Electronic Supplementary Information

Ionic liquid-based aqueous biphasic systems as one-step clean-up, microextraction and preconcentration platforms for the improved determination of salivary biomarkers

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Synthesis and characterization of the C_4Gu-Cl IL

The C_4Gu-Cl IL was synthesized according to the procedure described in the literature [29] with slight modifications. Briefly, 1H-pyrazole-1-carboxamidine hydrochloride (19.8 mmol, 2.90 g) and butylamine (19.8 mmol, 1.96 mL) were mixed in EtOH at 35 ºC for 48 h. Then, the solvent was evaporated (50 ºC, $1.50 \cdot 10^4$ N·m^{-2}) and the product was dried under vacuum at 25 ºC for 24 h. Finally, the IL was successfully characterized by ^{1}H-NMR.

Fig. S1 shows the synthesis procedure followed to prepare the IL, together with its chemical structure, and the ^{1}H-NMR spectrum obtained in DMSO-d_6.

Proteins assays

The Bradford Colorimetric Assay (BCA) was performed in the following samples: *i)* non-diluted healthy male saliva; *ii)* 1:2 diluted male saliva; *iii)* 1:10 diluted male saliva; *iv)* salt-rich phase of the ABS/TPP; *v)* IL-rich phase (diluted up to 1 g) of the ABS/TPP. The calibration standards were prepared within 0–2000 μg·mL^{-1} range by diluting the commercial BSA solution. Each well of the plate was filled with 250 μL of the Bradford standard solution and 5 μL of each sample. Finally, the well-plate was read at 595 nm, and the concentration of proteins in each sample were obtained using the measured absorbance values.

The solutions to run in the SDS-PAGE were prepared by mixing 45 μL of each sample or phase (depending on the experiment) with 15 μL of the Laemmli buffer. Non-diluted male saliva, 1:2 diluted male saliva, and diluted IL-rich phase, were measured by duplicate. Each solution was stirred at 100 ºC for 5 min before running the gel. An 80 V potential was initially applied and then increased up to 150 V for performing the electrophoresis. This procedure lasted 45 min, followed by the tinction of the gel with the Coomasie solution overnight. Finally, the gel was decolored using an aqueous solution of acetic acid/MeOH (10/30, v/v).

Tie-lines determination

The following system of equations was solved for the determination of TLs (Eqs. S1–S4):

$$[\mathrm{IL}]_{\mathrm{IL}} = A \cdot \exp[(B \cdot [\mathrm{salt}]_{\mathrm{IL}}^{0.5}) - (C \cdot [\mathrm{salt}]_{\mathrm{IL}}^{3})] \quad \text{(S1)}$$

$$[\mathrm{IL}]_{\mathrm{salt}} = A \cdot \exp[(B \cdot [\mathrm{salt}]_{\mathrm{salt}}^{0.5}) - (C \cdot [\mathrm{salt}]_{\mathrm{salt}}^{3})] \quad \text{(S2)}$$

$$[\mathrm{IL}]_{\mathrm{IL}} = \frac{[\mathrm{IL}]_{\mathrm{M}}}{\alpha} - \frac{1 - \alpha}{\alpha} \cdot [\mathrm{IL}]_{\mathrm{salt}} \quad \text{(S3)}$$

$$[\mathrm{salt}]_{\mathrm{IL}} = \frac{[\mathrm{salt}]_{\mathrm{M}}}{\alpha} - \frac{1 - \alpha}{\alpha} \cdot [\mathrm{salt}]_{\mathrm{salt}} \quad \text{(S4)}$$

where $[\mathrm{IL}]_{\mathrm{IL}}$ and $[\mathrm{salt}]_{\mathrm{IL}}$ are the weight percentages of the IL and the salt, respectively, in the IL-rich phase; $[\mathrm{IL}]_{\mathrm{salt}}$ and $[\mathrm{salt}]_{\mathrm{salt}}$ are the weight percentages of the IL and the salt, respectively, in the salt-rich phase; and $[\mathrm{IL}]_{\mathrm{M}}$ and $[\mathrm{salt}]_{\mathrm{M}}$ are the weight percentages of both the IL and the salt in the mixture point selected for the determination of the TL. α is the ratio between the IL-rich phase weight and the total mixture weight. A, B and C are fitting parameters of the regression. The TLL describes the difference between the composition of both the IL-rich and the salt-rich phases, and it was calculated applying the Eq. S5:

$$\mathrm{TLL} = \sqrt{([\mathrm{salt}]_{\mathrm{IL}} - [\mathrm{salt}]_{\mathrm{salt}})^2 + ([\mathrm{IL}]_{\mathrm{IL}} - [\mathrm{IL}]_{\mathrm{salt}})^2} \quad \text{(S5)}$$

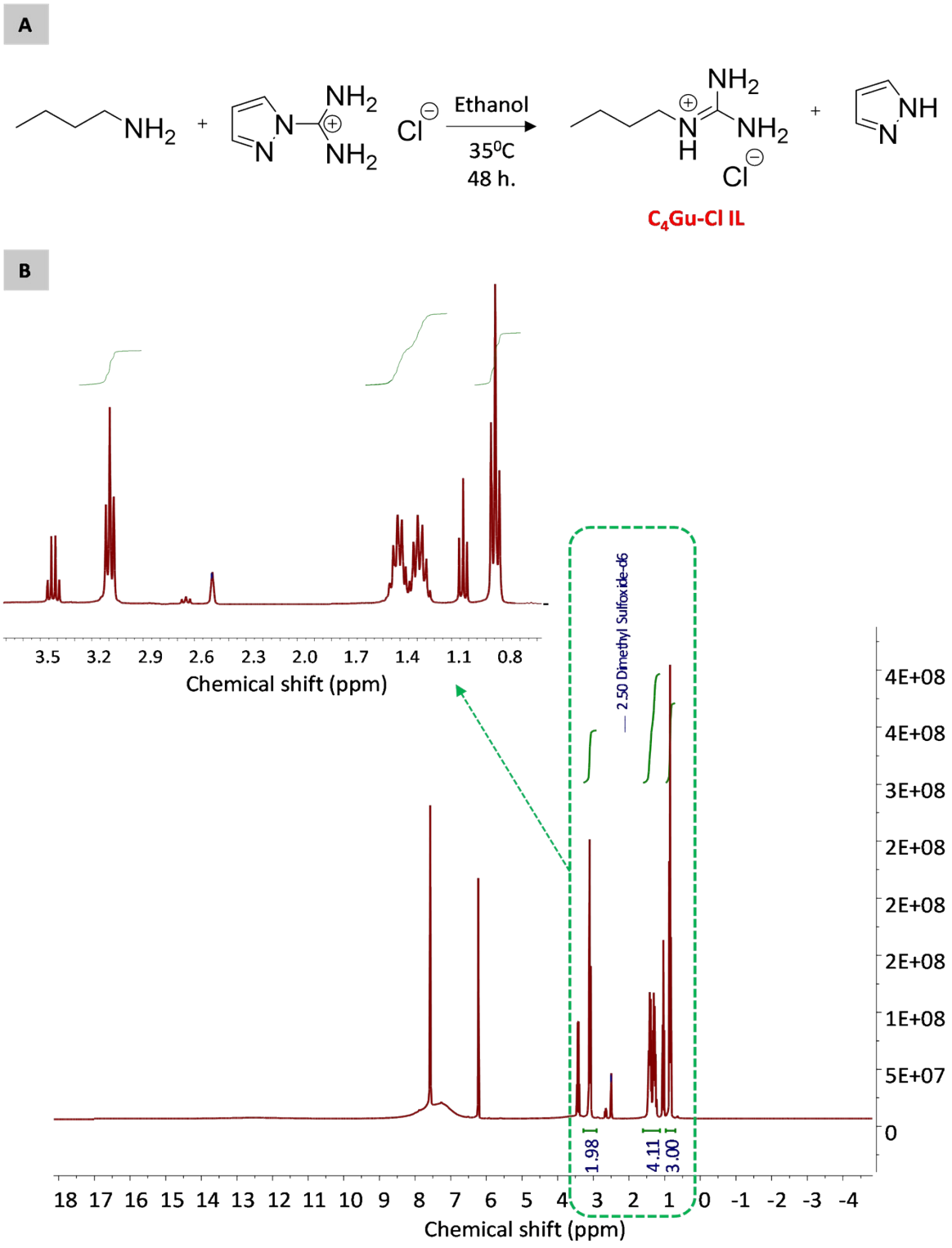


Figure S1. Synthesis and characterization of the C_4Gu-Cl IL: (**A**) Synthetic reaction scheme to form the C_4Gu-Cl IL; (**B**) ^{1}NMR spectrum (300 MHz, DMSO-d_6) of the synthesized C_4Gu-Cl IL:

- δ 3.18 – 2.98 (m, 2H), 1.51 – 1.20 (m, 4H), 0.84 (t, J = 7.2 Hz, 3H)
- Ethanol residual signals: 1.11 – 0.99 (t, 3H), 3.50 – 3.35 (q, 2H).

