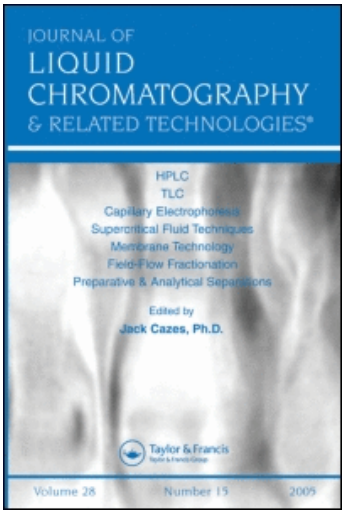

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Solid-Phase Extraction (SPE) and HPLC Analysis of Toxic Compounds and Comparison of SPE and Liquid-Liquid Extraction. I. Analysis of 4, 4'-Methyl-Enedianiline in Serum. II. Analysis of the Components of Dental Materials

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To cite this Article Shintani, H.(1992) 'Solid-Phase Extraction (SPE) and HPLC Analysis of Toxic Compounds and Comparison of SPE and Liquid-Liquid Extraction. I. Analysis of 4, 4'-Methyl-Enedianiline in Serum. II. Analysis of the Components of Dental Materials', Journal of Liquid Chromatography & Related Technologies, 15: 8, 1315 — 1335
To link to this Article: DOI: 10.1080/10826079208018290
URL: http://dx.doi.org/10.1080/10826079208018290

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(8), 1315-1335 (1992)

SOLID-PHASE EXTRACTION (SPE) AND HPLC ANALYSIS OF TOXIC COMPOUNDS AND COMPARISON OF SPE AND LIQUID-LIQUID EXTRACTION. I. ANALYSIS OF 4,4'-METHYL-ENEDIANILINE IN SERUM. II. ANALYSIS OF THE COMPONENTS OF DENTAL MATERIALS

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ABSTRACT

In this paper, we discuss two subjects concerned with solid phase extraction (SPE) and HPLC analysis of toxic compounds eluted to serum from materials used for medical devices. SPE and liquid-liquid extraction for serum toxic compounds were compared. Firstly, an analysis of a toxic and carcinogenic compound, 4,4'-methylenedianiline (MDA), from polyurethane sterilized by gamma-ray irradiation was studied. Serum MDA was analyzed to assess the potential MDA risk to patients exposed to PU medical devices. MDA was determined by reverse-phase HPLC using an eluent of a mixed solution of ammonium acetate and acetonitrile. An ODS column was used. Detection was carried out by an electrochemical detector (ECD). As liquid-liquid extraction was complex due to deproteinization followed by centrifuge separation and concentration, we substituted SPE. Serum MDA was satisfactorily recovered using Bond Elut® C-18, Phenyl and Cyclohexyl columns. The recovery of serum MDA from Bond Elut® C-1, C-2, C-8 and Silica was unsatisfactory. Elution was carried out using methanol containing 1M NH_4OH. SPE was superior to a liquid-liquid extraction due to an unnecessary of deproteinization, centrifuge separation and concentration.

Secondly, we analyzed the toxic compounds, methyl methacrylate (MMA), N,N-dimethyl p-toluidine (DMPT), and benzoylperoxide (BPO), all of which are components of the dental material of polymethyl methacrylate (PMMA). PMMA is widely used as the composite resin for the dental plate. BPO was converted to benzoic acid (BA) when in contact with DMPT or serum, therefore BA

analysis is necessary for BPO determination. As these compounds exhibit a potential for residue as well as toxicity, determination method and safety evaluation are required. Analysis was by HPLC combined with SPE using a C-18 column in both methods.

INTRODUCTION

I. Polyurethane (PU) is widely used for medical devices due to its biocompatibility (1-2). There are two types of medical use PU: thermoplastic PU and thermosetting PU. Thermoplastic PU is, for instance, fabricated by reacting polytetramethylene glycol (PTMG) with 4,4'-diphenylmethane diisocyanate (MDI). 1,4-butanediol (BU) is added as a chain-extender. N-butanol terminates the polymerization reaction. PTMG quantitatively exceeds MDI (1-2). Thermosetting PU is used as a potting material to connect blood dialysis fibers to a device module. Thermosetting PU is fabricated by reacting MDI with partially saponified castor oil in substitute for PTMG (3). No terminating reagent is required. A greater amount of MDI than castor oil is used, therefore the residue of MDI, a toxic compound, is evident in the thermosetting PU potting material (1-2).

