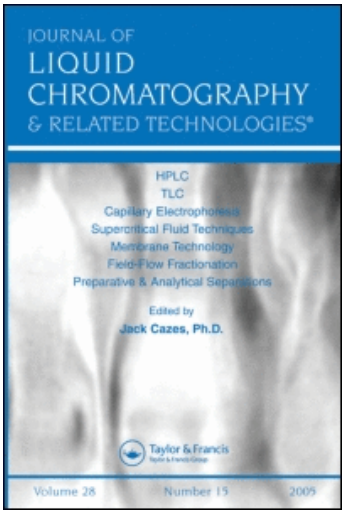

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LIQUID CHROMATOGRAPHY AND PURIFICATION OF DENTAL MONOMERS

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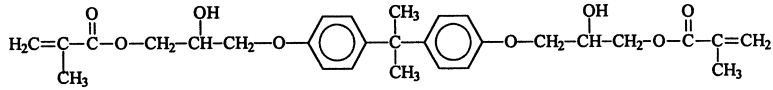
ABSTRACT

Preparative scale, normal phase liquid chromatography has been used to purify the major components of three dental monomers, Araldite GY281, BisGMA, and Cyacure™ UVR-6105. A silica column and a mobile phase consisting of a mixture of CH_2Cl_2 and ethyl acetate were used. Reverse phase HPLC with UV detection and proton NMR were used to analyze the raw materials and the purified compounds. The purified materials are being used in toxicology testing and metabolic studies.

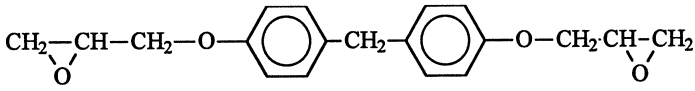
INTRODUCTION

Araldite GY281 and BisGMA are used as monomers to prepare dental restoratives and fissure sealants. The major ingredient in BisGMA is 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]propane.



Major Component of BisGMA

The major ingredient of GY281 is BisPhenol A Diglycidyl Ether (BFDGE)



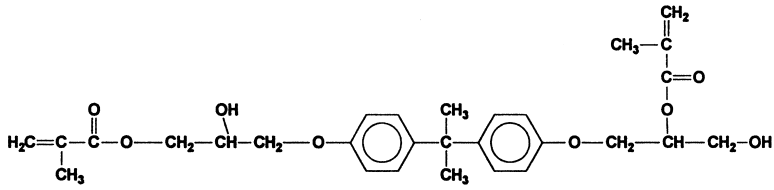
BFDGE, Major Component of GY281

The polymers produced with them have acceptable mechanical properties, chemical stability, and are able to simulate natural tooth color. However, in a case report BisGMA was shown to cause recurrent facial dermatitis.[1] Genotoxicity of BisGMA was observed in HeLa cells.[2] Also, it is possible that BisGMA may be metabolized by esterase enzymes to produce 2,2-bis-(4-hydroxy phenyl)propane, which is more commonly known as BisPhenol A (BPA), a possible endocrine disruptor.[3–5] It is also possible that compounds similar to BPA could be produced by the metabolism of BisGMA by mixed function oxidases.

In recent studies, Howdeshell and coworkers, along with Nagel and coworkers, showed that mice exposed to environmental levels (2.4 μg/Kg) of BPA on days 11–17 of gestation reached puberty sooner and males had an increase in the weight of their prostate.[6–7]

HPLC has been used by previous workers to separate BisGMA from other compounds in dental materials.[8,9] Semi-preparative, normal phase HPLC was used to purify BisGMA from Adaptic dental resin using a silica column and a mobile phase consisting of 4:1 methylene chloride:ethyl acetate (v/v). Other resin components, bisphenol A dimethacrylate and triethylene glycol dimethacrylate, eluted before BisGMA, while the following isomer of BisGMA eluted after the BisGMA.[8]

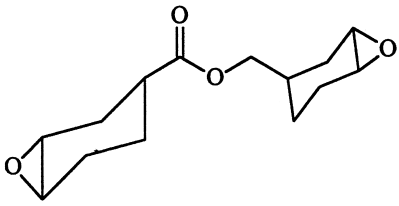
Proton NMR was used to distinguish between this branched isomer and BisGMA. Both compounds produced two methyl peaks, at 1.66 and 2.04 ppm, two phenyl doublets at 6.96 and 7.2 ppm, and a set of peaks due to the propoxy group. The branched isomer, though, produced a small triplet, at 5.2 ppm, due to the –C**H**-O-C=O hydrogen.