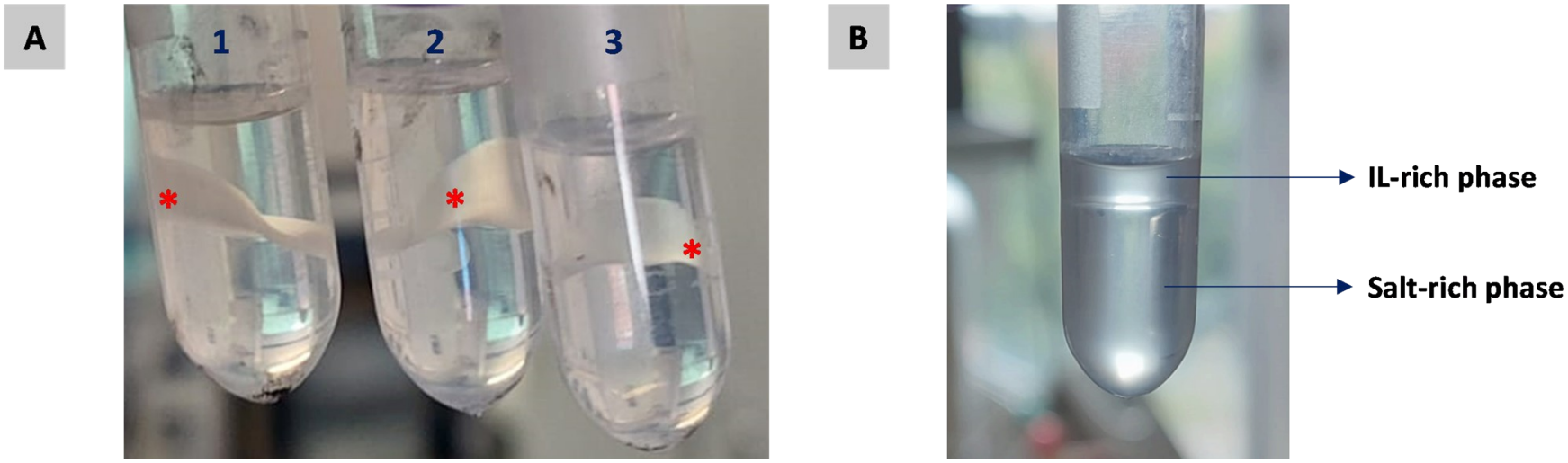


Figure S2. Development of ABS-TPP systems resorting to ABSs composed of 30 wt% of C_4Gu-Cl and 25 wt% of K_2HPO_4: (**A**) ABSs formed using real male saliva and different dilutions of the sample, i.e., **1)** non-diluted saliva, **2)** 1:2 diluted saliva, **3)** 1:10 diluted saliva (with the solid interphases marked with *); (**B**) ABS formed using ultrapure water, in which there is no formation of a solid interphase between the liquid phases.

