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3 **Supramolecular solvent-based microextraction probe for**
4 **fast detection of bisphenols by ambient mass spectrometry**

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20 **Abstract**

21 In this study, we investigated for the first time the suitability of supramolecular solvent
22 (SUPRAS)-based microextraction probe for the development of generic and fast sample
23 treatment prior to qualitative analysis by ambient mass spectrometry (AMS) based on ASAP
24 (atmospheric solids analysis probe). SUPRAS are nanostructured liquids formed by the self-
25 assembly of amphiphilic aggregates with multiple binding sites and microenvironments of
26 different polarity for the efficient extraction of multiple compounds. Different types of
27 SUPRAS were evaluated as a simple and single step sample treatment for ASAP. The method
28 was applied to the screening of bisphenol A and structural analogues in thermal paper. Optimal
29 results were achieved with SUPRAS synthesized with 1-decanol in mixtures of ethanol:water.
30 SUPRAS (1.1-2 μL) were loaded onto glass probes and placed in contact with samples for 10
31 seconds before ASAP analysis. AMS signal peaks (width: 0.2-0.5 min) were easily integrated
32 and normalized with internal standards (RSD: 2-25%). The method was applied to 62 samples
33 of thermal paper. BPA and BPS were the most widely used, this highlighting the progressive
34 industrial replacement of BPA by BPS.

35 **Keywords:** bisphenols; SUPRAS; ambient mass spectrometry; atmospheric solid analytical
36 probe; thermal paper.

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44 **1. Introduction.**

45 Ambient mass spectrometry (AMS) consists in modified atmospheric pressure ionization
46 sources, where solid or liquid samples are directly introduced, so that analytes desorb from the
47 matrix and enter the MS detector. AMS diversified rapidly since the first techniques appeared
48 in 2004 (desorption electrospray ionization, DESI, by Takats et al. 2004) and 2005 (direct
49 analysis in real time, DART, by Cody et al. 2005). AMS comprises a variety of techniques,
50 many times with 2D and 3D imaging possibilities (Awad et al. 2015, Laskin et al. 2016, Lu et
51 al. 2018, Perez et al. 2019), which have been applied in very different fields, such as
52 pharmaceutical, polymer, forensic, food and biological tissue analysis (Aszyk et al. 2018, Lu et
53 al. 2018, Paine et al. 2014, Xiao et al. 2020).

54 The atmospheric-solids-analysis probe (ASAP) technique was first reported in 2005 by
55 McEwen et al. In ASAP, the sample is loaded onto a disposable glass capillary which is placed
56 against the hot stream of the nebulizer gas (N_2) and near the corona needle in an atmospheric
57 pressure chemical ionization (APCI) source. Analytes are desorbed by high temperature and
58 ionized through the corona discharge reactions (McEwen, 2010). ASAP offers advantages over
59 other AMS techniques, such as simplicity, speed and solvent-free operation (avoiding solubility
60 limitations and the need of flow-rate optimization) (Blokland et al. 2020, Cechová et al. 2019,
61 Cvijović et al. 2019, Gaiffe et al. 2018, McCullough et al. 2020, Wójtowicz et al. 2019).

62 The number of studies dealing with the development of sample preparation strategies coupled
63 to AMS is increasing fast with the aim of improving the reproducibility, selectivity and
64 sensitivity of these techniques. Modified electrospray tips (Deng et al. 2017a, Liu et al. 2019,
65 Vasiljevic et al. 2019, Wong et al. 2013) and solid-phased microextraction (SPME) fibers or
66 coated inlet probes (Gómez-Ríos and Pawliszyn, 2014, Wang et al. 2020, Zhao et al. 2019)
67 have been proposed. The application of solvent-based approaches, mainly slug-flow
68 microextraction (SFME), is more limited but it is also gaining attention in the last years. In
69 SFME, plugs of immiscible liquids (usually the extraction solvent and the liquid sample) are in

70 contact in a thin capillary. Turbulences due to the movement of the plugs inside the thin probe
71 ensure the mass transfer at the interface (Deng et al. 2017b, Ren et al. 2014, Zhang et al. 2019).
72 SFME based on ethyl acetate was recently proposed for the analysis of polar compounds in
73 biofluids with nanoESI-AMS. A pipette was used to force the movement of the liquid plugs and
74 enhance recoveries (Zhang et al. 2019). In the same context, a multi-phase system based on two
75 cationic ionic liquids (ILs) and a dichloromethane (DCM) layer was employed for the
76 determination of perfluorinated compounds in waters. The sample was sandwiched between the
77 two ILs and the DCM phase, which was less viscous and it allowed direct analysis by sonic-
78 spray ionization (Lv et al.2019).