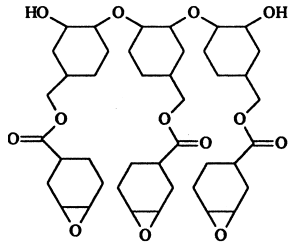
Branched Isomer of BisGMA

In the other study, reverse phase HPLC was used to separate BisGMA from compounds in other dental resins.[9] They found that hydroxyethyl methyacrylate (HEMA), BPA, and triethylene glycol dimethacrylate (TEGDMA) all eluted before BisGMA on an octadecyl-silica (C_{18}) column and a gradient elution. A gradient was required because the resins contained weakly and strongly retained compounds.

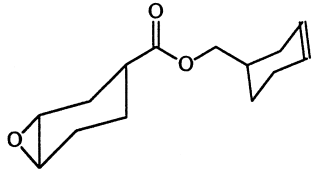
Cyacure™ UVR-6105 is a new monomer being evaluated for use in preparing dental materials. Currently, used dental resins made of dimethacrylates suffer polymerization shrinkage, leaving gaps that are susceptible to the formation of dental carries and clinical failure. To avoid this problem, spiro ortho carbonate expanding monomers have been developed.[10] Polymerization occurs through a ring opening, which causes the expansion. When copolymerized with the diepoxide, Cyacure™ UVR-6105, there is no net expansion or contraction. The mixture was found to have little or no cytotoxicity.[11] Also, UVR-6105 was copolymerized with the polyol, poly(tetrahydrofuran) and found to have low cytotoxicity.[12] The major ingredient (91–97%) of UVR-6105 is 7-oxabicyclo[4.1.0]hept-3-ylmethyl ester, or



Minor ingredients listed in the MSDS include soluble oligomers (<4%) and the monoepoxide of 3-cyclohexyenylmethyl-3-cyclohexene carboxylate (3–5%)



Trimer of UVR6105



Monoepoxide

To investigate the toxcicity and possible metabolism of UVR 6105, it is necessary to purify its main component as much as possible. Because of the lack of conjugated double bonds, none of these compounds absorb strongly in the UV, making quantitative HPLC analysis with UV detection difficult. Impurities that

absorb strongly would produce large peaks, whereas the major ingredient would produce a relatively small peak. Proton NMR analysis avoids this problem, since it has the same sensitivity for all types of protons.

In this report, a preparative scale silica column and a mobile phase consisting of 85:15 CH_2Cl_2:ethyl acetate (v/v) were used to purify 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane from BisGMA. For UVR6105, the mobile phase was 95:5 CH_2Cl_2:ethyl acetate (v/v), and for BFDGE it was 98:2 CH_2Cl_2:ethyl acetate (v/v). Proton NMR and analytical scale, reverse-phase HPLC were used to analyze the raw materials and the purified compounds.

EXPERIMENTAL

Deionized water (Milli-Q, Waters Corporation) and HPLC grade acetonitrile, methylene chloride and ethyl acetate (Aldrich Chemical Company) were used. For small scale preparations (~100–200 mg), normal phase liquid chromatography was performed using a glass column (50 cm long, 5 cm i.d.) packed with 60–200 mesh silica from J.T. Baker.

For larger scale purifications, a larger glass column (50 cm long, 5 cm i.d.) packed with 60–200 mesh silica from J.T. Baker was used. The mobile phase was 85:15 CH_2Cl_2:ethyl acetate (v/v) for bisGMA, 95:5 CH_2Cl_2:ethyl acetate (v/v) for UVR6105, and 98:2 CH_2Cl_2:ethyl acetate (v/v) for GY281.