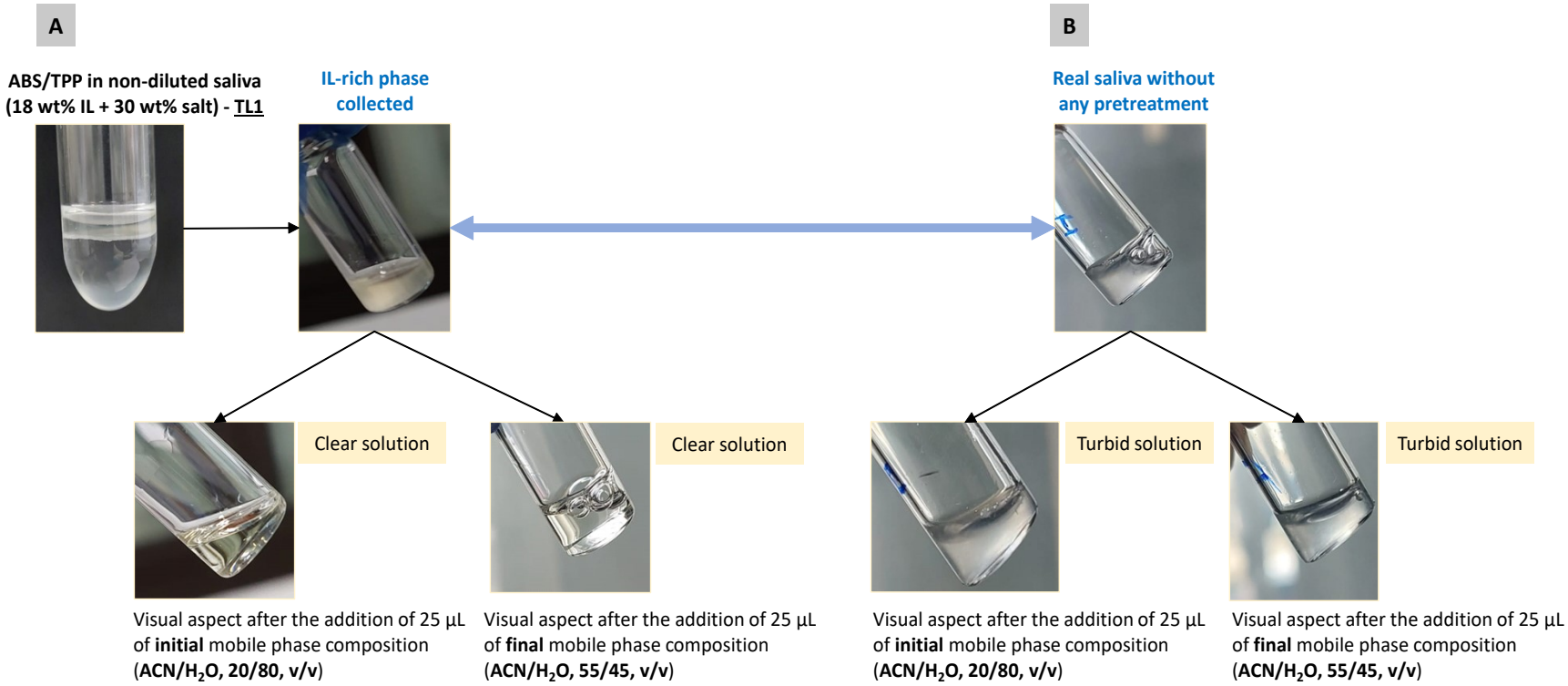


Figure S3. Solubility studies in both the initial and the final composition of the ACN/H_2O HPLC mobile phase of: (**A**) the IL-rich phase collected after performing an ABS/TPP using non-diluted saliva, using a mixture point belonging to the TL1 of the ABS composed by C_4Gu-Cl + K_2HPO_4 (18 wt% IL + 30 wt% salt); (**B**) real human saliva without any pretreatment.

