ml of freshly distilled ethyl acetate and the extracts pooled for each sample. The seven pooled extracts were transferred to seven 10.5 ml glass vials (Karl Hecht, Germany), evaporated at 60 °C to approximately 200 μl and transferred to sample vials.

2.5. Separation by gas chromatography

The analyses were performed by using combined GC/MS. The instrument was a Thermo Quest Trace GC connected to a Finnigan MD 800 quadropol mass spectrometer. The GC was further equipped with an autosampler (Finnigan AS-800, Thermo Quest). For chromatographic separation, we used a capillary column with following specifications: CP-SIL 8 CB wall-coated open tubular (WCOT) low bleed fused silica MS column with length 30 m, i.d. of 0.25 mm and a film thickness of 0.25 μm (Chrompack, Middelburg, The Netherlands). The carrier gas was helium with a flow rate of 1 ml/min, constant flow. Splitless injection was used, injector temperature was 250 °C and purge flow of helium gas was 70 ml/min. The temperature program for the oven: start point at 50 °C, with a rate of 50 °C/min up to 120 °C, hold time at 120 °C for 5 min, from 120 to 280 °C with rate of 10 °C/min, hold time at 280 °C for 1 min. The syringe was rinsed with ethyl acetate five times before and after every injection. A hole with diameter of 3 mm was made in the rubber septum of the sample vial, to prevent septum particles to contaminate the sample following needle perforation. Evaporation of the sample was prevented with aluminum foil as a seal between the septum and the vial. The oven program and analyses were performed using the software package Xcalibur (Xcalibur™, Finnigan Corp.).

2.6. Mass spectrometric detection

Identification and quantification of the analytes were performed by using the mass spectrometer in full scan mode, scanning from 50 to 350 *m*/z. The identification of the different compounds was then based on comparison of the obtained full scan spectra with spectra in the NIST library (National Institute of Science and Technology, Gaithersburg, MD, USA), retention time (Table 3) and spectra of the reference substances. The quantifications were performed by constructing mass fragmentograms of abundant ions characteristic for each different analyte (Table 3), and comparing the area under each peak with the area of the internal standard peak.

2.7. Calibration curves

Calibration curves and response factors were computed with reference substances analyzed with the previously described method in five different concentrations for each compound; from 0.7 to 30 μg/ml with diethyl phthalate, 2 μg/ml, as internal standard.

2.8. Blanks and recovery

Ethanol, ethyl acetate and water were distilled twice to eliminate contaminants. The solvents were then subjected to the same treatment and extraction procedure as the samples, and analyzed by GC/MS. No peaks were found, and the blanks were considered to be without compounds interfering with the analysis. To avoid contamination from other polymer-based materials and plastics, gloves were not used, and all procedures were performed with metal instruments and glassware. Glassware and instruments were rinsed in distilled ethyl acetate twice, wrapped in aluminum foil and kept at 100 °C for at least 12 h before use. The foil was washed with ethyl acetate before wrapping the equipment. Foil and polyester film were tested for leachables, and no contaminating peaks were found.

The relative recovery of each analyte compared to the I.S. was tested with a solution of ethyl acetate with reference substances in concentrations 1 and 10 μg/ml and I.S. of 2 μg/ml. The ratios of amounts were compared before and after evaporation. Furthermore, reference substances in concentrations 1 and 10 μg/ml were added to Ringer's solution and measured after extraction and evaporation. The ratios to I.S. were compared to the ratios from the initial solution in ethyl acetate.