Reverse phase HPLC was performed using a Waters 600E system controller and 484 tunable UV absorbance detector set at 277 nm for bisGMA and BFDGE, and at 205 nm for UVR6105. The mobile phase was 7:3 CH_3CN:H_2O (v/v) for BisGMA, and 6:4 CH_3CN:H_2O (v/v) for UVR-6105 and GY-281. The flow rate was 1.0 mL/min.

An Alltima C_{18} column, 250 x 4.6 mm, 5 μ particle size, from Alltech, was used. The injection volume was 20 μL. Data were collected and analyzed using Turbochrom version 4.1.

Proton NMR spectra of samples in $CDCl_3$ were obtained using a Bruker AC250 with NTNMR software from TecMag. A 90° pulse width, 4.096 sec acquisition time and 2 sec. pulse delay were used.

RESULTS AND DISCUSSION

Proton NMR of the unpurified BisGMA produced the expected peaks (8) due to 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane, along with the triplet at 5.2 ppm due to the branched isomer. These peaks were integrated and the relative amount of branched isomer to 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane was 3:22 (12%).

There were also small doublets at 3.9 and 4.2 ppm, due to ethoxylated BisGMA, previously described by Noda and coworkers.[9] Instead of a -O-CH_2-CHOH-CH_2-O- group, it has a–O-CH_2-CH_2-O- group. The purified 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane produced neither the 5.2 nor the 3.9 or 4.2 ppm peaks.

Reverse phase HPLC of the unpurified BisGMA produced a major peak eluting at 2.3 min. along with some smaller peaks (Fig. 1). The partly purified bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane produced the same large peak and only one smaller peak (due to the branched isomer), which represented 3.0% of the total peak area (Fig. 2). Because the structures are so similar, it is expected that the branched isomer will have almost the same UV absorbance as bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl]-propane. Thus, it is about 97% pure and is more suitable for metabolism studies than the unpurified BisGMA.

Also, the small earlier eluting peaks at 1.8 and 2.2 min were removed. They were due to the hydrolysis of the ester groups in bisGMA. The peak at 2.2 min

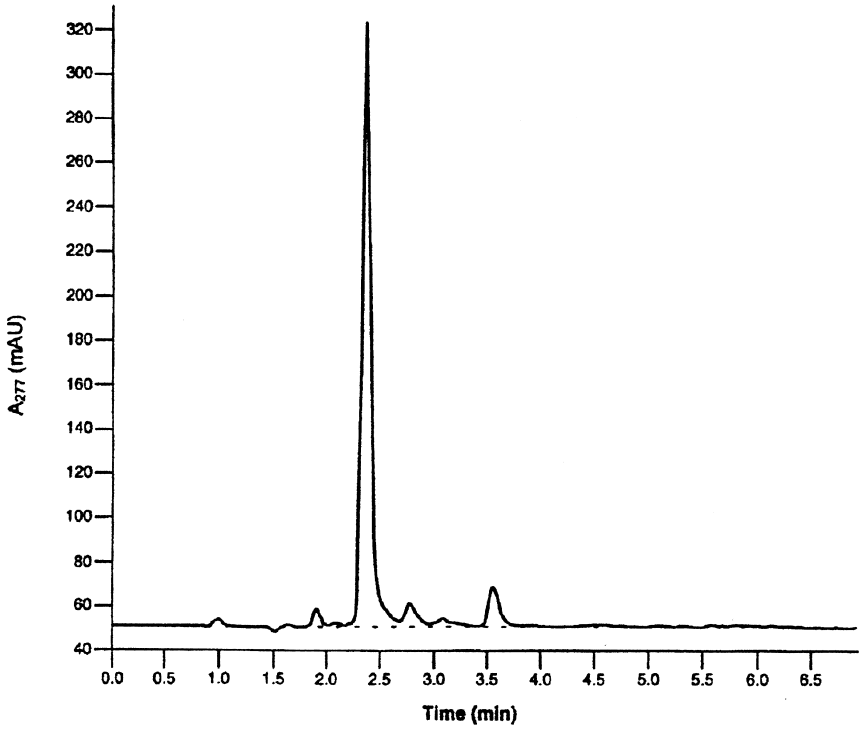


Figure 1. HPLC of unpurified BisGMA, using a C_{18} column and a mobile phase of 7:3 CH_3CN:H_2O (v/v) and UV detection (277 nm).

