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Magnet Integrated Fabric Phase Sorptive Extraction for the Extraction of Resin Monomers from Human Urine Prior to HPLC Analysis

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Abstract: In this work, a method for the simultaneous determination of four resin monomers: Bisphenol A, bisphenol A methacrylate glycidate, triethyleneglycol-dimethacrylate, and urethane dimethacrylate, from human urine using magnet integrated fabric phase sorptive extraction (MI-FPSE), followed by high performance liquid chromatography (HPLC) diode array detection (HPLC-DAD), is presented. MI-FPSE is a novel configuration of FPSE that incorporates the stirring and extraction mechanism into one device, resulting in an improved extraction kinetic factor. FPSE is a green sample preparation technique that uses a flexible surface, such as cellulose, coated with a polymeric material using sol–gel technology. Poly(tetrahydrofuran) (PTHF) material was selected, due to its higher efficiency in terms of recovery rate among the studied MI-FPSE membranes. Optimization of the extraction process was performed based on several extraction and elution parameters. The method was validated for its linearity, selectivity, accuracy, precision, and stability of the samples. For the four compounds, the LOD and LOQ were 0.170 ng/μL and 0.050 ng/μL, respectively. The relative standard deviation of the method was less than 9.8% and 11.9%, for the within-day and between-day precision, respectively. The relative recoveries were between 85.6 and 105.2% in all cases, showing a good accuracy. The effectiveness of the proposed method was confirmed through successful application to the bioanalysis of real urine samples.

Keywords: resin monomers; FPSE; bisphenol A; human urine; bioanalysis; HPLC-DAD

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1. Introduction

Composite dental resins are still considered representative materials in both preventive and restorative methods. They are a mixture of dental resins and various inorganic fillers. The resins consist of a mixture of two or more monomers, in order to achieve balanced functions in the applicable rheology, as well as to have the desired mechanical properties before and after curing. The components used to form composite resins have endocrine or cytotoxic properties, and they can potentially cause minimal or severe damage to the human body when found at certain concentrations [1]. The composite methacrylate resins used to date have been based on bisphenol A methacrylate glycidate (BisGMA) monomer, but due to the release of bisphenol A (BPA), there is a desire to replace it. In this way, various modifications can be made to the phases of the composite resin, such as the organic phase and reinforcing agents, in order to address the polymerization contraction [2,3].

The most widely used basic monomer is BisGMA, which is a bifunctional aromatic molecule bearing two terminal methacrylic groups capable of reacting to form a covalent

bond [4]. BisGMA's hazardousness mainly occurs in the synthetic route, because the release of by-products of BPA causes serious problems. Due to the hydrogen bonds formed between its hydroxyl groups, this monomer has an extremely high viscosity at room temperature, and because of its high molecular weight, it becomes difficult both to incorporate the reinforcing substances into its mass and to handle it. Thus, the addition of a second monomer (co-monomer) with a lower molecular weight for clinical use of the corresponding polymer is inevitable [5–7].

BPA and its derivatives, including BisGMA, have been identified as endocrine disruptors, which means that they can mimic and interfere with the function of various hormone receptors, affecting body systems. BPA behaves similarly to the natural estrogen 17-beta-estradiol and can bind to estrogenic or androgenic receptors. However, its activity is much lower and can potentially cause a decrease in fertility, oxidative stress, heart disease, diabetes, as well as problems related to the proper functioning of the immune system [8,9]. It has been found that BisGMA can degrade to BPA, with a conversion rate of 82.5% in 24 h. Due to the undesirable effects of BPA on the human body, strict regulatory limits have been set regarding its use. The tolerable daily intake (TDI) of BPA was reduced from 50 μg/kg to 4 μg/kg body weight in 2015 by the European Food Safety Authority (EFSA) [10,11].