As all medical devices require sterilization before human application, sterilization procedures should not be detrimental to the device. In spite of PU's biocompatibility, it has been reported that toxic MDI is evident in PU (1-2). 4,4'-methylenedianiline (MDA), a carcinogen, is reportedly produced by hydrolyzing residual MDI or by PU degradation by heat, by irradiation or by chemical reaction (4-10). MDA is reported to be toxic (11), mutagenic (12), teratogenic (13), and carcinogenic compound (1-2,14-15). It is necessary to evaluate the possibility of MDA production in sterilized medical devices and the subsequent migration of MDA into the human body. Thus, we evolved a method of HPLC analysis and a pretreatment method of blood serum MDA.

MDA is unstable upon heat, therefore GLC was inferior to HPLC (16-17). Previously we have reported liquid-liquid extraction of serum MDA, which shows a satisfactory recovery rate, but is complex due to centrifuge separation and concentration after deproteinization (16-17). An alternative liquid-liquid extraction has been reported describing the extraction of n-heptane-isoamyl alcohol (99:1), diethyl ether or benzene from alkalified serum. However, the recovery rate is low (from 70 to 80%) (18-20). SPE is easier to handle, to prepare for an automated system and requires less solvent. Deproteinization, centrifuge separation, and concentration are unnecessary. Therefore, we selected SPE of serum MDA. To date this method has not been reported. SPE of basic drugs using C-18 column has been reported (21). The comparison of the eluents from the reverse phase columns in SPE was discussed.

II. Polymethylmethacrylate (PMMA) is widely used as the composite resin for the dental plate (22). In accordance with the current PMMA fabrication method, benzoylperoxide (BPO) and N,N-dimethyl p-toluidine (DMPT) are added as the initiator and the stimulator, respectively. If insufficiently polymerized, methyl methacrylate (MMA), DMPT and BPO exhibit a residue potential. BPO was converted to benzoic acid (BA) when in contact with DMPT or serum, therefore BA analysis is necessary for BPO determination. BA is not an original component for the fabrication of PMMA. As these compounds are reportedly toxic (23), representing a degree of risk. In order to evaluate the risk factor to the recipient, the authors quantitatively analyzed residue in composite resins using serum extraction. MMA and BPO are unstable upon heat, therefore HPLC is considered to be superior to GLC. Determination was carried out by HPLC combined with SPE using a C-18 in both methods. The comparison of the eluents from a C-18 column in SPE was discussed. The comparison of SPE and liquid-liquid extraction was also discussed.

EXPERIMENTAL

I. Analysis of MDA in Serum

The chemical structures of chain-extended thermoplastic PU and that of MDA are presented (Figure 1).

Sample of Thermosetting PU Potting Material

Thermosetting PU is fabricated by reacting MDI with partially saponified castor oil (3,17). Thermosetting PU is used as a potting material to connect blood dialysis fibers to a device module of artificial dialyzers and plasma separators (17). The potting material portion was separated and the round sheet (diameter and thickness are 5 cm and 0.1 cm, respectively) was immersed into 20 ml of serum at room temperature. Serum was replaced daily over seven consecutive days. The serum extract was subjected to SPE followed by HPLC analysis. Five specimens were tested (n=5).

Equine serum is supplied from Flow Co. Ltd. and other reagents commercially available are special grade.

Preparation of Standard Sample of MDA in Aqueous Solution or in Serum

As a standard MDA solution, 10 mg of MDA was dissolved with 10 ml of methanol and diluted with water to the desired MDA concentration. Fifty μl of 2-200 μg/ml standard MDA aqueous solution was added to 950 μl of serum to prepare a serum sample containing MDA at a concentration of 0.1-10 ppm.

HPLC Analysis of MDA

A conventional and insufficiently endcapped ODS column, ODS 120T-1251 (4.6X250 mm) from Toso Co. Ltd., Tokyo, Japan, was used

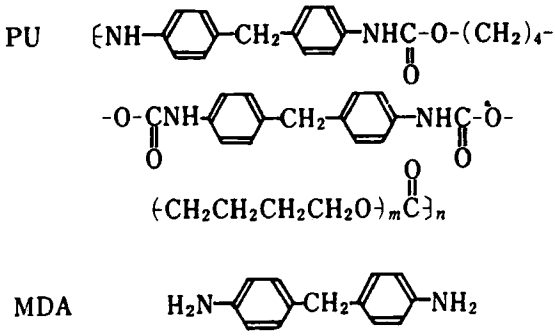

FIGURE 1 Chemical structure of chain-extended PU and that of 4,4'-methylenedianiline (MDA)

with an eluent of a mixed aqueous solution of 50 mM ammonium acetate and acetonitrile at a ratio of 7/3. The flow rate is 1.2 ml/min. Detection was carried out using ECD and UV simultaneously. 900 mV was used for ECD detection. The ECD working electrode was a glassy carbon. The reference electrode was Ag/AgCl. UV detection was carried out at 250 nm, the maximum wavelength of MDA.