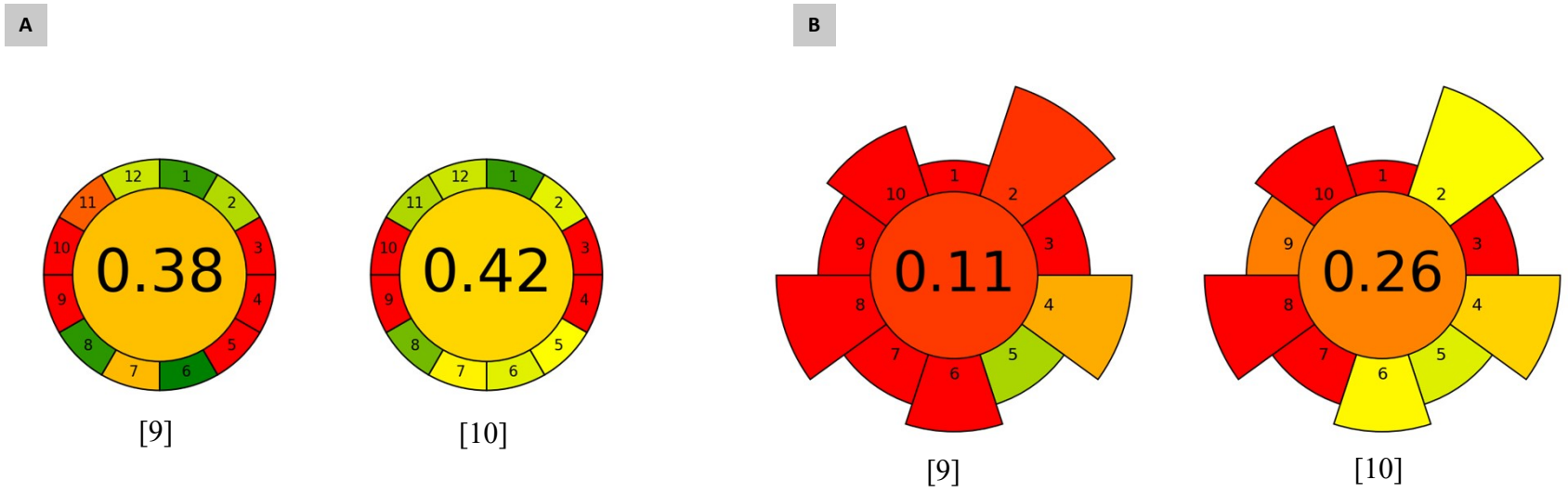
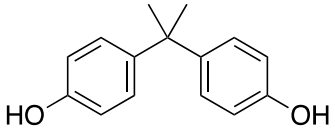
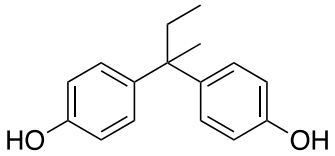
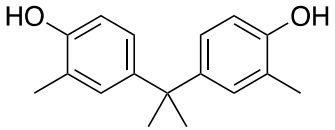
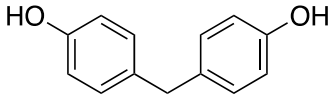
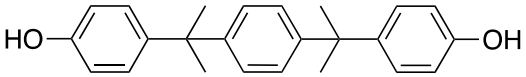


Figure S4. Diagrams obtained in the greenness assessment of 2 representative reported methods for the determination of BPs in human saliva [9,10]: **(A)** AGREE diagrams for the entire method; **(B)** AGREEprep diagrams for the sample preparation procedure in such studies.

Table S1. Physicochemical properties of the target bisphenols, obtained from SciFinder® 2023 database.

Bisphenol (abbreviation)	Chemical structure	CAS number	Molecular weight (g·mol^{-1})	pK_a	Log K_{ow}*
Bisphenol A (BPA)		80-05-7	228.29	10.29	3.641
Bisphenol B (BPB)		77-40-7	242.31	10.27	4.150
Bisphenol C (BPC)		79-97-0	256.34	10.45	4.643
Bisphenol F (BPF)		620-92-8	200.23	9.91	2.764
Bisphenol P (BPP)		2167-51-3	346.46	10.31	6.564

* Logarithm of octanol/water partition coefficient at 25 ºC.

Table S2. Preparation of citrate and phosphate buffers.