The composite methacrylate resins used to date are based on the BisGMA monomer, but due to the release of bisphenol A, there is a tendency to replace them. Triethyleneglycol-dimethacrylate (TEGDMA) has been widely used as a diluent is methacrylate derivatives and is usually found at a ratio of 25% versus 75% BisGMA. However, TEGDMA can penetrate the cell membrane and react with intracellular molecules, causing oxidative stress [12,13]. A monomer that can be used either as an alternative to BisGMA or in combination with it, in order to improve the properties of synthetic resins, is urethane dimethacrylate (UDMA) [7]. The negative effects of this monomer are mainly the induction of cytotoxicity and genotoxicity, as with TEGDMA. However, the simultaneous combined use of UDMA and TEGDMA reduces the number of cells undergoing apoptosis and has less influence on the cell cycle, whereas the stand-alone use of each monomer shows higher cytotoxicity and genotoxicity [4,14]. Thus, there is an increased risk associated with BisGMA, TEGDMA, UDMA, and BPA, and their monitoring in biological matrices is considered important.

Nowadays, a highly important idea in green sample preparation is the integration of several processes in fewer steps, for the selective extraction of the target analytes. Bearing this in mind, and taking into consideration the beneficial role of stirring, submerging the extraction sorbent material unit and stirring it in the same device appears to be a novel improvement. On the basis of this concept, various unique extraction and stirring integrated procedures have been developed and thoroughly appraised [15–17]. At the same time, various novel sorbent-based microextraction techniques have been developed [18–20]. Magnet integrated fabric phase sorptive extraction (MI-FPSE) is a recently introduced extraction technique, which has been used in environmental, biological, and food sample analysis [21–23]. In MI-FPSE, two FPSE membranes are joined together, and a magnetic rod is embedded in the device. This creates a customizable extraction surface that is easy to handle, enables exploiting a wide variety of sol–gel adsorbents, and can be used to develop simple, fast, environmentally friendly, and sensitive methods. In this way, extraction devices consisting of FPSE membranes with different polarities can be combined to extract chemical compounds with a wider range of polarities [24].

In this work, for the first time, MI-FPSE was used in combination with HPLC-DAD to monitor oral resin monomers (TEGDMA, UDMA, BisGMA, and BPA) and an endocrine disruptor (BPA) in human urine samples. Following the selection of the most efficient sol–gel MI-FPSE membrane, the main extraction parameters were thoroughly investigated and optimized. Accordingly, the proposed method was validated and successfully used to determine target analytes in real human urine samples.

2. Materials and Methods

2.1. Instrumentation

The chromatographic quaternary low-pressure gradient HPLC- PDA system (Shimadzu, Kyoto, Japan) consisted of an FCV-10ALVP mixing system, an LC-10ADVP pump, a Shimadzu SCL-10ALVP System Controller, a Rheodyne (Cotati, CA, USA) 7725i injection valve with a 20 μL loop, and an SPD-M10AVP PDA detector controlled by Lab Solutions-LC software (Shimadzu (Kyoto, Japan)). A DGU-10B degassing unit with helium was used for the degassing of the mobile phase. Antistatic plastic tweezers (IDEAL-TEK SA, 6828 Balerna, Switzerland) free of oral resin monomers and BPA were used for MI-FPSE extraction device handling. A ReactiVap 9-port evaporator model 18,780 by Pierce (Rockford, IL, USA) was applied for sample evaporation. Q-Max RR syringe filters (0.22 μm nylon membrane) obtained by Frisenette ApS (Knebel, Denmark) were used for sample filtration prior to HPLC analysis. The deionized water purification and production system was a Milli-Q Plus (Millipore, Bedford, MA, USA). BPA-free polypropylene containers were used for the collection of real urine samples.

2.2. Reagents, Chemicals and Samples

BPA, TEGDMA, UDMA, and BisGMA standards were purchased from Sigma-Aldrich-LLC (Taufkirchen, Germany). HPLC grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). The two applied FPSE membranes, namely the sol–gel Carbowax 20M that was based on a polyethylene glycol polymer and the sol–gel poly(tetrahydrofuran) coated on cellulose fabric substrate, were produced via a sol–gel coating process, as described (i.e., synthesis and characterization) elsewhere [21,23,25,26].

Stock standard solutions were prepared in water at a concentration of 100 μg mL^{-1} in water. The stock aqueous solutions were found to be stable for almost 2 months when stored at +4 °C. The working solutions were derived from the stocks by appropriate dilutions at various concentrations, covering the whole linear range from 0.5 to 15 ng/μL. The working standard solutions were replaced every ca. five days. Human urine was collected in polypropylene containers from healthy volunteers, who were fully informed about the experimental procedure after obtaining their written consent.