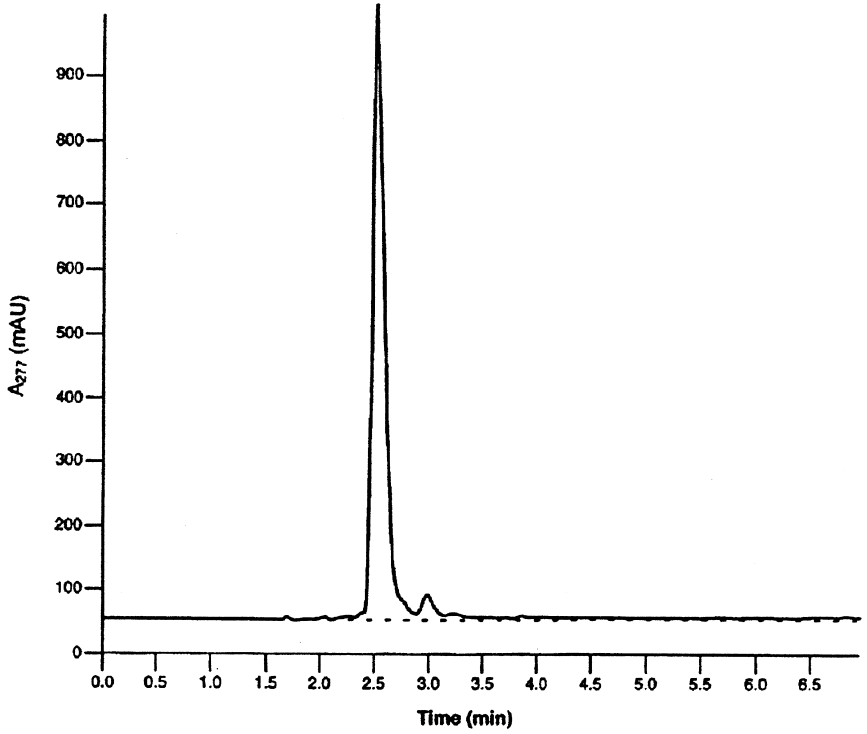


Figure 2. HPLC of BisGMA, purified on a silica column (50 cm long, 5 cm i.d.) with a mobile phase of 85:15 CH_2Cl_2:ethyl acetate (v/v).

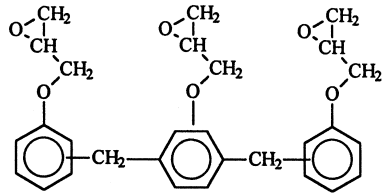
was the first to appear, and is probably due to the hydrolysis of the first ester to produce a diol. The peak at 1.8 min appeared later, and is due to the hydrolysis of the second ester to produce a tetraol. These peaks reappeared in the purified bisGMA after storage for 1–2 days at room temperature in a mixture of 1:3 CH_3CN:H_2O (v/v), containing 0.2 M potassium phosphate buffer, pH 7.4.

Proton NMR analysis of unpurified UVR 6105 showed the expected multiplets from 0.9 - 2.5 ppm, due to the cyclohexyl –C**H**$_2$- protons, the –C**H**$_2$-O protons at 3.099 ppm and the oxirane (or epoxy) protons from 3.7–3.9 ppm. There were no detectable phenyl or alkenyl protons. The absorbance spectrum of UVR 6105 in acetonitrile shows no peak, but instead, just a rapid increase in absorbance below 210 nm. Analysis of a 1.16 mg/mL solution by reverse-phase HPLC using UV detection at 205 nm produced several peaks, as shown in Fig. 3a.