SPE of MDA

Reverse phase columns (Bond Elut^R C-1, 2, 8, 18, Phenyl and Cyclohexyl with a resin weight and void volume 100 mg and 120 µl, respectively) and Bond Elut^R Silica with identical resin weight and void volume were conditioned with 2 ml of methanol and rinsed with 2 ml of water. Thereafter, 1 ml of serum sample containing MDA at a concentration of 0.1-10 ppm was applied to the conditioned columns and rinsed with water. The columns trapping MDA were eluted with 250 µl of methanol containing 1M NH_4OH. The drain was trapped and 10 µl applied to HPLC. Conditioning, rinsing and elution were carried out by a Vac Elut^R SPS24 vacuum system from AnalytiChem, USA.

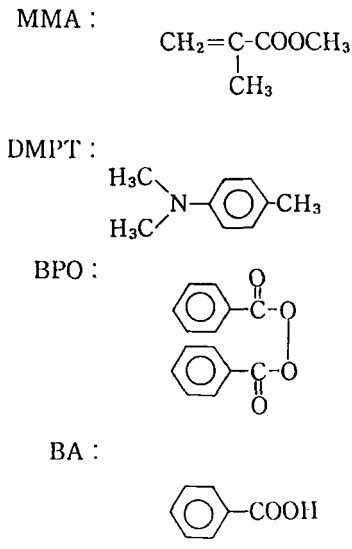

FIGURE 2 Chemical structure of methyl methacrylate (MMA), N,N-dimethyl p-toluidine (DMPT), benzoylperoxide (BPO), and benzoic acid (BA)

II. Analysis of MMA, DMPT and BPO, the Components of the Dental Material, in Serum

The chemical structure of MMA, DMPT, BPO and BA is presented in Figure 2. BPO was converted to BA when in contact with serum or DMPT, therefore BA analysis is necessary for BPO determination.

Sample of Dental Materials

Two individual PMMA composite resins of dental material (Yunifast^R and Acron^R for samples A and B, respectively) were analyzed. Sample A was polymerized at room temperature and sample B at 100 ^oC. Each sample (3X 3X 0.1 cm sheet of approximately 3 g) was immersed into 10 ml of serum at room temperature. Serum was replaced daily over three consecutive days. The serum extract was

subjected to SPE followed by HPLC analysis. For each sample, three specimens were tested (n=3).

Preparation of Standard Aqueous Solution and Serum Sample of MMA, DMPT, BA and BPO

Preparation was as follows: one to 100 mg each of MMA and DMPT were added to 50 ml of a mixed aqueous solution of water and acetonitrile at a ratio of 20/1. 100 µl was added to 1900 µl of water or serum. BA and BPO solutions were individually prepared identical to this procedure, because DMPT and BPO were reacted when mixed. They were converted to BA and others (24-25).

MMA, DMPT, BPO and BA Analysis by HPLC

The column for MMA, DMPT and BPO analysis was Capcell Pak^R C-18 SG-120 from Shiseido Co. Ltd., Tokyo, Japan. The eluent was a mixed solution of water and acetonitrile at a ratio of 1/1. The flow rate was 1.2 ml/min. Detection wavelength was 235 nm. The retention time (t_R) of MMA, DMPT, BPO was 5 min, 15 min, and 24 min, respectively.

BA analysis is as follows: a Capcell Pak^R C-18 AG-120 column was used with an eluent of a mixed aqueous solution of water and acetonitrile at a ratio of 4/1. Detection wavelength was 250 nm. The t_R of BA was 5.8 min. The rest of the procedure was identical to MMA, DMPT and BPO analysis.

SPE of MMA, DMPT, BPO and BA

The compounds with the exception of DMPT and BA are neutral. DMPT and BA are basic and acidic compounds, respectively. SPE of BA is carried out separately from SPE of MMA, DMPT and BPO.

In order to successfully retain DMPT on a reverse-phase column (C-18 column), it is necessary to depress ionization. Thus, the authors used 50 mM phosphate buffer at pH 7.5 for column conditioning. The column was Bond Elut^{R} C-18 with a resin weight of 100 mg. SPE of MMA, DMPT and BPO is as follows: the C-18 column was conditioned with 2 ml of acetonitrile and 2 ml of 50 mM phosphate buffer at pH 7.5. Thereafter, one ml of the standard aqueous solution or the serum sample containing one to 100 ppm of MMA, DMPT and BPO were applied to the conditioned column, vacuumed, rinsed with 0.5 ml of 50 mM phosphate buffer at pH 7.5 and eluted with one ml of acetonitrile. The drain was trapped and 20 µl applied to HPLC.