2.3. Chromatographic Conditions

The examined oral monomers were separated in a Perfect Sil Target ODS-3 (5 μm, 250 × 4.6 mm) column working at 35 °C, within 10 min, in gradient mode. The binary mobile phase consisted of H_2O (A) and acetonitrile (B). Gradient conditions were 0–15 min, 70% A, 1 mL/min, 6–7 min, 70–75% A, 1–1.1 mL/min, 7–15 min, 75% A, 1.1 mL/min. A 2 min equilibration period proved sufficient and was followed between runs. The inlet back pressure ranged between 110 bar at the beginning and 117–118 bar at the end of analysis. Peak monitoring and integration were both carried out at 222 nm, which was the appropriate wavelength for all four compounds. Peaks were identified from their retention times, as well as by the spectra provided by the diode array detector. Retention times were 3.9 min for BPA, 5.2 min for TEG-DMA, 7.3 min for UDMA, and 9.0 min for BisGMA. The column was operated under a constant temperature of 35 °C.

2.4. MI-FPSE

The MI-FPSE procedure consisted of the following steps: (a) immersion of the sol–gel PTHF coated MI-FPSE device in 2 mL of ACN:MeOH (50:50 *v*/*v*) for 5 min, to remove any residual components and to activate the sorbent, (b) rinsing with deionized water to wash out any remaining organic solvents, (c) immersion of the extraction device into the sample (1 mL, 900 μL of urine and 100 μL of standard solution for spiked samples or water for blank samples) and extraction under magnetic stirring (390 rpm) for 20 min, (d) elution for 5 min in a vial containing 500 μL MeOH, (e) filtration and evaporation until dry under

gentle nitrogen stream, (f) reconstitution in 100 μL of MeOH, and (g) injection into the HPLC-PDA system.

After every pretreatment, the extraction device was washed with 2 mL of ACN: MeOH (50:50 *v*/*v*) for 5 min and left to air dry. Subsequently it was stored in an airtight sealed vial. Under these conditions, it could be reused up to 10 times, with no substantial loss of extraction capacity, when a 10% recovery loss criterion was applied and no carryover phenomenon was noticed.

2.5. Method Validation

The MI-FPSE HPLC-PDA method was validated using spiked urine samples, in terms of the linearity, sensitivity, selectivity, stability, precision, and accuracy. Calibration curves were constructed by plotting the peak area versus concentration. Least square linear regression analysis was adopted for the calculation of slopes, intercepts, and coefficients of determination.

Accuracy was expressed as percentage of relative recoveries (%RR = mean concentration found/added concentration × 100) using regression data. Precision was estimated in terms of the relative standard deviation (%RSD = calculated standard deviation/mean found concentration × 100) with repeated measurements. Intra-day accuracy and precision were assessed using three measurements carried out on the same day as each of the five selected quantity levels, namely at 0.100 and 0.500 ng μL^{-1}. Inter-day accuracy and precision were determined with a triplicate analysis at the same concentration levels on four consecutive days.

2.6. Greenness Assessment of the Proposed Method

As a final step, ComplexGAPI index [27] was used for the evaluation of the green character of the proposed method. This index incorporates the assessment of the original GAPI index (five pentagons) [28], while it can additionally evaluate the synthetical procedure for the preparation of sol–gel PTHF coated MI-FPSE media (additional hexagon). In this context, the sample collection, storage, transport, preservation, the sample preparation, the reagents and chemicals, the method type, and the instrumentation were evaluated. A green color shows compliance with the principles of green analytical chemistry (GAC) [29]. Yellow and red colors show medium and high environmental impacts, respectively.