79 In this study, we investigated the suitability of supramolecular solvent (SUPRAS)-based
80 microextraction probe for ASAP screening of organic contaminants in solid materials in a single
81 step. SUPRAS are nanostructured liquids produced by self-assembly and coacervation of
82 amphiphiles in aqueous or hydro-organic media. SUPRAS have a high number of available
83 binding sites (amphiphile concentration $\sim 0.1-1$ mg/ μ L) and high surface area due to their
84 discontinuous character what is beneficial for the efficient extraction of compounds at low
85 volumes and under short extraction times. SUPRAS are also suitable for wide screening
86 purposes and for the obtainment of MS fingerprints since they offer regions of different polarity
87 within their aggregates and they can establish mixed interactions for extraction (polar, ionic or
88 hydrogen bonds with the polar groups of amphiphiles and the aqueous pools and dispersive
89 interactions with the hydrocarbon chains layers). They also feature certain restricted access
90 properties for clean-up and have been proven to exclude protein and polysaccharides in
91 extraction processes (Ballesteros-Gómez and Rubio, 2012). These properties, together with
92 their high surface tension and low volatility (that facilitate their confinement inside the glass
93 capillary during extraction) and their low toxicity, make them excellent candidates for the
94 proposed microextraction probe format which is operated under simple contact with the sample
95 for few seconds.

96 As proof-of-principle, the developed SUPRAS-based microextraction probes in combination
97 with ASAP-MS/MS were applied to the screening bisphenol A and six replacements in thermal
98 paper. BPA replacements were 4,4'-Sulfonyldiphenol (bisphenol S, BPS), 4,4'-
99 Methylenediphenol (bisphenol F, BPF), 4-(4-phenylmethoxyphenyl)sulfonylphenol (BPS-
100 MAE), 4-(4-propan-2-yloxyphenyl)sulfonylphenol (D-8), 4-(4-hydroxy-3-prop-2-
101 enylphenyl)sulfonyl-2-prop-2-enylphenol (TGSA) and N-(p-Toluenesulfonyl)-N'-(3-p-
102 toluenesulfonyl-oxyphenyl)urea Pergafast 201. SUPRAS made up of inverse aggregates of
103 simple alcohols, diols and carboxylic acids prepared in THF:water or ethanol:water mixtures
104 (Ballesteros-Gómez and Rubio, 2012, González-Rubio et al., Ruiz et al. 2007) were
105 investigated in terms of compatibility, sensitivity and reproducibility with ASAP-MS/MS
106 analysis of target compounds. The optimal method was applied to the analysis of 62 samples of
107 thermal paper in order to investigate the extent of the recent BPA replacement in the Spanish
108 market.

109

110 **2. Material and methods.**

111 *2.1. Chemicals and reagents.*

112 Solvents were methanol (MeOH), ethanol and tetrahydrofuran (THF), obtained from VWR –
113 Prolabo Chemicals (Bois, France). Ultra-high-quality water was obtained from a Milli-Q water
114 purification system (Millipore, Madrid, Spain). 1-hexanol, 1-decanol (98%), 1-tetradecanol,
115 1,2-decanediol (98%) and 1-decanoic acid (98%) were from Sigma–Aldrich Co. (St. Louis,
116 USA) and hydrochloric acid (37%) was supplied by Merck (Darmstadt, Germany).

117 Internal standards (IS) Bisphenol A-d₁₆ (BPA-d₁₆) and bis(4-hydroxyphenyl) Sulfone-d₈ (BPS-
118 d₈) were acquired from Toronto Research Chemicals (Toronto, Canada). Stock solutions of IS
119 (BPS-d₈ and BPA-d₁₆) were prepared in MeOH (5 mg·mL⁻¹) and stored at -20°C. Intermediate
120 and working solutions were prepared by appropriate dilution in MeOH and also stored at -20°C.

121 Table S1 shows the full names, CAS numbers, molecular formula and physical-chemical
122 properties of the target compounds.

123

124 2.2. Apparatus.

125 Determination of BPA and replacements was carried out using a 6420 Triple Quadrupole mass
126 spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source from
127 Agilent Technologies (Palo Alto, California) modified with an ASAP unit (Ionsense Inc., see
128 Figure S1). The source was operated in negative mode. Optimal source parameters
129 recommended for ASAP were: gas temperature, 325°C; gas flow, 4.0 L·min⁻¹; vaporization
130 temperature, 400°C, nebulizer gas pressure, 20 psi; capillary voltage, -1000 V; corona voltage,
131 10 µA. After the probe was inserted in the ASAP unit, the MS signal was recorded for 1 min.

132 *Qualitative analysis MassHunter workstation* software from Agilent Technologies (Palo Alto,
133 California) was used for determination of bisphenols, registering characteristics transitions for
134 each analyte (see Table S2, according to Dueñas-Mas et al. 2019).