BA is an acidic compound with terminal carboxylate. In order to successfully retain BA on a reverse-phase column, it is necessary to depress ionization. Thus, an acetic acid aqueous solution at pH 3 was added to the sample solution at a ratio of 1/1 and mixed well prior to the application of SPE column. The column was Bond Elut^{R} C-18 with a 100 mg resin weight. SPE of BA is as follows: the C-18 column was conditioned with 2 ml of acetonitrile and 2 ml of an acetic acid aqueous solution at pH 3. One ml of the standard aqueous solution or the serum sample containing one to 100 ppm of BA were applied to the conditioned column. These were two-fold diluted with an acetic acid aqueous solution at pH 3 prior to application to the conditioned column. Thereafter, they were vacuumed, rinsed with 0.5 ml of an acetic acid aqueous solution at pH 3 and eluted with one ml of acetonitrile acidified with acetic acid to pH 2.5. The drain was trapped and 20 µl applied to HPLC. Conditioning, rinsing and elution were carried out by a Vac Elut^{R} SPS24 vacuum system.

RESULTS AND DISCUSSION

I. Analysis of MDA in Serum

HPLC Analysis

MDA is detectable by ECD due to the presence of two primary aromatic amino groups (Figure 1). The use of an insufficiently endcapped ODS column, ODS 120T, is required to add 50 mM ammonium acetate into the eluent thus attaining a common ion effect, achieving fast elution and preventing MDA peak tailing. The addition of more than 50 mM salts is essential for sensitive ECD detection.

MDA is detectable by ECD with an application of more than 500 mV. Sensitivity increased and selectivity decreased with increasing electrical voltage. More than 1000 mV voltage was found to deteriorate the glassy carbon working electrode, therefore 900 mV voltage was applied. MDA was detected by UV at 250 nm, the maximum wavelength of MDA. There was a significant difference of selectivity and sensitivity between UV and ECD detection for MDA analysis. UV indicating a greater baseline variation caused by impurities in ammonium acetate in the eluent (16-17). ECD was clearly superior being 50 times more sensitive (16-17). The detection limit of ECD and UV was 3 and 150 ppb, respectively.

Liquid-Liquid Extraction of MDA in Serum

Liquid-liquid extraction of serum MDA has been reported (18-20). In order to suppress ionization of MDA, serum was alkalified and extracted repeatedly with hydrophobic organic solvents such as heptane, ether or benzene (18-20). These methods are complex procedures, requiring repeated extraction, and large volume

consumption of organic solvents. Reported recovery rates at 70-80% are unsatisfactory (18-20).

In our liquid-liquid extraction, one part of alkalified serum was extracted twice with seven parts of a mixture of chloroform and methanol at a ratio of 3/1, a satisfactory recovery of serum MDA was attained (98%, n=3). We have developed a more efficient method, in which two parts of acetonitrile is added to one part of serum. This procedure requires only single extraction and shows a satisfactory recovery of serum MDA (99%, n=5) (17,26). Most liquid-liquid extraction procedures need repeated extraction, extract condensation and excessive organic solvent consumption. The condensation by evaporation is undesirable due to MDA instability upon heat (16-17), therefore we selected SPE of serum MDA.

SPE of MDA in Serum

Bond elut^{R} C-1, C-2, C-8, C-18, Phenyl, Cyclohexyl and Silica columns were tested. Addition-recovery experiment of serum MDA was carried out as follows: fifty µl of 21 µg/ml MDA spiked to one ml serum to prepare one µg/ml serum MDA and applied to the conditioned columns. Following SPE procedure describes in the Experimental section. The recovery rate is calculated on the average of 5 specimens. The C.V. is less than 1.4% in every case.

The recovery rate of serum MDA from C-1, C-2, C-8 and Silica were 56%, 75%, 90% and 12%, respectively. Residue MDA was determined in the drain. The recovery rate of C-18, Phenyl and Cyclohexyl was 100%. A lower recovery rate for C-1, C-2, C-8, and Silica and a higher rate for C-18, Phenyl and Cyclohexyl indicated that the predominant factors for retaining MDA on reverse phase columns were van der Waals binding and pi-pi binding between benzene rings. The binding of MDA to silanol in reverse-phase

columns was not predominant due to a lower recovery of Silica. This is due to water in serum interfering the combination of MDA to silanol. A lower recovery of C-1 to C-8 was due to insufficient capacity of van der Waals binding. Recovery rate among C-1, C-2 and C-8 columns increased with increasing column hydrophobicity due to increase of van der Waals capacity.