3. Results and Discussion

3.1. Selection of an Appropriate Sol–Gel Coated MI-FPSE Device and Mechanism of Extraction

The choice of the most favorable membrane is commonly based on experimental tests, since the polarity of the target analytes differs over a wide range. A decisive factor for FPSE extraction is the intermolecular interactions, which depend on the polarity of the monomers, but also of the sorbent and therefore the MI-FPSE device. Adsorbent coatings are crafted using the sol–gel method, either on a hydrophilic cellulose fabric or on a hydrophobic polyester fabric. In summary, the choice is determined by the chemical structure of the targeted analytes, the ability of the sol–gel materials to form intermolecular interactions with them, the environment of the sample (aquatic or non-aquatic), and other factors. Two different sol–gel coated on cellulose substrate membranes were tested, namely a medium polarity sol–gel PTHF membrane, and a polar Carbowax 20M (CW 20M) membrane. The benchmark for the best selection was the absolute recovery of the four compounds after extracting the target analytes from 1 mL of sample spiked at a final concertation of 0.5 ng mL^{-1} under magnetic stirring (390 rpm) for 20 min, and elution for 5 min under by an aliquot of 500 μL MeOH. As shown in Table 1, both MI-FPSE devices proved to be biocompatible with the matrix of human urine. The sol–gel PTHF coated on cellulose substrate MI-FPSE membrane yielded slightly higher recoveries. Thus, it was considered the most effective for all target analytes. Optimization of the extraction procedure was further applied.

Table 1. Selection of the MI-FPSE extraction device, characteristics, and extraction performance of the applied sorbents membranes. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

Sol–Gel Coating	Sorbent Loading (mg/cm^2)	Fabric Substrate	Polarity	Absolute Recovery Values (%)			
				BPA	TEG	UDMA	BisGMA
PTHF	3.96	Cellulose	Medium Polar	76.0	72.2	77.2	60.2
CW 20M	4.71	Cellulose	Polar	65.5	64.7	62.9	54.6

3.2. Investigation of the Effect of Elution Solvent Composition, Sample Volume, Extraction and Elution Time, and Stirring Rate on the Extraction Efficiency

Initially, three different sample quantities (i.e., 500 μL of undiluted urine, 1000 μL of undiluted urine, and 1000 μL of urine diluted 1-fold with water) were compared. The three sample types resulted in similar absolute recovery values. Thus, the 1000 μL undiluted urine sample was chosen, to ensure high preconcentration of the analytes, sufficient sensitivity, and adequate sample consumption. Then, the type of the eluent was studied. Due to the polarity differences among the four monomers, it was necessary to experimentally investigate and select the appropriate solvent for eluting the target analytes. Considering the above, 500 μL of various solvents or solvent mixtures, such as ACN, MeOH, MeOH:ACN (50:50 v/v), and acetone, as well as the mobile phase of HPLC, were used to elute the four selected compounds from the PTHF membrane. In all cases the solvents demonstrated satisfactory elution, with minor differences in the absolute recoveries (Figure 1). The solvent methanol stood out compared to the other solvents, both in terms of recoveries, but also considering the environmental and economic costs. Four volumes were tested, to select the most appropriate sample volume, with the volume of 500 μL being optimum.

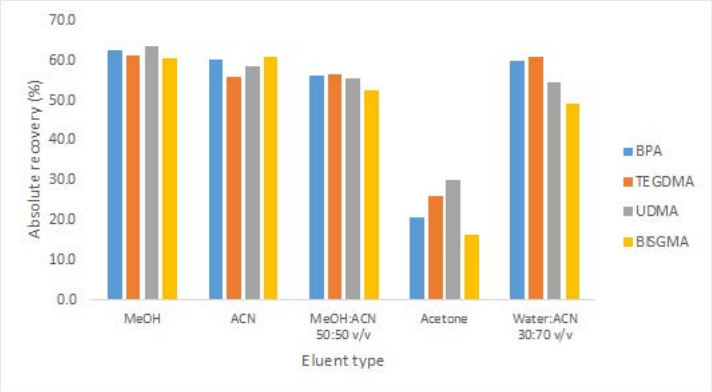

Figure 1. Evaluation of different elution solvents/solvent mixtures for the extraction of the target analytes. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

According to FPSE principles, the intervention time, both during the extraction and elution under potential constant stirring, is the most important parameter of the whole procedure, due to the equilibrium mode of action. The original MI-FPSE protocol was designed for a 40 min duration extraction step. Two scenarios were equally possible. Either the equilibrium would not be reached until the 40 min time limit and more compounds could be extracted, or it would be achieved earlier. Thus, six different time intervals were applied in the range of 10 to 60 min. It is clearly confirmed in Figure 2 that the extraction of analytes was favored from 10 to 20 min. After that time interval and when 30, 40, 50, and 60 min of extraction were applied, the results indicated that smaller quantities were extracted. In fact, 20 min were adequate for the equilibrium to be reached under a constant stirring rate.