135 Glass melting point capillaries (0.8-1.1 i.d., 90 mm length) were obtained from Pyrex (Thermo
136 Fisher Scientific, USA). For optimization experiments and SUPRAS production we employed
137 a vortex-shaker REAX Top (Heidolph, Schwabach, Germany) equipped with a head (ref. 549-
138 01000-00) with 10 microtubes from Heidolph (Schwabach, Alemania) and a 36 x 2.2/1.5 mL
139 angle rotor (ref. 1162) MPW350R high speed centrifuge from MPW Med-Instruments
140 (Warschaw, Polonia).

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142 2.3. SUPRAS sample treatment optimization.

143 SUPRAS were synthesized from ternary solutions of 50 mL containing the amphiphile (5%
144 v/v), organic solvent (10-30% v/v) and water (65-85%). Various amphiphiles (1-hexanol, 1-
145 decanol, 1-tetradecanol, 1,2-decanediol and 1-decanoic acid) and two organic solvents (THF

146 and ethanol) were tested. Milli-Q water was employed as coacervating agent (poor solvent for
147 the amphiphile) and was acidified at pH ~2.5 for SUPRAS made up of 1-decanoic acid in order
148 to ensure the protonated form of amphiphile, which is needed for SUPRAS formation. Synthetic
149 solutions were vortex-stirred for 5 min and centrifuged for 5 min at 2.500 rpm to accelerate
150 phase separation. The upper SUPRAS phase was transferred to a closed glass bottle and stored
151 at 4°C until used (within 1 week).

152 Tickets containing BPA, BPS, Pergafast 201 and TGSA were used as representative samples
153 for optimization. Due to the limited amount of material and to prevent contamination after
154 manipulation of the samples, different tickets were used for different batches of experiments
155 along the optimization process. SUPRAS composition was first optimized by carrying out the
156 sample treatment in 2 mL Eppendorf microtubes by simple contact of the SUPRAS phase (400
157 μL , ISs 1 mg/L) with the sample aliquots (20 mg) during 1 h without stirring. Extraction
158 experiments were done in triplicate. Glass probes were then immersed (open end) into the
159 SUPRAS phase and immediately analysed ($n=5$) by ASAP-MS/MS. SUPRAS volume loaded
160 on the open side of the probe was calculated by weight difference before and after probe loading
161 and ranged from 1.1 to 2 μL . Solid samples were also directly analysed by scratching the surface
162 of the paper sample with the open end of the probe so that solid particles (few milligrams)
163 remained on it (Ballesteros-Gómez et al. 2014). Statistical comparisons were performed with
164 Minitab software Ver. 18 (Minitab Inc, State College, Pennsylvania, USA) using one-way
165 analysis of variance (ANOVA) and Tukey's tests ($p\text{-value} < 0.05$).

166

167 *2.4 SUPRAS-based microextraction probe-ASAP-MS/MS optimal method for analysis of BPA*
168 *and replacements in thermal paper.*

169 SUPRAS of inverse aggregates of 1-decanol were prepared with 50 mL solutions of 5% v/v
170 amphiphile, 10% ethanol and 85% water. The open end of a disposable glass probe was
171 immersed in SUPRAS (with 1 mg/L ISs) to be loaded with 1.1-2 μL . Then, the SUPRAS-based

172 microextraction was carried out by immediately putting the loaded probe in contact with the
173 sample surface for 1-60 s. The position of the probe during extraction was horizontal and
174 perpendicular to the sample. The probe was then injected in the ASAP unit for MS analysis.
175 Experiments were done 5 times per sample. Blanks were injected between samples to ensure
176 lack of cross-contamination.

177 *2.5. Thermal paper samples.*

178 Samples were collected in Córdoba (Spain) from October 2019 till January 2020. Thermal paper
179 samples ($n=62$) were classified in four groups: food stores and restaurants ($n=23$), ATMs ($n=7$),
180 petrol stations and public transport ($n=10$) and other stores (clothes, cosmetics, stationer's, etc.,
181 $n=22$).

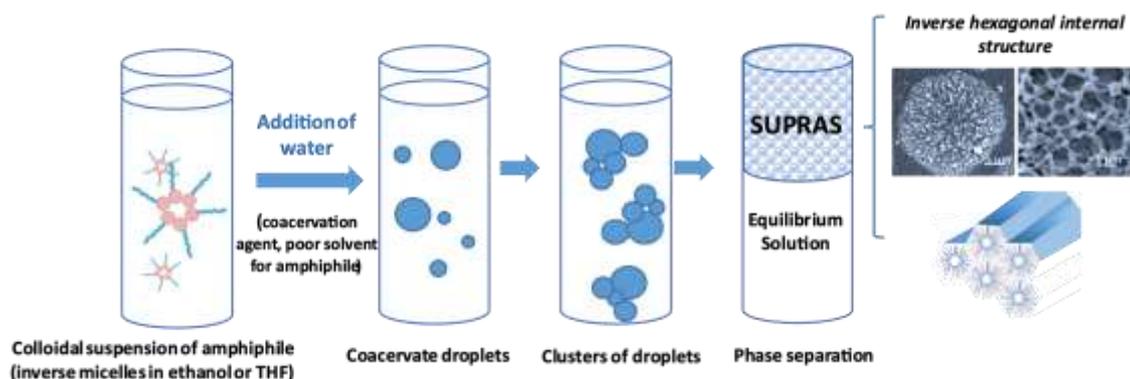
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183 **3. Results and discussion.**