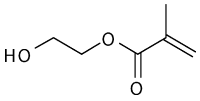
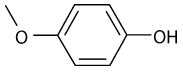
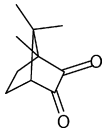
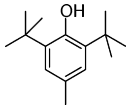
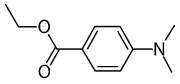
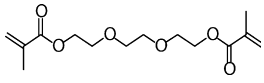
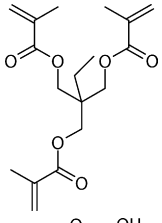
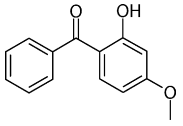
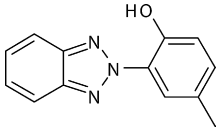
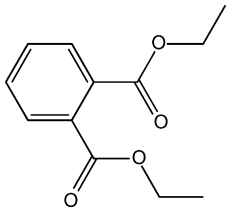
Lowest limit of detection, LOD, ≥3 S/N (signal to noise), and lowest limit of quantification, LOQ, ≥10 S/N, was found by analyzing reference compounds in concentrations from 0.001 to 10 μg/ml. Precision was tested with a reference cocktail in two concentrations, 1 and 10 μg/ml, and given as the standard deviation (S.D.) and relative standard deviation (R.S.D.) between repeated measurements for within-day and between-day measurements.

From the full scan spectra of each reference substance, one or two characteristic mass fragment was selected, preferably the base peak and/or the molecule ion (Table 3). The peak areas of these specific fragments in each sample of the reference substances were integrated and all integrations manually adjusted if necessary. Area ratios and response factors were incorporated in the calculation procedure, and the amounts of each eluate in each sample were computed.

3. Statistical methods

The results are presented as mean values with associated standard deviations (Fig. 2A and B). The Student's Independent-Samples *t*-test was used to test if

Table 3
Reference substances given with their function within the material, the molecular ion, characteristic ions, structure formula and retention time (The same parameters are given for I.S.)

Reference substance	Function	Molecular ion, *m/z*	Characteristic ions, *m/z*	Structure formula	RT (min)
HEMA	Monomer	130	69[a], 87		3.12
MEHQ	Inhibitor	124	109[a], 124		5.97
CQ	Initiator	166	95[a], 138, 166		8.21
BHT	Inhibitor	220	205[a], 220		11.71
DMABEE	Accelerator	193	148[a], 164, 193		14.60
TEGDMA	Monomer	286	69, 113[a]		16.33
TMPTMA	Monomer	338	69[a], 253		17.80
HMBP	UV stabilizer	228	151[a], 227		18.19
TIN P	UV stabilizer	225	225[a]		18.58
Internal standard:					
Diethyl phthalate	I.S.	222	149[a], 177		12.96

[a] Illustrates base peak.

observed differences in mean values of each compound were significant; in both solutions, and between each compound eluting from the various materials. The significance levels were expressed as two-tailed values and significance level was set at 0.05. The calculations were performed by using SPSS software (SPSS Inc., Chicago, USA).

4. Results and discussion

4.1. Materials

Composites, compomers and resin modified glass ionomer cements all contain an organic polymer matrix with inorganic filling particles embedded. Yet, ingredients differ greatly

Table 4
Eluates and given ingredients from the four materials

Eluates/ingredients	TC		FZ		DY		FU	
	Detected[a]	MSDS	Detected[a]	MSDS	Detected[a]	MSDS	Detected[a]	MSDS
HEMA	*x*		*x*		*x*		*x*	*x*
MEHQ	*x*		*x*		*x*		*x*	
CQ	*x*		*x*		*x*		*x*	*x*
BHT			*x*		*x*		*x*	*x*
DMABEE	*x*		*x*		*x*			
TEGDMA	*x*	*x*	*x*	*x*				
TMPTMA					*x*	*x*		
HMBP					*x*			
TIN P	*x*							

In the column marked MSDS, *x* represents ingredients given in the MSDS from the manufacturer.
[a] Represents eluates detected in amounts higher than limit of quantification.

concerning types and amounts [5]. The Material Safety Data Sheets (MSDS) are known to be incomplete and sometimes misguiding [22,23]. In the MSDS, the manufacturers are obliged to give information about the main ingredients (≥1%). Most additives and some monomers are present in concentrations below 1% and therefore information about these compounds is not given (Table 4). Furthermore, some compounds found in the materials are not purposely added by the producers, but are remnants from the synthesis of the raw materials, like stabilizers and catalysts, i.e. triphenyl antimony [2,3,5]. The exposure to components in minor amounts (less than 1%), however, cannot be excluded to be responsible for allergic or other adverse effects.