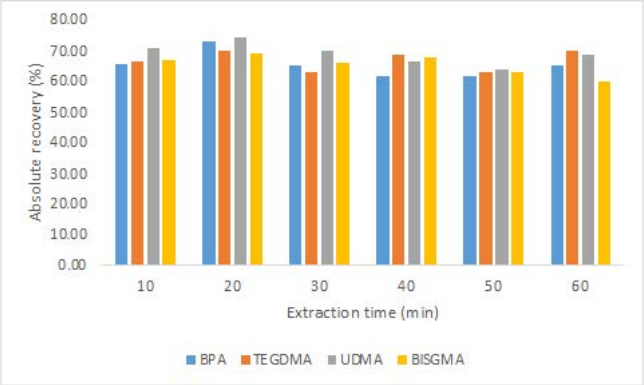

Figure 2. Investigation of the effect of extraction time on the extraction efficiency. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

Regarding the elution time, three time periods were tested, i.e., 5, 10, and 15 min, under stirring. The same intervals were investigated without stirring. It was observed that 5 min of elution was sufficient time for the quantitative desorption of all analytes, while no agitation was required during this step. The stirring rate during the adsorption of the analytes was studied with medium stirring conditions (i.e., 260 rpm) and high stirring conditions (i.e., 390 rpm). In the latter case, the absolute extraction recoveries were higher and, thus, this stirring rate was employed for the subsequent experiments.

Then, the ionic strength of the sample was studied. The addition of salt to a liquid sample solution causes two competing effects, in terms of ionic strength. On the one hand, the salting out effect leads to a reduction of the water solubility of all analytes. On the other hand, however, the viscosity of the solution increases at the same time, preventing the diffusion of compounds and reducing the mass transfer rate from the sample to the surface of the extraction medium. As a result, a smaller quantity is extracted in the same extraction time. Thus, the effect of ionic strength was studied, by adding different amounts of sodium chloride (NaCl); namely 5%, 10%, and 15% w/v NaCl were investigated for the urine samples. The addition of salt was not beneficial for any of the extraction process optimization studies, while a NaCl content higher than 5% caused an average decrease in the calculated recoveries ranging from 8% to 10%. As shown in Figure 3, NaCl addition did not have any beneficial effect on the recoveries, especially for TEGDMA, where the percentages were about 20% lower [22].

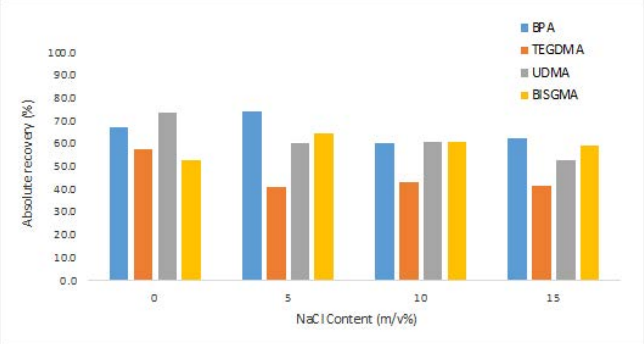

Figure 3. Investigation of the effect of ionic strength on the extraction efficiency. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

3.3. Method Validation

The MI-FPSE HPLC-PDA method was validated in terms of linearity, sensitivity, selectivity, stability, precision, and accuracy using spiked urine samples. Linearity was

assessed with triplicate analysis in a working range from 0.050–0.500 μg mL^{-1}. The correlation coefficients were 0.991, 0.9995, 0.9990, and 0.997 for BPA, TEGDMA, UDMA, and BisGMA, respectively. Limits of detection (LODs) and limits of quantification were estimated using the formulas LOD = 3 S/N and LOQ = 10 S/N, where S = signal and N = noise. For the four analytes, the LOD and LOQ rates were 0.017 and 0.050 ng μL^{-1}, respectively. Selectivity was evaluated by analyzing blank urine samples (n = 6) and comparing the outcomes with the chromatographic data. The selectivity was verified by the absence of interference in the same chromatographic window of the analytes of interest.

The intra-day and inter-day accuracy and precision results are summarized in Table 2.