Buffers[a]	pH	Amount of $C_6H_8O_7$[b] (g)	Amount of $C_6H_5K_3O_7$[b] (g)	Amount of KH_2PO_4[b] (g)	Amount of K_2HPO_4[b] (g)
Citrate buffers	5	7.3258	17.6782	-	-
	6	3.6750	21.3197	-	-
	7	0.9623	24.0422	-	-
	8	0.1163	24.8807	-	-
Phosphate buffer	7	-	-	7.2415	12.3282

[a] The total mass of all the buffers solutions was 50 g, being at ca. 50 wt% of $C_6H_5K_3O_7$ + $C_6H_8O_7$ for citrate buffers and at ca. 40 wt% of KH_2PO_4 + K_2HPO_4 for phosphate buffer.

[b] Amounts of non-hydrated compounds.

Table S3. Chromatographic retention times obtained in the HPLC for target analytes under optimum conditions, as well as optimum fluorescence conditions set in the FD for the identification of the target bisphenols.

Bisphenol	Retention time (min) ± SD[a]	λ_{ex}[b] (nm)	λ_{em}[c] (nm)
BPF	10.48 ± 0.01	235	300
BPA	13.53 ± 0.01	275	305
BPB	16.09 ± 0.02	275	305
BPC	18.34 ± 0.02	275	305
BPP	27.04 ± 0.04	235	300

[a] Standard deviation for n = 10.

[b] Excitation wavelength.

[c] Emission wavelength.

Table S4. Ability of the C_4Gu-Cl IL to form biphasic systems in water with different salts and buffers.

Salts		Is the ABS formed?
Citrate salts	Citrate buffer[a] pH 5	No
	Citrate buffer[a] pH 6	Yes
	Citrate buffer[a] pH 7	Yes
	Citrate buffer[a] pH 8	Yes
	$C_6H_5K_3O_7$	Yes
Phosphate salts	KH_2PO_4	No
	Phosphate buffer[b] pH 7	Yes
	K_2HPO_4	Yes
	K_3PO_4	Yes

[a] Buffer prepared by mixing $C_6H_8O_7$ and $C_6H_5K_3O_7$ in water at different ratio depending on the pH (see Table S2).
[b] Buffer prepared by mixing KH_2PO_4 and K_2HPO_4 in water (see Table S2).

Table S5. Correlation parameters for the experimental binodal data of the ABSs composed of C_4Gu-Cl and salt according to Eq. (1).

Salt		A ± SD [a]	B ± SD [a]	(C ± SD[a])·10^5	R^2 [b]
Citrate salts	Citrate buffer pH 6	114 ± 39	-0.2 ± 0.1	1.8 ± 0.9	0.984
	Citrate buffer pH 7	126 ± 6	-0.28 ± 0.01	2.7 ± 0.1	0.998
	Citrate buffer pH 8	113 ± 7	-0.26 ± 0.02	3.2 ± 0.2	0.996
	$C_6H_5K_3O_7$	119 ± 6	-0.27 ± 0.02	3.3 ± 0.2	0.997
Phosphate salts	Phosphate buffer pH 7	123 ± 15	-0.32 ± 0.04	3.7 ± 0.5	0.994
	K_2HPO_4	131 ± 13	-0.33 ± 0.03	5.4 ± 0.4	0.994
	K_3PO_4	206 ± 29	-0.53 ± 0.05	9 ± 1	0.990

[a] Standard deviation.
[b] Determination coefficient.

Table S6. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + citrate buffer ($C_6H_8O_7$ / $C_6H_5K_3O_7$) at **pH 6 (2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**
55.25	9.64
43.49	15.36
35.62	19.49
23.39	27.07
19.84	29.52
16.01	32.25
13.14	34.77

Table S7. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + citrate buffer ($C_6H_8O_7$ / $C_6H_5K_3O_7$) at **pH 7 (2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