The weight percent of resin components in resin-based dental restorative materials is higher in composites than in compomers. Furthermore, the presence of resin components is lower in the resin modified glass ionomer materials. Monomers, the main organic ingredients, range from small molecules to high molecular weight substances. During polymerization the monomers crosslink to create a polymeric matrix. The polymerization is however, not complete, leaving up to 10% of residual monomers capable of leaching out [24]. Residual low molecular weight monomers, like HEMA and TEGDMA, are relatively mobile, and may diffuse through the matrix into an immersion medium. High molecular weight monomers like Bis-GMA (2,2-bis[4-(2′-hydroxy-3′-methacryloxypropoxy)phenyl]propane) are rigid and hydrophobic, and not likely to diffuse out from the materials [24]. The various additives, besides being of low molecular weight, are often not included in the polymerized network and are therefore easily eluted.

4.2. Immersion media

The type of immersion media has a substantial effect on the rate of elution of the elutable molecules. To simulate an oral environment, we have used Ringer's solution to extract the eluates from the cured specimens. Ringer's solution was used because it has previously been employed as a physiological saline solution [25]. Aqueous solvents are not able to extract the total amount of eluates from the cured specimens. Therefore, we have also used ethanol as an extracting solvent to be able to estimate the total amount of compounds that might leak from the actual filling materials. As expected, higher amounts of eluates were found in ethanol solutions compared to in Ringer's solutions, Fig. 2A and B. This is especially pronounced from the resin modified glass ionomer cement specimens. The choice of elution time was based on pilot studies, which demonstrated that elution in ethanol was close to completed within the first 24 h, whereas in Ringer's solution the elution was considered completed after 7 days. Ferracane and Condon found 75–100% of the leachable components to be eluted from composite specimens into ethanol within the first few hours, and component release was considered essentially complete after 24 h both in 75% ethanol and in water [26]. In some studies, the specimens are allowed to desiccate for 24 h to 7 days prior to immersion in media [27,28]. However, in our experiment the specimens were polymerized and immediately immersed in media, since this is clinically more relevant. A strong correlation between the surface area of the specimens and the amounts of eluted TEGDMA in short time elution was demonstrated by Pelka et al. [29]. This is the reason why we express the elution in μg/mm^2. In the study the mass/volume ratio between the specimens and the test solutions were at least 1:10, and the specimens were fully immersed in the test solutions according to ISO 10993-13 [30].

4.3. Evaporation, extraction and recovery

The extraction into ethyl acetate of eluatable compounds from Ringer's solution was performed three times. By use of GC/MS, we confirmed in pilot studies that eluates were present in the first and in the second extraction. The analysis of the third, fourth and fifth extract, however, displayed no detectable compounds; indicating that three extractions should be sufficient. Recovery test after extraction and evaporation, and evaporation exclusively, showed 72–103% and 86–107% recovery, respectively. This was performed for all analytes in two different concentrations, 1 and 10 μg/ml. In the analysis of specimens, it is possible that a portion of the detected HEMA might be a result of the decomposition of larger monomers like UEDMA (Urethane modified Bis-GMA). To confirm this, we analyzed standard samples of the most

common monomers in dental composites; UEDMA, Bis-EMA (2,2-bis[4-(2′-methacrylyloxyethoxy)phenyl]propane) and Bis-GMA and found minor amounts of HEMA decomposed from UEDMA, in accordance with findings of Spahl et al. [2,31].

4.4. GC/MS

The monomers in resin-based dental restorative materials range from low to high molecular weight (MW) substances. In the analysis of low MW monomers and additives GC/MS based methods are to be preferred [32]. Analysis of the high molecular weight monomers Bis-GMA, Bis-EMA and UEDMA are better performed by use of high performance liquid chromatography, HPLC [2,31]. All analytes investigated here can be classified as low MW compounds, therefore, all analysis were performed by the use of GC/MS.