Table 2. Intra-day and inter-day accuracy and precision for the MI-FPSE method. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

Analyte	Added (ng μL^{-1})	Intra-Day (n = 5)			Inter-Day (n = 4 × 3)		
		Found (ng μL^{-1})	RSD%	RR%	Found (ng μL^{-1})	RSD%	RR%
TEGDMA	0.100	0.093 ± 0.003	3.2	93.0	0.090 ± 0.008	8.9	90.0
	0.500	0.462 ± 0.011	2.4	92.4	0.450 ± 0.045	10.0	90.0
BPA	0.100	0.086 ± 0.004	4.7	86.0	0.088 ± 0.005	5.7	88.0
	0.500	0.510 ± 0.017	3.3	102.0	0.478 ± 0.047	9.8	95.6
UDMA	0.100	0.092 ± 0.009	9.8	92.0	0.099 ± 0.005	5.1	99.0
	0.500	0.503 ± 0.011	2.2	100.6	0.526 ± 0.039	7.4	105.2
BisGMA	0.100	0.103 ± 0.004	3.9	103.0	0.097 ± 0.006	6.2	97.0
	0.500	0.433 ± 0.007	1.6	86.6	0.428 ± 0.051	11.9	85.6

Intra-day precision and accuracy were very satisfactory, given that the RSD% rates were lower than 9.8% and the RR% values ranged between 86.0 and 103.0% for all target analytes at all the concentration levels. Inter-day precision and accuracy results were also very satisfactory, as the respective RSD% rates were lower than 11.9 and the RR% values ranged between 85.6 and 105.2%.

3.4. Freeze–Thaw Cycles

Freeze–thaw stability was examined in six consecutive freeze–thaw cycles (% Stability = $C_i \times 100/C_{fresh}$, C_i = measured concentration of each analyte in stored spiked urine sample, C_{fresh} = measured concentration of each analyte in the freshly spiked urine sample) [30]. The freeze–thaw cycle stability study revealed that all four compounds were stable for two circles, using the criterion of a 10% recovery loss.

3.5. Reusability

Reusability was studied for the extraction device under a 10% extraction efficiency loss criterion, based on the absolute recovery values of the analytes in sequential extractions with the same device (% Extraction capacity = $C_i \times 100/C_{initial}$, C_i = measured concentration after each specific reuse of the device, i = 1, 2, . . . 10, $C_{initial}$ = measured concentration after the first extraction). The sol–gel PTHF coated MI-FPSE device could be reused at least ten times without significant loss of its extraction capability.

3.6. Real Sample Analysis

The developed and validated method was used for the extraction of the target analytes from five human urine samples collected from healthy volunteers. In these cases, the four monomers were not detected. A blank urine sample (A) subjected to the MI-FPSE protocol and a spiked urine sample at 0.1 ng μL^{-1} are provided in Figure 4.

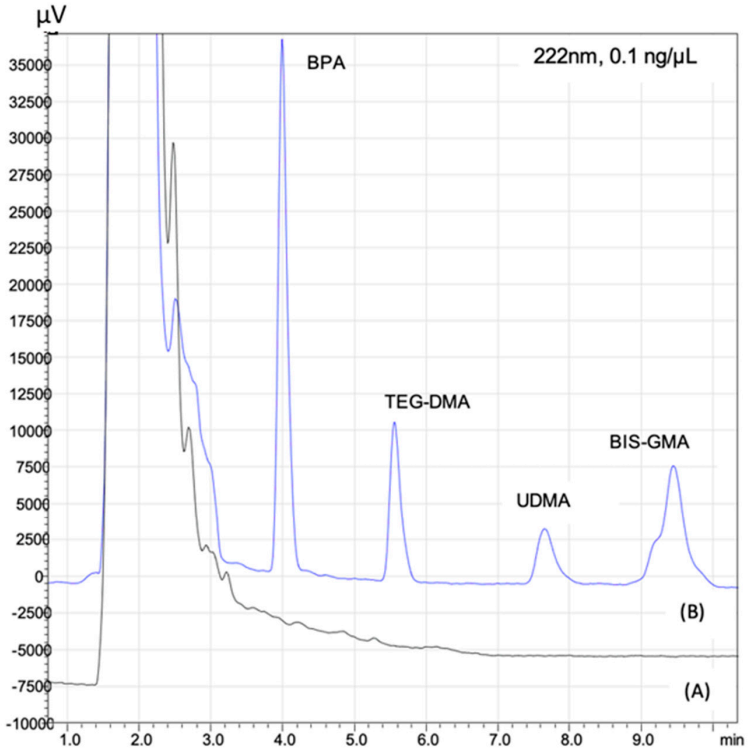

Figure 4. Typical HPLC chromatograms of Blank urine sample (A) and spiked urine sample at 0.1 ng μL^{-1} (B). Peaks at (B) are: 1: BPA, 2: TEGDMA, 3: UDMA, and 4: BisGMA. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane methacrylate).