184 *3.1. SUPRAS composition optimization.*

185 SUPRAS formation occurs in two consecutive stages. First, tridimensional aggregates (micelles
186 or vesicles, generally) are spontaneously formed by self-assembly of amphiphilic compounds
187 when they reach a critical micellar/vesicular concentration. Then, these aggregates increase
188 their size and/or are reorganized by the action of a coacervate agent (pH or temperature change,
189 or addition of a salt or a poor solvent for the amphiphile) so that a new liquid phase (SUPRAS)
190 is formed and separated from the bulk solution. This new phase presents a complex internal
191 nano- or microstructure, such as bilayers, inverse hexagonal phase, sponge phase, etc. (Romera-
192 García and Ballesteros-Gómez, 2020). Figure 1 shows the formation process of SUPRAS of
193 inverse aggregates in organic solvent by the addition of water (poor solvent for the amphiphile,
194 coacervating agent). They have been successfully employed in the extraction of a wide variety
195 of organic contaminants in liquid and solid samples (Ballesteros-Gómez and Rubio, 2012,

196 including bisphenols and analogues in biological samples (Romera-García et al. 2019),
197 wastewater (Ruiz et al. 2008) and indoor dust (Dueñas-Mas et al. 2019).



198

199 **Figure 1.** Schematic picture of the SUPRAS formation and its expected microstructure.

200 First, we investigated the influence of the functional group of the amphiphile forming the
201 SUPRAS on the ASAP-MS/MS analysis. SUPRAS were made up from solutions of 5% v/v of
202 1-decanol, 1,2-decanediol or 1-decanoic acid, 20% v/v of THF and 75% of milli-Q water
203 (acidified in the case of 1-decanoic acid at pH ~2.5) and containing 1 mg/L of ISs. The MS
204 peaks areas of the quantifier ions of each analyte and of ISs were recorded and results were
205 expressed as absolute areas or as relative areas ($\text{area}_{\text{analyte}}/\text{area}_{\text{IS}}$).

206 Results with SUPRAS made up of 1-decanol were similar to those synthesized with the diol (in
207 terms of sensibility and reproducibility). The ratio $\text{area}_{1\text{-decanol}}/\text{area}_{1,2\text{-decanediol}}$ (absolute areas) of
208 BPS, BPA, Pergafast and TGSA were 0.8, 1.2, 1.3 y 1.6, respectively. These values seem to be
209 related with the higher amount of water in SUPRAS of 1,2-decanediol (~30% w/w) in
210 comparison with that of SUPRAS of 1-decanol (~5% w/w) under the same synthesis conditions
211 (Ballesteros-Gómez and Rubio, 2012, González-Rubio et al. 2022). So, the lower the water
212 solubility of the compounds ($\text{TGSA} < \text{Pergafast} < \text{BPA} < \text{BPS}$, see table S1) the better they were
213 extracted with 1-decanol-based SUPRAS. Due to the fact that all the analytes were better
214 extracted with 1-decanol-based SUPRAS (except the most water soluble compound, BPS) this
215 was selected as optimal. Furthermore, SUPRAS formation diagrams were wider for 1-decanol
216 than for 1,2-decanediol (Ballesteros-Gómez and Rubio, 2012, González-Rubio et al. 2022), thus

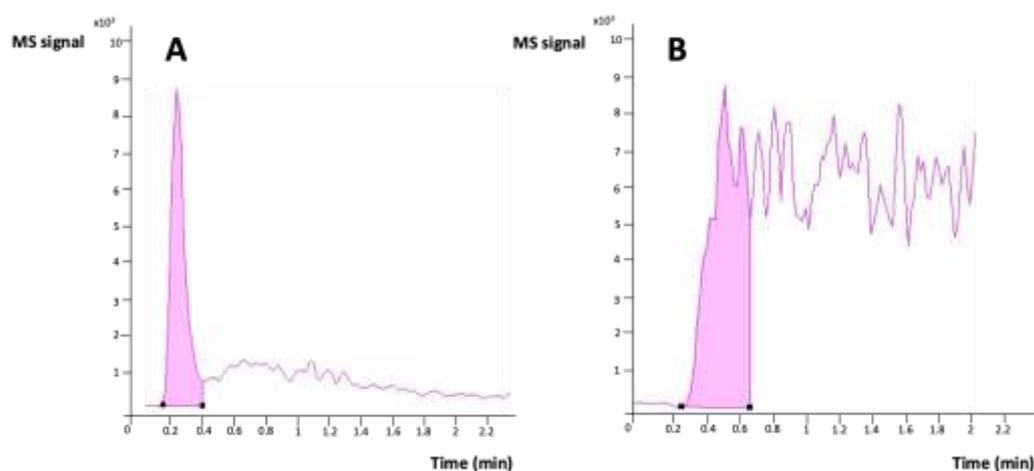
217 allowing the synthesis of SUPRAS in a wider composition range. SUPRAS of 1-decanoic acid
218 were discarded due to strong matrix suppression effects.