Fig. 1 displays chromatograms of elution from the specimens immersed in Ringer's solution. All peaks were sharp and symmetrical, except from MEHQ, which gave broader peaks. Ethyl acetate was injected between the samples series from each material to check if there were any carry-over effects during analysis. No peaks were observed above LOD.

4.5. Limit of quantification and limit of detection

In our analysis, the limit of detection, LOD, varies between the different substances, and was between 0.01 and 1 μg/ml. Low weight molecules needed higher concentrations to be detected. Limit of quantification, LOQ, was between 0.1 and 1 μg/ml. Lower amounts can be detected by using the GC/MS in selected ion monitoring (SIM) mode. However, at this stage we wanted to ensure a reliable identification of the eluted compounds, therefore, the mass spectrometer was used in full scan mode.

4.6. Precision

Within-day precision between injections of reference substances was measured as the standard deviation (S.D.) from 0.150 to 0.002, and the relative standard deviation (R.S.D.) from 0.451 to 0.018%. Between-day variation calculated during 2 days, was between 0.159 and 0.003 (S.D.) and R.S.D. was between 0.512 and 0.019% for all compounds, with a slightly higher S.D. and R.S.D. for the higher concentrations investigated (Table 5).

4.7. Calibration curves

The linearity was good for all substances in our test concentrations. Furthermore, the concentrations of eluted substances were well inside the linearity range, except for TEGDMA from TC and TMPTMA from DY in ethanol which both eluted slightly higher concentrations. The concentrations could preferable have been calibrated up to 50 μg/ml. R^2 was 0.99 for HEMA, MEHQ, DMABEE, TMPTMA and TIN P, 0.98 for BHT and TEGDMA, 0.94 for HMBP and 0.76 for CQ. The low R^2 for CQ is probably due to low sensitivity for this compound.