The peaks eluting from 8.4–9.4 min. were due to the oligomeric impurities. They were removed in the purified UVR6105, as shown in Fig. 3b. Also, the

small, earlier eluting peaks were removed. In the unpurified UVR6105, they were due to hydrolysis products in which either one or both the epoxy groups have been hydrolyzed to produce a diol or tetraol. These peaks reappear within 1–2 days of storing UVR6105 in a mixture of 1:3 CH_3CN:H_2O (v/v), containing 0.2 M potassium phosphate buffer, pH 7.4.

Proton NMR analysis of unpurified GY281 showed the expected multiplets due to the –C**H**$_2$-O protons at 3.099 ppm and the oxirane (or epoxy) protons from 3.7–3.9 ppm. The –CH_2- group between the phenyls produces a singlet at 3.95 ppm. There were also overlapping peaks due to aromatic protons from 6.8–7.2 ppm, indicating that there was a mixture of o-, m- and p- isomers. However, the largest phenyl peaks were the two doublets expected for the p-isomer. Also, there was a trace (1.7%) of methyl protons, which produced a singlet at 1.63 ppm, indicating only a small contamination due to bis phenol A diglycidyl ether (BADGE). Analysis of a 0.43 mg/mL solution of GY281 by reverse-phase HPLC produced several peaks, confirming the lack of purity and the presence of trace BADGE as shown in Fig. 3a. Purified BFDGE produced a major peak at 5.5 min, with a small overlapping peak, as shown in Fig. 3b. The small peak accounted for 9% of the total peak area. The peaks eluting from 6.5–7 min were due to o-, m- and p-isomers of a triepoxide.