219 After selection of SUPRAS based on simple alcohols, we investigated the influence of the
220 amphiphile alkyl chain length. SUPRAS constituted by 1-hexanol, 1-decanol and 1-
221 tetradecanol were compared. The solid sample was also directly measured in order to verify
222 that the sample preparation step with SUPRAS was beneficial.

223 The MS signal as a function of time was clearly different with and without SUPRAS treatment.
224 MS signals from SUPRAS-based microextraction probe-ASAP-MS/MS resulted in signal
225 peaks of 0.2-0.4 min, which could be easily integrated for data processing. Contrarily, the direct
226 injection of solid samples did not show a clear peaks and MS signals kept almost constant
227 during several minutes before starting to gradually drop. As an example, figure 2 shows the
228 registered MS signal (BPA quantifier) of a representative sample with (2A) and without (2B)
229 SUPRAS treatment.

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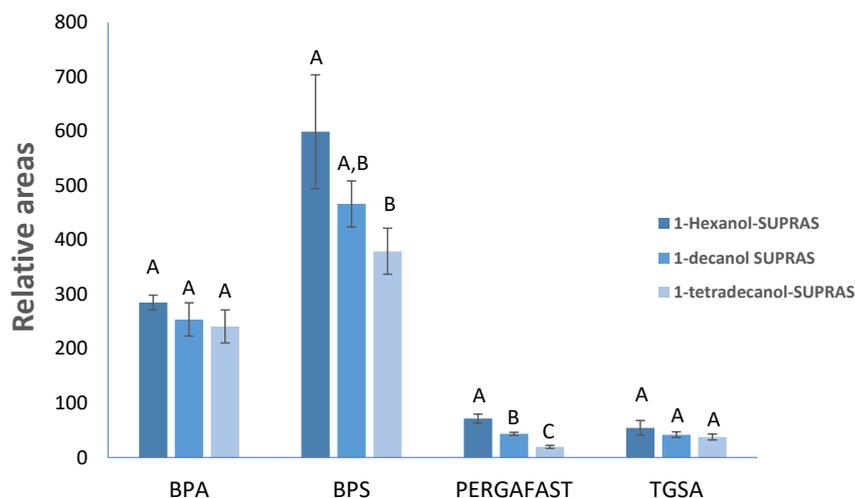
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233 **Figure 2.** Absolute AMS peak areas of BPA (quantitative transition) in a thermal paper sample
234 analyzed by (A) previous extraction with SUPRAS of 1-decanol for 1 h (SUPRAS synthesis mix

235 *composition: 5% v/v amphiphile, 75% v/v water and 20% THF v/v) and (B) direct analysis of*
236 *the solid.*

237 Other advantage of the use of SUPRAS treatment was that during the direct analysis of solids,
238 particles were easily released from the probe and deposited onto the APCI source, thus
239 generating cross-contamination and the need of cleaning the source between injections. With
240 the direct analysis of the solid, values of relative standard deviation (RSD, %) were very high
241 (50-90%), most probably due to variations in the loaded volume on the probe and the lack of
242 correction by ISs. Furthermore, the signal from the less volatile compounds (TGSA and
243 Pergafast 201, see vapor pressure values in Table S1) were considerably lower (around 2-4
244 times) than those observed from SUPRAS treated samples (results not shown). By adding ISs
245 to SUPRAS, variations due to differences in probe loading (and instrument fluctuations) could
246 be corrected down to 25% RSD, values that can be considered acceptable for screening
247 purposes.

248 When comparing the performance of SUPRAS constituted by 1-hexanol, 1-decanol and 1-
249 tetradecanol (relative AMS peak areas) we could observe that the extraction efficiency of the
250 target compounds slightly improved with the lower chain length, as it is shown in figure 3
251 (differences were only significant among the three alkanols for Pergafast 201 and for BPS with
252 1-tetradecanol). This was not due to differences in the ionization process since average absolute
253 areas of ISs did not change significantly among the different SUPRAS treatments. In contrast,
254 RSDs slightly improved with the higher chain length and values were 4-25% with 1-hexanol,
255 2-16% with 1-decanol and 3-14% with 1-tetradecanol. The SUPRAS synthesized with 1-
256 decanol was selected as an optimal compromise between sensibility and reproducibility.