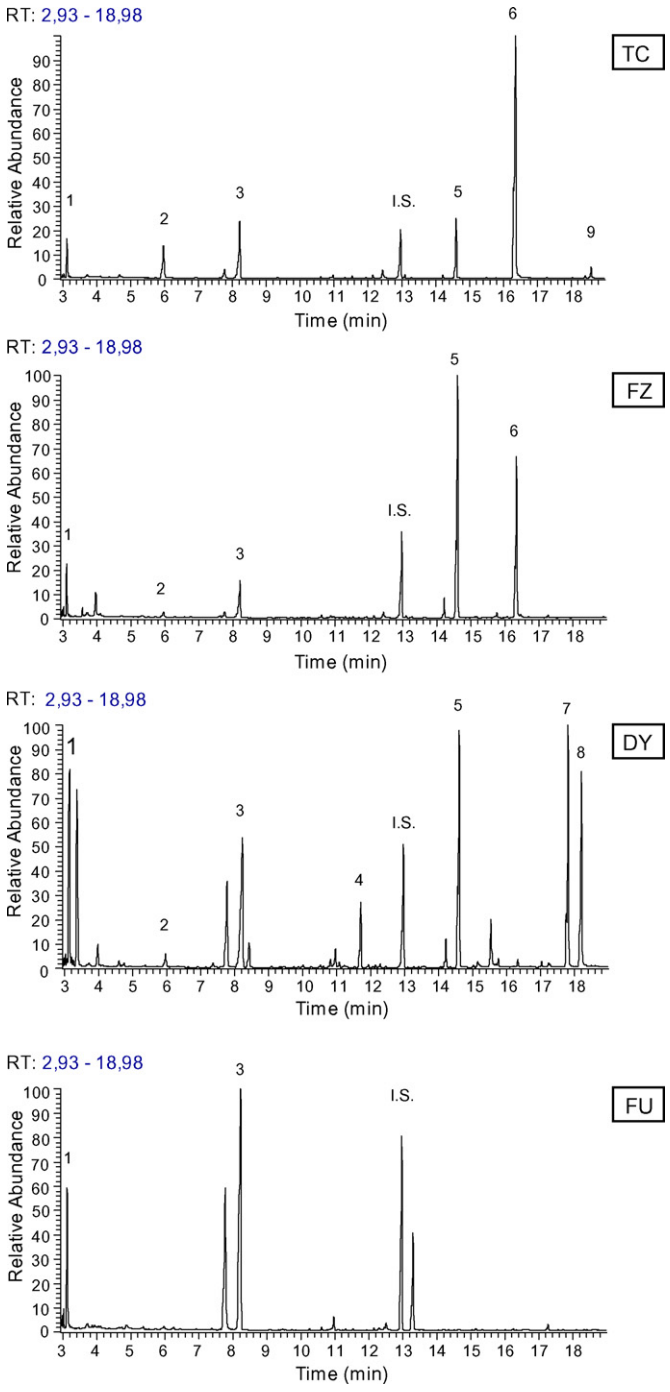


Fig. 1. Chromatograms of elution in Ringer's solution from TC, FZ, DY and FU. Sections from RT 3 to 19 are displayed. 1 = HEMA, 2 = MEHQ, 3 = CQ, 4 = BHT, 5 = DMABEE, 6 = TEGDMA, 7 = TMPTMA, 8 = HMBP, 9 = TIN P.

4.8. Internal standard

In previous studies on resin-based materials, monomers have been quantified by using external standard curves with reference substances [28,33–37]. Very few reports have applied internal standards (I.S.) in the quantification procedure [2,21,27,32]. In two studies caffeine was used as I.S. [2,32] and the amounts of elutable compounds was given as a percentage of the response of caffeine. Caffeine may be chosen because of a suitable reten-