Concerning SPE eluent, methanol or a mixed solution of 10 mM ammonium acetate and methanol (at a ratio of 1/1) indicated an insufficient recovery (Figure 3). A satisfactory recovery of MDA was attained using a strongly alkalified methanol, methanol containing 1M NH_4OH (Figure 3). Acidified methanol resulted in insufficient MDA recovery, as the acidified effect was depressed by alkalified serum.

MDA Production and Elution to Serum from Thermosetting PU Potting Material Sterilized by Gamma-Ray Irradiation

MDA was produced and eluted to serum at a few ppm (μg/sample g) from thermosetting PU potting material sterilized with gamma-ray irradiation at 10 Mrad. Less than one ppm was produced and eluted to serum at 2.5 Mrad irradiation. 2.5 Mrad irradiation is an officially approved level to medical device sterilization. The regression between MDA amount and irradiation dose is a second order equation.

According to the risk factor estimation using reference 15, this quantity of MDA formation and elution is "not significant".

II. Analysis of MMA, DMPT and BPO, the Components of the Dental Material, in Serum

HPLC Analysis of MMA, DMPT, BPO and BA

In order to successfully analyze a basic compound DMPT by the reverse-phase column, free silanol effect should be sufficiently

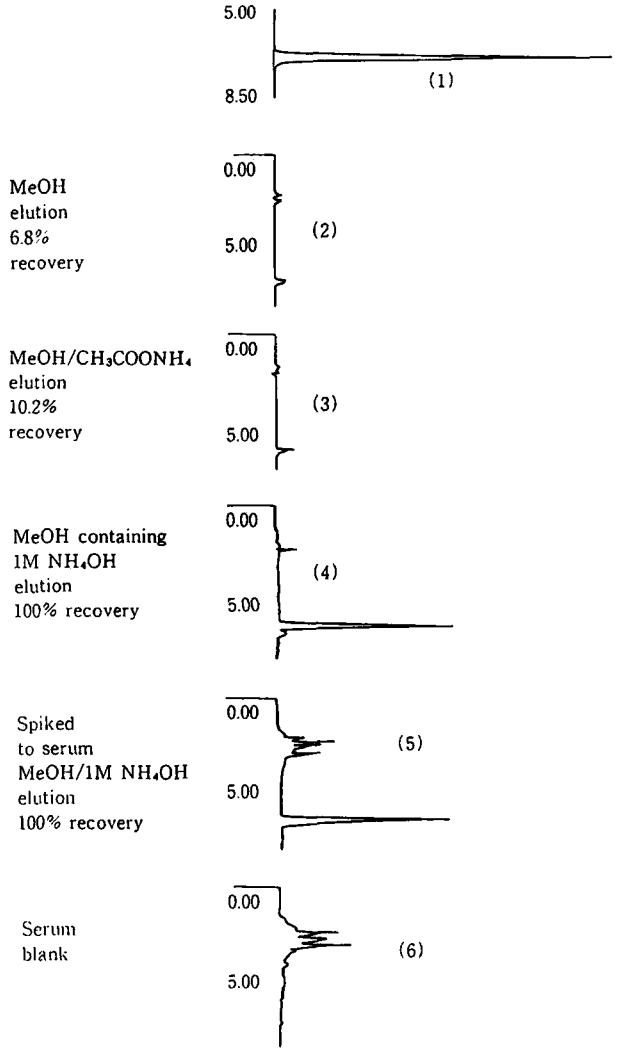

FIGURE 3 HPLC chromatograms of MDA treated with different eluents in SPE using C-18 column
HPLC conditions: C-18 column, Eluent: methanol and an aqueous solution of 10 mM ammonium acetate at a ratio of 1/1, Flow rate: 1.2 ml/min, Detection: 254 nm. MDA peak has a retention time at 5.95 min.
(1) 105 ng/10 µl MDA standard solution, 10 µl applied to HPLC.
(2) 100 µl of 105 ng/10 µl MDA solution applied to C-18 resin with 100 mg and 120 µl, respectively, resin weight and void volume, and eluted with 250 µl of methanol. 200 µl collected and 10 µl applied to HPLC. When 100% is recovered, the theoretical concentration of MDA would be 52.5 ng/10 µl.

depressed to prevent DMPT tailing. By employing a Capcell Pak^{R} C-18 SG-120, simultaneous analysis of MMA, DMPT and BPO was successfully attained without DMPT tailing.