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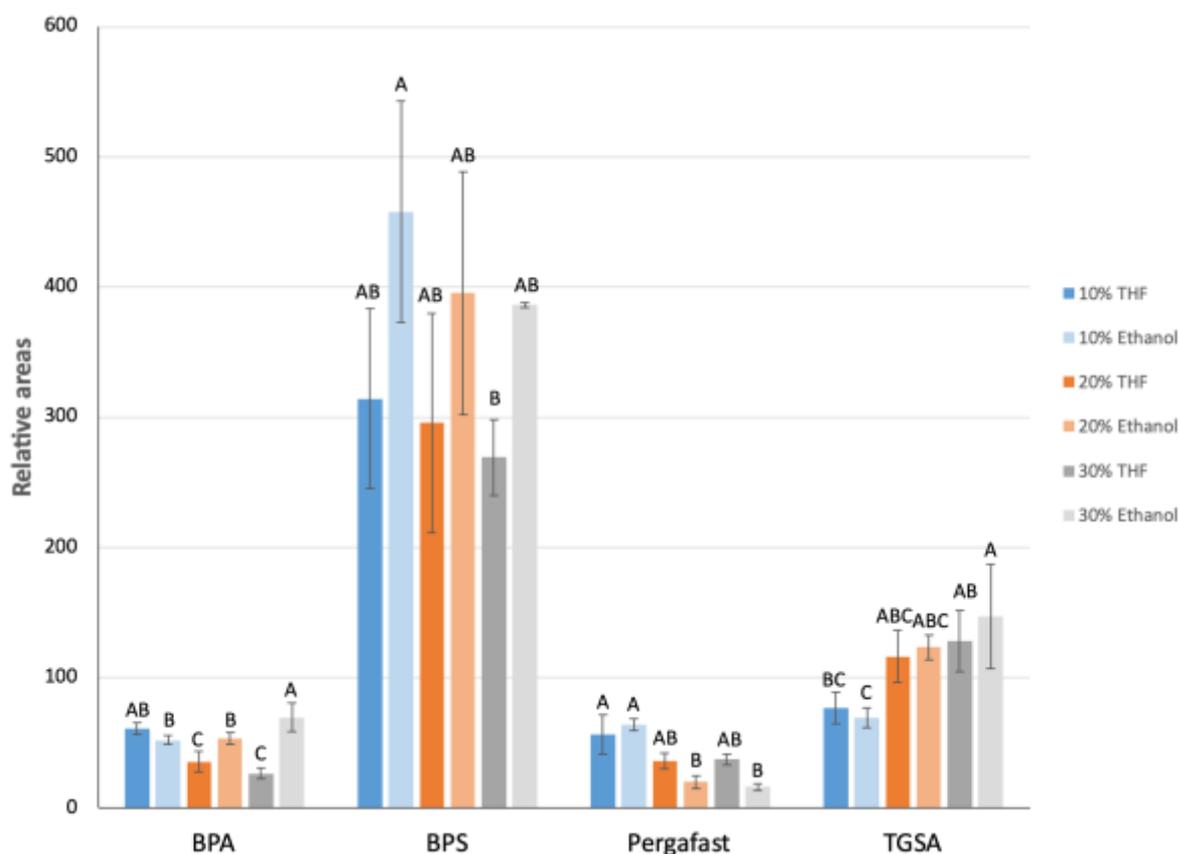
258 **Figure 3.** Relative AMS peak areas of BPA, BPS, Pergafast 201 and TGSA in thermal paper
 259 samples analysed after 1 h extraction with SUPRAS made up of 1-hexanol, 1-decanol and 1-
 260 tetradecanol (SUPRAS synthesis mix composition: 5% v/v amphiphile, 75% v/v water and 20%
 261 THF v/v). Significant differences are indicated by different letters on the top of the bars (Tukey
 262 tests).

263 SUPRAS of 1-decanol were then synthesized in different organic solvent:water mixtures (THF
 264 and ethanol at 10, 20 and 30% v/v). Table S3 shows the solubility and volatility parameters of
 265 THF, ethanol and water for discussion. SUPRAS of inverse aggregates are formed in mixtures
 266 of water and a wide variety of protic and aprotic organic solvents (Romera-García and
 267 Ballesteros-Gómez, 2020). THF:water has been the most employed synthesis solvent mixture.
 268 Due to the low dielectric constant and predominance of dispersive binding forces, THF
 269 enhances the extraction of non-polar compounds. When it is mixed with water (of high
 270 dielectric constant and predominance of hydrogen bonds binding forces) this results in mixtures
 271 of wide polarity and solubility that favor SUPRAS formation in a wider range of composition.
 272 We also investigated the use of ethanol:water mixtures. Ethanol as a protic and more polar
 273 solvent, provided a more balanced contribution of dispersion, polar and hydrogen bonds forces

274 to improve the extraction of polar and moderately polar compounds. Furthermore, it is less
275 volatile and toxic than THF.