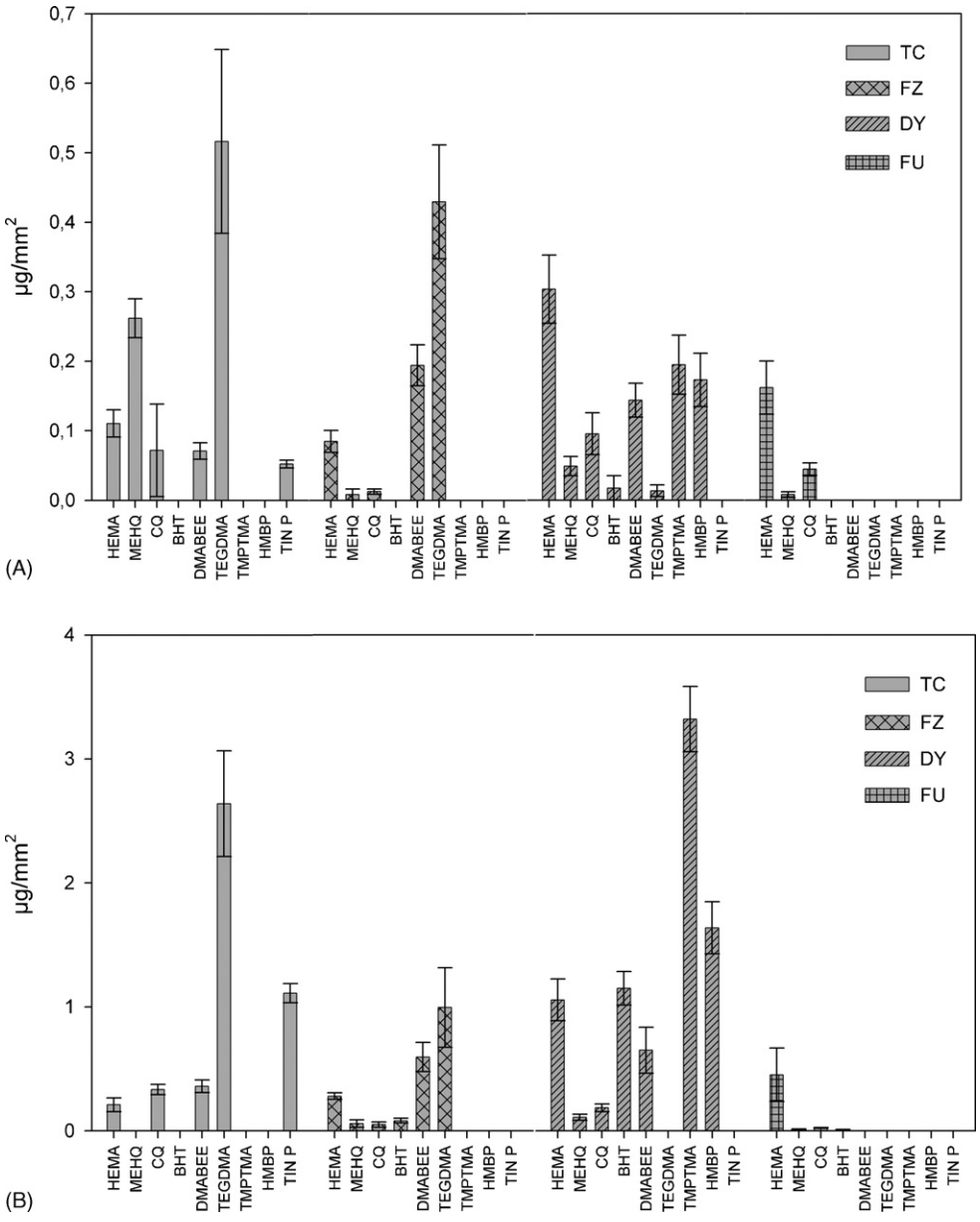


Fig. 2. Eluates from Ringer's solution (A) and from ethanol (B), quantities given in µg/mm^2 of sample surface. Mean values (bars) and S.D. (vertical lines) are given.

tion time. However, for *in vivo* analysis of saliva the potential of caffeine as a contaminating substance is high. In our study, we planned for further *in vivo* analysis, and accordingly, diethyl phthalate was used as an I.S. Diethyl phthalate has a high stability, easily detectable mass fragments and a retention time which is not interfering with the elutable compounds. Stable isotopes labelled standards would be most preferable for the quantification procedure [38], because they behave identically during sample preparation, extraction and evaporation. Deuterated or ^{13}C labelled analogues were, however, not available for the nine substances in this study.

4.9. Quantities of eluates from specimens

The quantities of eluted compounds, showed a wide variation depending on the elution media as shown in Fig. 2A and B. In ethanol, the eluted amounts was statistically significantly higher compared to eluted amounts in Ringer's solution for all substances ($p < 0.05$) except for MEHQ from TC. The highest observed difference was measured from TC for the compound TIN P for which the eluted amount was 20 times higher in ethanol compared to in Ringer's solution. Monomers represented the dominating group of elutable compounds, in ethanol as in Ringer's solution. The highest amount of a single substance (TMPTMA) eluted from one specimen was 3.28 µg/mm^2 specimen surface, eluted in ethanol from DY.

However, the most interesting findings are the differences in amounts of eluted compounds depending on the composition of the materials. The variation of eluted amounts in Ringer's solution, which is clinically most relevant, is shown in Fig. 2A. The mean value of TEGDMA was higher in TC than in FZ, and lower from DY. However, the difference in detected amounts of

Table 5
Precision of the assay for all nine analytes in two concentrations for within-day and between-day measurements