BPO was converted to BA when contacting with DMPT or serum, thus BA analysis was necessary. BA was successfully analyzed without the use of an acidified eluent when using a Capcell Pak^{R} C-18 AG-120. Ion-suppression chromatography is not required.

Liquid-Liquid Extraction of MMA, DMPT, BPO and BA in Serum

Liquid-liquid extraction was carried out by adding an identical volume of acetonitrile to serum for deproteinization and MMA and DMPT extraction. The MMA peak showed an insufficient separation from serum admixtures and insufficient recoveries of MMA and DMPT (84% and 62% for MMA and DMPT, respectively, n=3). Thus, SPE was studied.

SPE of MMA, DMPT, BPO and BA

The following recovery rate is the average amount of 5 specimens. The C.V. is less than 1.5% in every case.

SPE of BA in food has been reported by Terada et al.(27). There have been no SPE with a satisfactory recovery of serum MMA, DMPT, or BA. When the authors applied Terada's method for serum BA, the recovery was insufficient (eluent: a mixed solution of water and acetonitrile at a ratio of 1/1).

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(3) The same procedure as above except for the elution, 250 µl of a mixed aqueous solution of 10 mM ammonium acetate and methanol at a ratio of 1/1.
(4) The same procedure as above except for the elution, 250 µl of methanol containing 1M NH_4OH.
(5) 50 µl of 21 µg/ml MDA was added to 1 ml serum to prepare 1 µg/ml serum. This was applied to C-18 resin, eluted with 250 µl of methanol containing 1M NH_4OH. 200 µl collected. Ten µl applied to HPLC. If 100% is recovered, the theoretical concentration would be 50.0 ng/10 µl.
(6) The same procedure as above but omitting MDA, the serum blank.

Acetonitrile, alkalified acetonitrile containing 50 mM sodium hydroxide, or acidified acetonitrile adjusted to pH 2.5 with acetic acid were compared. Acetonitrile showed an insufficient recovery (80%) (28). Alkalified acetonitrile and acidified acetonitrile indicated 85% or 100% recovery, respectively, therefore acidified acetonitrile was superior to alkalified (28). Acidified solution was used during conditioning, therefore alkalinity may be suppressed due to acidified condition.

In DMPT elution, alkalified acetonitrile was more effective than acetonitrile due to alkalified conditioning. Acetonitrile also produced a satisfactory recovery of MMA and DMPT, however acidified acetonitrile was inferior to alkalified acetonitrile due to alkalified conditioning (28-29).

In SPE of MMA, DMPT and BPO, 50 mM phosphate buffer at pH 7.5 was used for column conditioning. The use of water or more than 50 mM phosphate buffer resulted in a lower recovery. This is because an insufficient depression of DMPT ionization by water and excessive buffer ions more than 50 mM interfere with DMPT retention on the column.

Figure 4a, b, and c show HPLC chromatograms of a standard sample solution, serum sample containing 11 ppm of MMA and DMPT and a blank serum, respectively. Following SPE treatment, MMA and DMPT were 100% recovered with no significant difference in the standard sample and the serum sample, indicating that there was no interference by serum admixtures with the recovery of MMA and DMPT. MMA and DMPT did not significantly react with serum components. The blank serum chromatogram in Figure 4c shows no interference by serum admixtures.

Figure 5a shows that the standard sample solution of BPO at the concentration of 11 ppm was successfully (98%) recovered from C-18 column. When BPO was added to serum, BPO was rapidly converted to BA with no BPO elution (Figure 5b). The blank serum chromatogram was identical to Figure 4c.