276 Figure 4 shows results with SUPRAS made up of 1-decanol and different solvent mixtures at
277 10, 20 and 30% v/v. Relative AMS peak areas for BPA, BPS and Pergafast 201 generally
278 decreased with the THF percentage while the opposite trend was observed for TGSA. Contrarily
279 and with the exception of BPS and Pergafast 201, relative AMS peak areas increased with the
280 ethanol percentage. Since optimal solvent (and solvent percentage) could not be found for all
281 the compounds, we selected 10% v/v ethanol for further experiments on the basis of its lower
282 toxicity and minimal solvent consumption. Additionally, lower percentages of organic solvent
283 in the synthesis should result in SUPRAS of higher viscosity and cohesive forces and lower
284 volatility because of the higher content in amphiphile and lower content in organic solvent
285 (Ballesteros-Gómez and Rubio, 2012). These properties are beneficial to maintain the SUPRAS
286 structure and volume unaltered onto the glass capillary during the extraction. In this way,
287 analytes diffuse from the sample to the SUPRAS phase contained in the probe and the loss of
288 SUPRAS soaking the sample is minimized (as it would occur with conventional solvents) thus
289 being available for ASAP injection.

290



291

292 **Figure 4.** Relative AMS peak areas of BPA, BPS, Pergafast 201 and TGSA in thermal paper
 293 samples analysed after 1h extraction with SUPRAS made up of 1-decanol and different
 294 synthesis percentages of THF or ethanol (SUPRAS synthesis mix composition: 5% v/v
 295 amphiphile, 65-85% v/v water and 10-30% organic solvent v/v). Significant differences are
 296 indicated by different letters on the top of the bars (Tukey tests).

297 3.2. SUPRAS-based microextraction probe optimization.

298 Finally, we investigated the feasibility of the SUPRAS-based microextraction probe approach
 299 coupled to ASAP-MS/MS. For this purpose, SUPRAS synthesized with 5% v/v of 1-decanol,
 300 10% v/v of ethanol and 85% of water were tested.

301 SUPRAS (1.1-2 μ L) were loaded inside probes (open end) by simple immersion. The open end
 302 of glass probes containing SUPRAS were placed in contact with the sample surface for 1-60 s.
 303 The probe was placed in horizontal position and perpendicular to the sample. An extraction
 304 time of 10 seconds was proposed as optimal. Longer extraction times led to significant losses

305 of SUPRAS by adsorption onto the sample, while shorter times originated irreproducible
 306 results. At this time period, ISs areas kept similar when compared with the extraction performed
 307 in Eppendorf tubes during 1 h contact this suggesting that the most of the SUPRAS volume
 308 remained in the glass capillary and that there were not significant losses during the extraction
 309 process. Relative AMS peak areas (see figure S2) neither dropped drastically when using the
 310 SUPRAS loaded probes (1.3, 1.1, 1.4 and 2.3 times lower for BPA, TGSA, Pergafast 201 and
 311 BPS). This suggests a fast mass transfer process approaching equilibrium after 10 s and similar
 312 SUPRAS/sample ratios in both extraction procedures. It must be noted that the ratio SUPRAS
 313 volume/sample amount was similar in both cases with values of 20 mL/g sample for extractions
 314 performed in Eppendorf tubes and in the range ~24-43 mL/g sample for the SUPRAS probe
 315 approach (considering the approximate i.d. of the probe as 1 mm and the mean sample weight
 316 as 5.9 mg/cm² in order to estimate the amount of extracted sample aliquot).

317 *3.3. Analysis of thermal paper samples.*

318 Optimal conditions (section 2.4.) were applied for the microextraction probe-ASAP-MS/MS
 319 analysis of 62 samples of thermal paper collected in Córdoba (Spain). Table 1 shows the
 320 screening results (presence of major and secondary compounds).

Table 1. Bisphenols found in thermal paper samples.

	Sample	Major bisphenol	Secondary bisphenols with lower abundance
Shops (clothes, cosmetics, libraries, etc.)	1	BPS	
	2	BPS	BPA
	3	BPS	
	4	BPA	BPS
	5	BPS	
	6	BPA	BPS
	7	BPS	BPA
	8	BPA	BPS
	9	BPS	Pergafast, BPA
	10	BPS	D-8
	11	BPA	BPS
	12	BPA	BPS
	13	BPS	BPA

	14	BPS	BPA, D-8
	15	BPS	BPA, D-8
	16	BPA	BPS
	17	BPA	BPS
	18	BPS	BPA
	19	BPS	BPA, D-8
	20	BPA	BPS
	21	BPS	BPA, D-8
	22	TGSA	BPS, BPA
Supermarket and restaurants	1	Pergafast	BPS, BPA
	2	BPS	D-8
	3	BPA	
	4	BPS	D-8
	5	BPA	
	6	BPA	
	7	BPA	
	8	BPS	
	9	BPA	
	10	BPS	
	11	BPA	
	12	BPS	
	13	BPS	D-8
	14	BPA	BPS
	15	BPA	BPS
	16	BPA	BPS
	17	BPS	D-8
	18	BPS	BPA
	19	BPS	BPS, D-8
	20	BPA	
	21	BPS	BPA
	22	BPA	BPS
	23	BPS	BPA
Transports and gas stations	1	BPS	BPA
	2	BPA	BPS, D-8
	3	BPA	BPS
	4	BPA	BPS
	5	BPS	BPA, D-8
	6	BPS	
	7	BPS	
	8	BPS	
	9	BPS	
	10	BPS	BPA, D-8
Banks	1	BPS	BPA, D-8
	2	BPA	BPS
	3	BPS	D-8
	4	BPA	BPS
	5	BPA	BPS, D-8