Precision of the assay[a]					
Analyte		Within-day		Between-day	
		S.D.	R.S.D. (%)	S.D.	R.S.D. (%)
HEMA	10 μg/ml	0.050	0.451	0.055	0.512
	1 μg/ml	0.016	0.070	0.011	0.049
MEHQ	10 μg/ml	0.024	0.236	0.074	0.297
	1 μg/ml	0.018	0.038	0.012	0.042
CQ	10 μg/ml	0.003	0.294	0.074	0.369
	1 μg/ml	0.010	0.043	0.012	0.047
BHT	10 μg/ml	0.017	0.156	0.021	0.153
	1 μg/ml	0.002	0.318	0.003	0.335
TEGDMA	10 μg/ml	0.062	0.079	0.074	0.091
	1 μg/ml	0.029	0.045	0.033	0.053
TMPTMA	10 μg/ml	0.150	0.018	0.159	0.019
	1 μg/ml	0.054	0.029	0.051	0.121
DMABEE	10 μg/ml	0.009	0.169	0.011	0.196
	1 μg/ml	0.005	0.138	0.005	0.158
HMBP	10 μg/ml	0.074	0.202	0.084	0.220
	1 μg/ml	0.030	0.162	0.030	0.164
TIN P	10 μg/ml	0.093	0.196	0.107	0.216
	1 μg/ml	0.045	0.178	0.046	0.180

[a] $n = 15$ for within-day precision and $n = 10$ for between-day precision for all concentrations.

TEGDMA in TC and FZ was not statistically significant. From FU no TEGDMA was found, on the other hand HEMA was the dominating monomer. The mean values of eluted HEMA were as follows: DY > FU > TC > FZ. The observed differences were statistically significant.

From the initiator system, the eluted DMABEE in Ringer's solution showed mean values from FZ > DY > TC. The observed differences were all statistically significant. Compared to TC, FZ eluted only about 25% of the amount of CQ in Ringer's solution. This might partly be explained by the presence in FZ of an additional initiator, di-phenyliodonium chloride (DPICl) [5]. DPICl in addition to TEGDMA, has been assumed to be the reason for the cytotoxic effect of the resin modified glass ionomer cement, Vitrebond [32]. Thus, the potential effect of each eluate has to be considered, not only the highest total amounts of eluates. Furthermore, the eluate mixture might also be of importance; in a resent study a higher experimental cell death inducing potential was indicated from mixtures of monomers than from the single monomers [14].

HMBP, an UV stabilizer, found to leach from the compomer (DY) in this study, was recently found to have estrogenic activity *in vitro* [39] in concentrations above 1 μmol/l (0.23 μg/ml). The mean amount found in our study was 0.17 $\mu g/mm^2$ eluted from DY specimens in Ringer's solution. From elution in ethanol we measured about ten times this amount (1.64 $\mu g/mm^2$) as an indication of possible maximum elution potential. The *in vivo* implication of the detected amounts of HMBP is difficult to assess. However, the results may indicate that the potential of estrogenic activity cannot be excluded. High quantities of this compound and potential long term biological effects should be carefully considered.

5. Conclusions

The applied GC/MS method seems well suited for analysis of small monomers and additives eluting from composites, compomers and resin modified glass ionomer cements. The analyzed eluates included several groups of ingredients; monomers, initiators, accelerators, inhibitors and stabilizers. The results allowed for a possibility to compare eluted amounts of organic compounds between various resin-based dental restorative materials. Our study has shown that the elution pattern from resin-based dental restorative materials differs, not only in the types of eluates, but also regarding the total and single components' amounts. For that reason the materials have different potential for causing adverse effects, thus in assessment of biocompatibility both quantitative and qualitative evaluations of the eluates has to be considered. Since the health hazard of several of the substances leaching is questioned, better knowledge of the exposure to the human body is important.

When an allergic reaction from an ingredient of these materials is revealed, better knowledge about the composition of the products may give an opportunity to select an appropriate filling material in clinical dentistry.

The results from this study represent *in vitro* elution in ethanol and Ringer's solution. Eluted amounts from resin-based dental restorative materials into human saliva might be quantified somewhere in between. In the oral environment, the process of leaching is affected by many factors, such as saliva's composition, pH and the amount of saliva secretion. Further studies will be addressed to *in vivo* situations by collecting saliva samples that has been in contact with resin-based dental restorative materials.

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