3.7. Greenness Assessment of the Proposed Method

As described in Section 2.6, the ComplexGAPI index was used for the evaluation of the green character of the proposed method. Figure 5 shows the ComplexGAPI pictogram of the developed method. It can be observed that many of the requirements were met (green color). Further suggestions for the improvement of the method's green character include the replacement of the organic solvents with greener solvents [31] and the utilization of ultra-high performance liquid chromatography instead of the conventional HPLC technique.

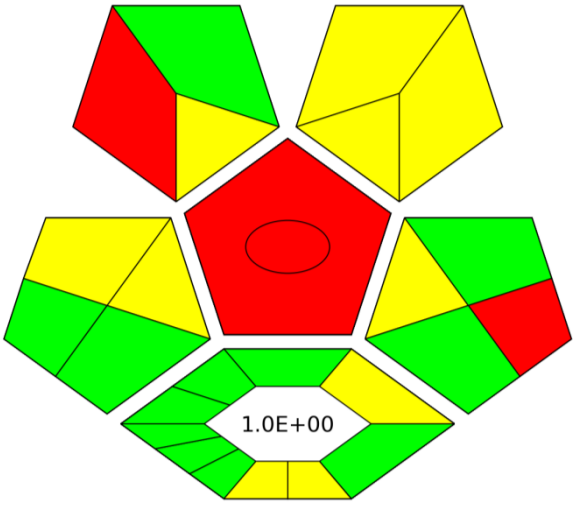

Figure 5. ComplexGAPI index for the developed method.

3.8. Comparison of the New Method

The determination of the selected monomers in human urine samples using an MI-FPSE sample pretreatment method prior to high pressure liquid chromatography (HPLC) coupled with a photodiode array detector (HPLC-PDA) was compared with other methods. Due to the originality of the subject matter, it was not possible to compare with many other works that used a human urine substrate to develop a method for the determination of the selected monomers. In the work described in Table 3, the pretreatment method involved protein precipitation with acetonitrile and drying, and unlike in the present proposed method, pretreatment was performed using the MI-FPSE technique followed by drying under N_2 stream and reconstitution with MeOH to a final volume of 100 μL. This was achieved without any negative impact on the performance of the analytical method, since the detection and quantification limits were particularly lower in the proposed method while, the recoveries were relatively similar, as shown in Table 3.

Table 3. Comparison of current study with a previously described method. (BPA: Bisphenol A, BisGMA: bisphenol A methacrylate glycidate, TEGDMA: triethyleneglycol-dimethacrylate, UDMA: urethane dimethacrylate).

Analytical Technique	Pretreatment Protocol	% RR	RSD %	LOD (ng/μL)	Reference
HPLC-UV	ACN and centrifugation at 3500 rpm for 15 min.	95.0–106.9%	<6.6%	BPA-1.1 TEGDMA-0.6 UDMA-0.6 Bis-GMA-0.6	[32]
HPLC-PDA	MI-FPSE	85.6–105.2%	<11.9%	BPA-0.017 TEGDMA-0.017 UDMA-0.017 Bis-GMA-0.017	Current Study

4. Conclusions

In the study herein, a simple and rapid MI-FPSE method was developed and validated for the determination of four monomers released from dental materials (i.e., BPA, TEGDMA, UDMA, and BisGMA) in human urine. It is a configuration that integrates the stirring and extraction mechanism into a single sample preparation device, while taking into account the advantages of improving the extraction kinetic factor, and more specifically the diffusion, for the performance of microextraction techniques. FPSE was the scaffold used to develop the new MI-FPSE-HPLC-PDA method for the determination of selected monomers in human urine. The method was simple, economical, and in accordance with green chemistry trends. The MI-FPSE method exhibited good accuracy and precision, a wide linear range, and low LOD and LOQ values. The sol–gel PTHF coated MI- FPSE membrane was found to be reusable at least 10 times.

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