321

322 BPA [$n=27$, detection frequency (DF)= 43.5 %], BPS ($n=33$, DF=53.2 %), Pergafast 201 ($n=1$,
323 DF= 1.6 %) and TGSA ($n=1$, DF=1.6 %) were detected as major color developers in thermal
324 paper samples. These results are slightly different from those found in previous studies in
325 Europe or in Spain. Thus, Verveliet et al. 2019 (sample collection years 2017 and 2018)
326 reported DFs of 67.6%, 15.1%, 12.6% and 0.84% for BPA, BPS, Pergafast 201 and TGSA,
327 respectively, in European countries. Björnsdotter et al. 2017 (sample collection year 2016)
328 revealed DFs of 55% BPA, 21% BPS and 21% Pergafast 201 in cash receipts from Europe
329 while in Spain DFs were 88% BPA followed by 8% BPS. Molina-Molina et al. 2019 (sample
330 collection year 2017) measured BPA and BPS in Spain, Brazil and France. While samples from
331 Spain and Brazil showed a major use of BPA (DFs $\geq 90\%$) values in France were 51% for BPA
332 and 21% for BPS. In the present study (sample collection in late 2019 and early 2020), we found
333 in Spain an increasing use of BPS (DF 53%) in detriment of BPA (DF 44%) with respect to
334 these previous studies. This suggests that legislative restrictions and growing concern by the
335 adverse effects of BPA have led to the replacement of BPA by BPS in the recent Spanish
336 market. The use of the other studied substitutes (Pergafast 201 and TGSA) remain still minor
337 in Spain with DFs similar to those reported by Verveliet et al. 2019. However, Pergafast 201
338 have been found at higher DF in other European countries, e.g. a value of 14% was reported in
339 the Netherlands (Björnsdotter et al. 2017). Similarly, Eckardt et al. 2017 reported DFs for
340 Pergafast 201 of 36% in 2017, 34% in 2018 and 49.5% in 2019 in thermal papers from
341 Germany.

342 Together with the main developer we detected the co-occurrence of secondary or trace
343 developers in many samples. BPS was detected at low abundance in 19 out of 27 samples with
344 BPA as main developer. Similarly, some samples containing BPS as major color developer

345 contained low abundance peaks of BPA (8 out of 33), of both BPA and D-8 (7 out of 33) or of
346 BPA and Pergafast 201 (one sample). D-8 was in total detected in 16 samples and always as
347 secondary compound. Finally, also the samples containing TGSA and Pergafast 201 contained
348 trace levels of BPA and BPS. These results are in agreement with previous studies where the
349 simultaneous presence of several bisphenols in the same thermal paper samples has been
350 described. Secondary color developers at trace levels have been frequently found in thermal
351 paper and may be due to cross-contamination during manufacturing or to the use of recycled
352 paper (Björnsdotter et al. 2017, Verveliet et al. 2019, Yang et al.2019). Furthermore, these
353 secondary compounds have been also reported at higher relative abundance than just trace
354 levels in some samples and this may be explained by the use of industrial mixtures (Björnsdotter
355 et al. 2017, Verveliet et al. 2019, Yang et al. 2019). As an example, Verveliet et al. 2019 found
356 these mixtures in 42 out of 308 samples, being D-8 the most frequently used secondary color
357 developer in Europe.

358 **3. Conclusions.**

359 A rapid and simple sample preparation strategy based on SUPRAS-based microextraction
360 probes for screening of BPA and replacements in materials prior to ASAP analysis is proposed.
361 SUPRAS made up of inverse aggregates of 1-decanol in ethanol:water mixtures (containing 1
362 mg/L ISs) were loaded in ASAP glass probes (1.1-2 μ L) and extraction was made by simple
363 contact with the sample surface during 10 seconds. Subsequent ASAP-MS/MS analysis (1 min)
364 generated MS peaks that could be processed as reproducible and integrable signals after IS
365 correction (0.2-0.4 min peaks, areas RSD of 2-25%). Contrarily, the direct analysis of solids
366 generated a continuous MS signal and common cross-contamination due to the release of
367 particles inside the MS source. Samples of thermal paper from South Spain were screened.
368 Results suggested that BPA has been quickly replaced by BPS, while the use of other
369 alternatives (TGSA, Pergafast 201, D-8, etc.) is still limited.

370

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