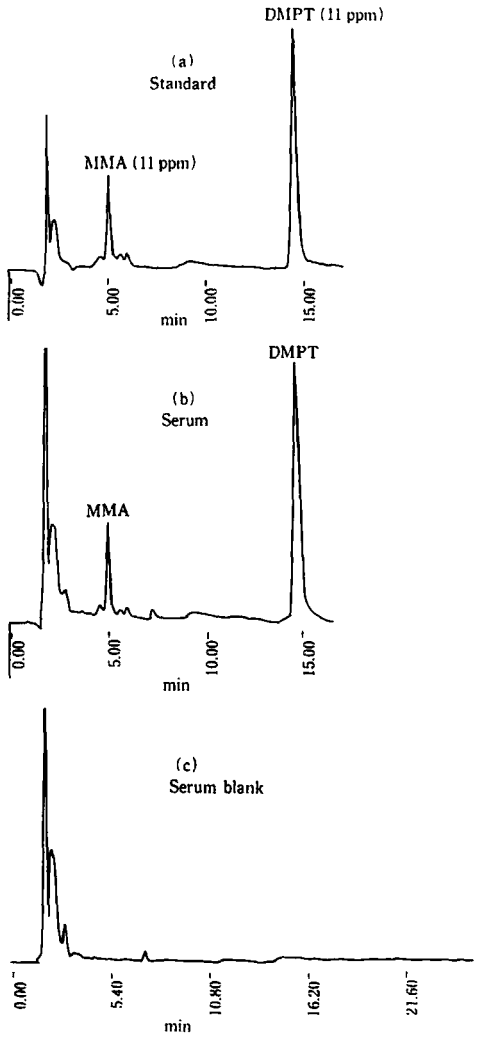

FIGURE 4 SPE of MMA and DMPT from C-18 column
(a) 11 mg of MMA and DMPT were added to 50 ml of a mixed aqueous solution of water and acetonitrile at a ratio of 20/1. 100 µl added to 1900 µl of water to obtain an 11 ppm (µg/ml) solution. One ml applied to the conditioned C-18 column with 100% recovery. HPLC conditions refer to the text.
(b) Identical to (a) excepting that 100 µl of solution were added to 1900 µl of serum in place of water to obtain 11 ppm (µg/ml) serum solution. One ml applied to the conditioned C-18 column with 100% recovery.
(c) One ml of a native serum was treated, recovered and applied to HPLC. The result indicated no interference by serum admixtures with the elution of MMA and DMPT.

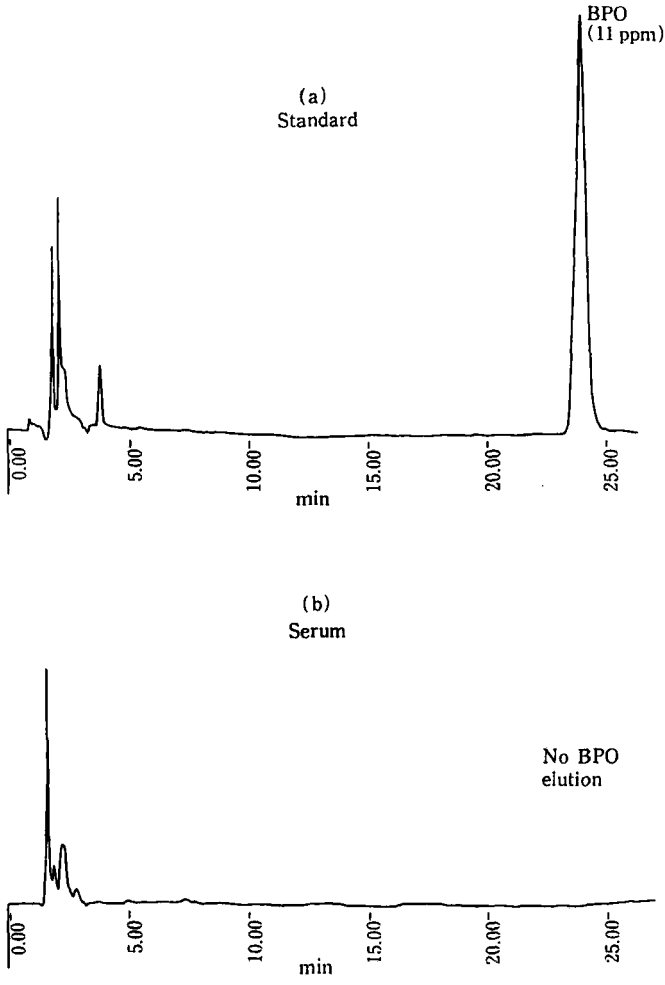

FIGURE 5 SPE of BPO from C-18 column
(a) 11 mg of BPO was added to 50 ml of a mixed aqueous solution of water and acetonitrile at a ratio of 20/1. 100 µl added to 1900 µl of water to obtain 11 ppm (µg/ml) solution. One ml applied to the conditioned C-18 column with 100% recovery. HPLC conditions refer to the text.
(b) Identical to (a) excepting that 100 µl of solution was added to 1900 µl of serum in place of water to obtain 11 ppm (µg/ml) serum solution. One ml applied to the conditioned C-18 column with no BPO recovery. This was due to the conversion of BPO to BA when in contact with serum. The blank serum was identical to FIGURE 4c.