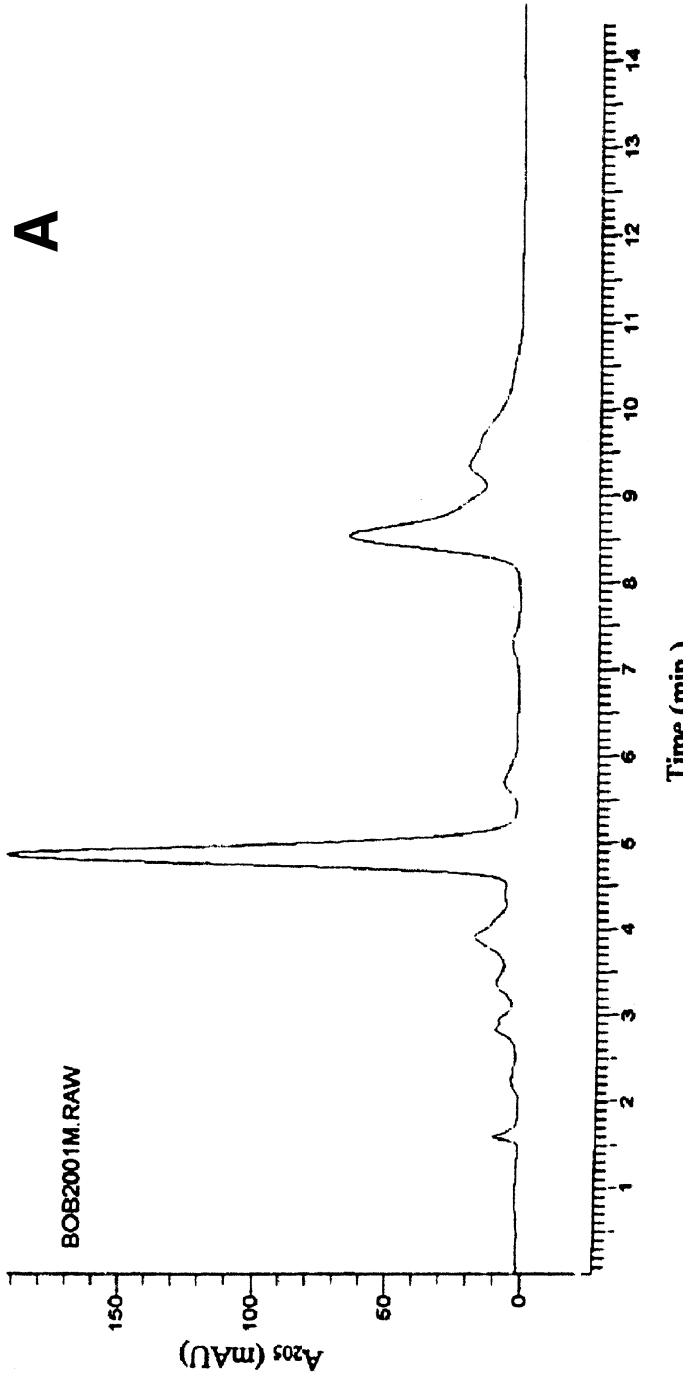


Triepoxide of BFDGE

Also, the earlier eluting peaks, due to hydrolysis products, were removed. As with bisGMA and UVR6105, they reappear in 1–2 day's storage at room temperature in a mixture of 1:3 CH_3CN:H_2O (v/v), containing 0.2 M potassium phosphate buffer, pH 7.4.

CONCLUSIONS

Normal phase liquid chromatography has been used to purify the major ingredients of three different dental materials. BisGMA was about 96% pure, with the 4% contaminant being the branched isomer. UVR6105 was about 99% pure, with higher molecular weight oligomers being removed. BFDGE was about 92% pure, with the 8% contaminant being m- and/or o-isomers.



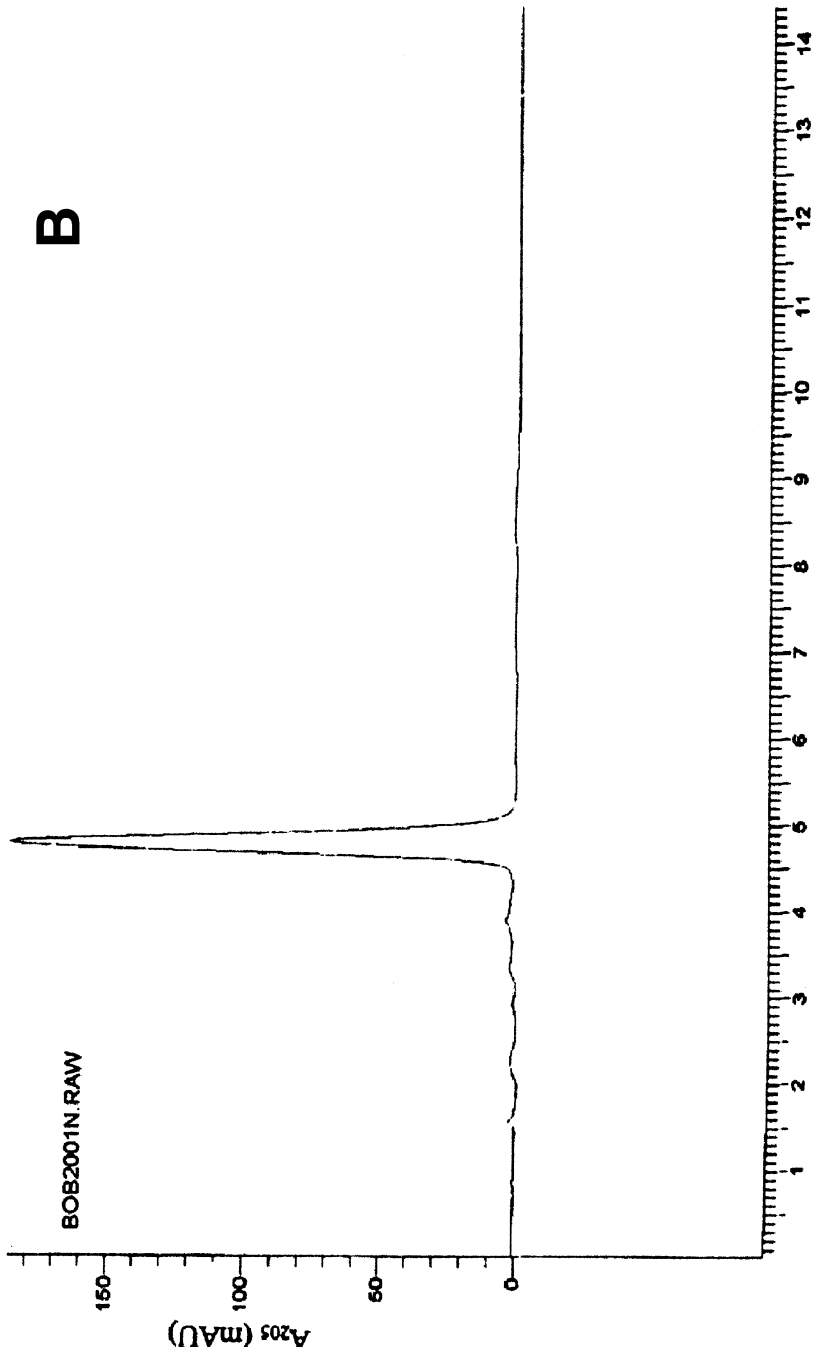
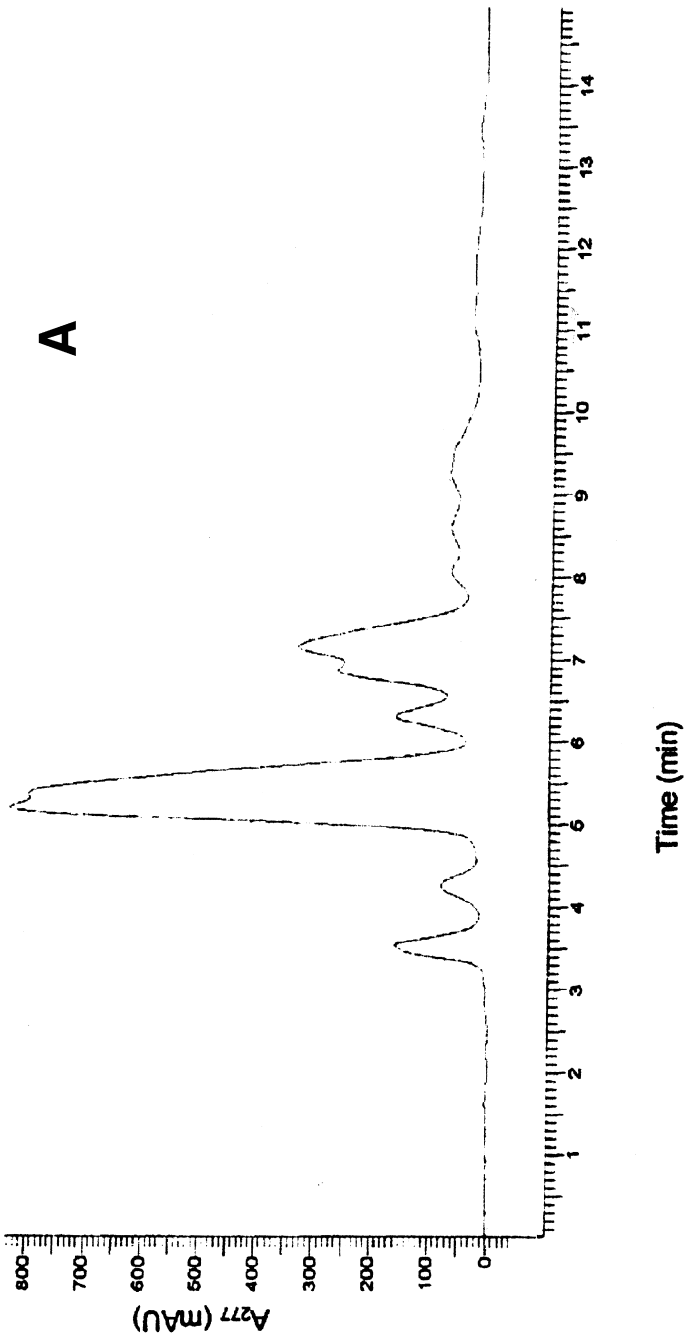


Figure 3. HPLC of unpurified UVR6105 (top) and purified UVR6105 (bottom); purified on a silica column (50 cm long, 5 cm i.d.) using a mobile phase of 95:5 CH_2Cl_2:ethyl acetate (v/v).