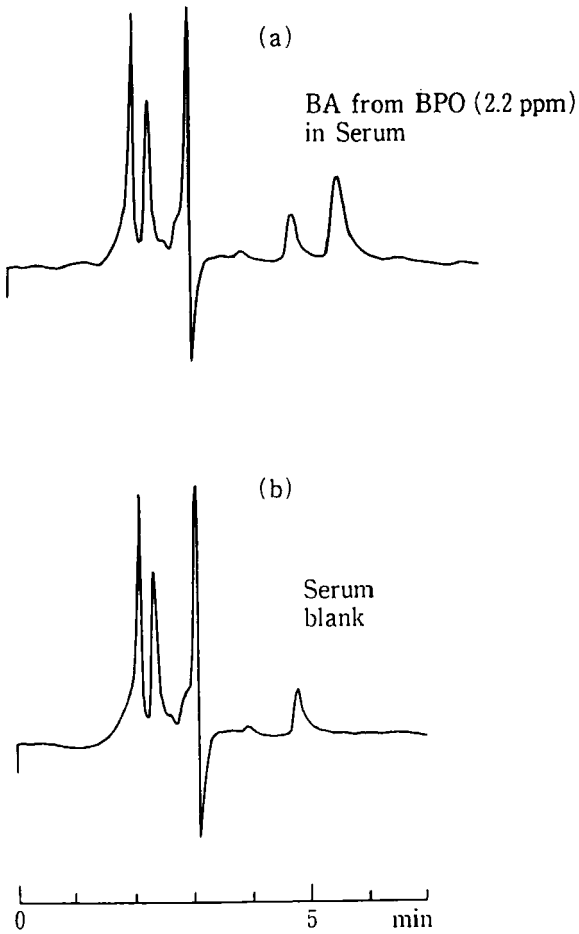

FIGURE 6 SPE of BA from BPO in serum using C-18 column
(a) 11 mg of BPO was added to 50 ml of a mixed aqueous solution of water and acetonitrile at a ratio of 20/1. 100 µl was added to 1900 µl of serum to obtain 11 ppm (µg/ml) serum solution. This was 5-fold diluted with an acetic acid aqueous solution at pH 3. One ml applied to the conditioned C-18 column with 100% recovery of BA from BPO. HPLC conditions refer to the text.
(b) One ml of 5-fold diluted native serum with an acetic acid aqueous solution at pH 3 was treated and recovered. This applied to HPLC. The result indicates no interference by serum admixtures with the elution of BA.

Figure 6a shows a HPLC chromatogram of serum sample containing 2.2 ppm of BPO, which was completely (100%) converted to BA and successfully (99%) recovered from C-18 column by the SPE of serum BA. Figure 6b shows serum blank.

The Eluted Amount of MMA, DMPT and BPO with Serum from Two Different PMMA Dental Materials

Two different samples with an almost identical weight (around 3 g) were immersed in 10 ml of serum and the leached amount was determined by SPE followed by HPLC. The serum was replaced daily over three consecutive days. The eluted amount was accumulated to evaluate the extractable quantity.

MMA and DMPT eluted with serum were 32.04 μg/g and 66.44 μg/g for sample A and 10.32 μg/g and zero for sample B, respectively (n=3). DMPT was not employed in the fabrication of sample B. A greater elution of MMA and DMPT from a more pliant sample A indicates that serum can more readily penetrate the interior of the softer material. Sample A is more pliant than sample B. BPO was determined as BA, because BPO was immediately converted to BA when in contact with serum. BPO elution to serum was negligible (2.3 and 0 μg/g for samples A and B, respectively, n=3). This was possibly due to excessive hydrophobicity of BPO. BPO in the surface of the material can be extracted with serum. More BPO was eluted from the more pliant material.

CONCLUSION

SPE of serum MDA was successfully carried out using C-18, Phenyl or Cyclohexyl column combined with the eluent of methanol containing 1M NH_4OH. HPLC of MDA was carried out using ODS column combined with the eluent of a mixed solution of 50 mM ammonium acetate and acetonitrile. Detection was by ECD. The eluted amount

of MDA from gamma-ray irradiated potting material was a few ppm at 10 Mrad irradiation. Less than one ppm of MDA was formed at 2.5 Mrad irradiation. Risk factor estimation indicates these amounts are "not significant".

SPE of MMA, DMPT, BPO and BA in serum were successfully accomplished using C-18 column combined with an ionization suppression of DMPT and BA. HPLC of DMPT and BA was successfully accomplished by the use of sufficiently endcapped C-18 column. The eluted amount of MMA, DMPT and BPO from PMMA was 10 to 100 ppm.

SPE was superior to liquid-liquid extraction due to the unnecessity of deproteinization, centrifuge separation and concentration. Less organic solvent consumption, easy handling, easy application for automated procedure and so on are other favorable factors in SPE.

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Received: October 28, 1991
Accepted: December 12, 1991