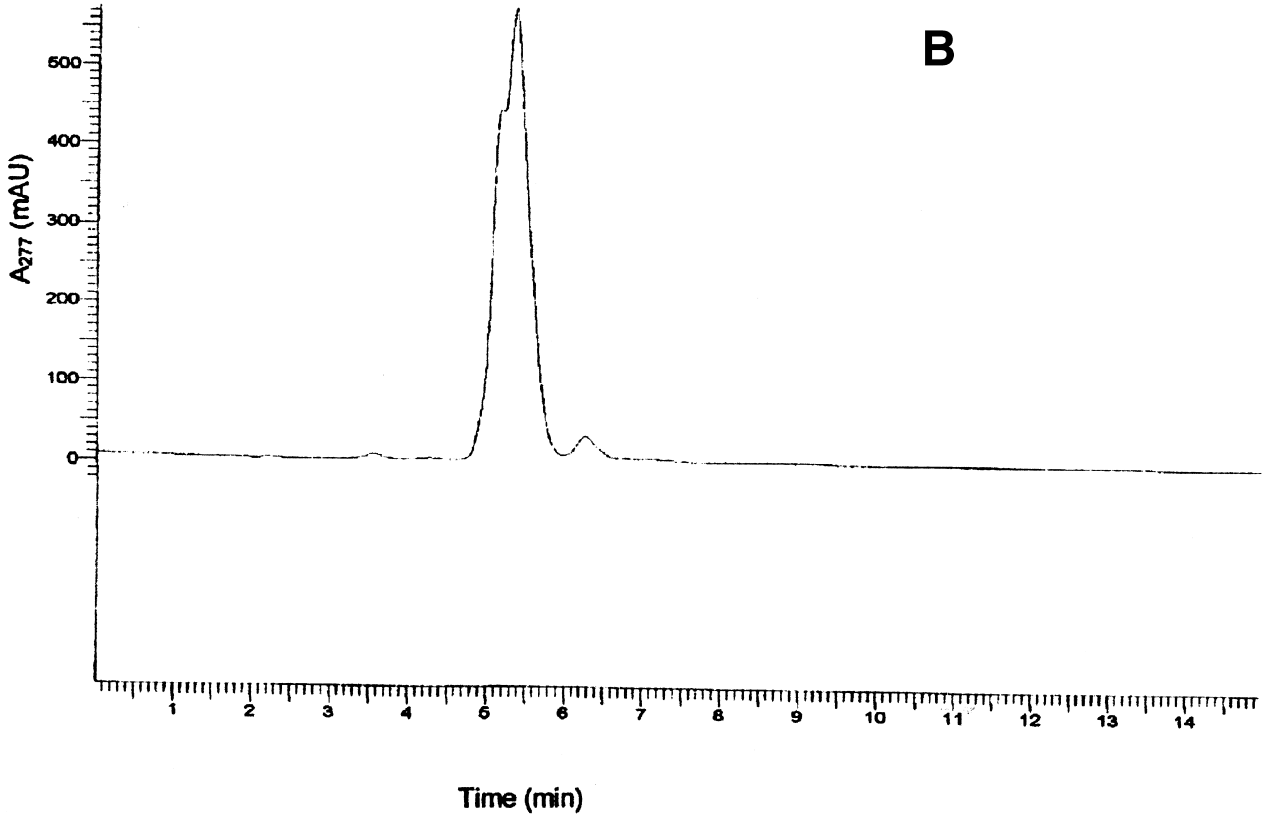


Figure 4. HPLC of unpurified GY281 (top) and purified BFDGE (bottom); purified on a silica column (50 cm long, 5 cm i.d.) using a mobile phase of 95:5 CH_2Cl_2:ethyl acetate (v/v).

Reverse phase HPLC was used to analyze the dental materials and their purified ingredients. Hydrolysis products appeared after 1–2 days in 1:3 CH_3CN:H_2O (v/v), containing 0.2 M potassium phosphate buffer, pH 7.4. They were diols and tetraols that were more polar than the main ingredients, and eluted earlier. The purified compounds are available for toxicology studies, but should be stored in a desiccator at room temperature.

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