$wt\%_1$	$wt\%_2$	$wt\%_1$	$wt\%_2$
55.89	8.54	15.82	28.04
51.21	10.56	15.10	28.56
48.35	11.21	14.46	29.06
44.48	12.84	13.83	29.58
41.08	14.37	12.93	30.13
38.11	15.67	12.42	30.59
35.46	17.01	12.06	30.89
32.80	18.58	11.55	31.34
31.03	19.41	11.07	31.75
29.39	20.23	10.31	32.34
27.49	21.29	9.87	32.76
26.34	21.88	9.47	33.13
25.11	22.42	9.10	33.49
23.62	23.40	8.66	34.05
22.70	23.82	8.28	34.49
21.22	24.81	7.92	34.87
20.52	25.11	7.57	35.30
19.84	25.49	7.24	35.72
19.25	25.85	6.97	36.06
18.67	26.20	6.68	36.45
17.88	26.69	6.40	36.84
17.15	27.14	6.10	37.25
16.37	27.64		

Table S8. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + citrate buffer ($C_6H_8O_7$ / $C_6H_5K_3O_7$) at **pH 8 (2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**	**wt%$_1$**	**wt%$_2$**
58.62	6.77	11.46	29.71
53.02	8.40	10.58	30.55
45.58	11.90	10.06	31.05
40.14	14.57	9.59	31.47
37.91	15.29	9.02	32.05
33.99	17.39	8.50	32.69
31.91	18.08	7.95	33.29
29.29	19.36	7.57	33.78
26.62	20.58	7.30	34.10
24.72	21.53	6.98	34.48
22.37	23.04	6.71	34.84
20.88	23.81	6.33	35.34
19.76	24.43	5.96	35.88
18.79	24.92	5.54	36.54
17.81	25.46	5.24	37.07
16.35	26.46	4.94	37.58
15.22	27.15	4.51	38.37
14.29	27.80	4.23	38.93
13.50	28.27	3.61	40.23
12.73	28.67	3.18	41.11
12.16	29.13		

Table S9. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + $C_6H_5K_3O_7$ **(2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**	**wt%$_1$**	**wt%$_2$**
56.81	7.52	16.09	26.19
50.92	9.66	15.69	26.43
44.71	11.83	15.12	26.82
41.18	13.38	14.56	27.24
37.97	15.00	14.19	27.48
36.52	15.42	13.81	27.73
34.40	16.47	13.39	28.07
32.74	17.26	12.95	28.42
31.04	18.04	12.55	28.80
29.46	18.91	11.89	29.29
28.08	19.72	11.47	29.64
26.83	20.26	10.86	30.12
25.66	20.78	10.26	30.63
24.51	21.32	9.58	31.28
23.14	22.17	9.00	31.87
22.22	22.62	8.44	32.43
21.38	23.06	7.77	33.20
20.96	23.15	7.39	33.66
20.03	23.77	6.91	34.23
19.43	24.12	6.76	34.38
18.76	24.44	6.49	34.79
18.17	24.78	6.17	35.22
17.37	25.31	5.59	36.09
16.65	25.81		

Table S10. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + phosphate buffer (KH_2PO_4 / K_2HPO_4) at **pH 7 (2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**
52.50	8.51
45.78	9.21
41.88	10.76
38.15	12.33
35.46	13.38
33.48	14.56
31.24	15.42
28.08	17.04
25.79	18.30
23.54	19.56
21.55	20.66
20.09	21.56
18.54	22.43
17.29	23.17
16.14	23.85
15.08	24.54
14.16	25.17
13.43	25.67
12.62	26.24
11.78	26.83
11.20	27.22
10.40	27.86
9.70	28.39
8.84	29.08

Table S11. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + K_2HPO_4 **(2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**	**wt%$_1$**	**wt%$_2$**
55.43	8.11	15.56	22.35
46.28	9.48	14.69	22.63
41.83	10.52	14.21	22.88
38.02	12.02	13.74	23.11
36.76	12.39	13.32	23.35
34.49	13.44	13.03	23.52
33.38	13.75	12.65	23.74
31.46	14.60	12.29	23.98
29.86	15.34	11.91	24.22
28.96	15.55	11.46	24.54
27.75	16.14	10.79	24.98
26.47	16.70	10.55	25.13
25.34	17.26	10.27	25.33
24.15	17.92	9.71	25.72
23.59	17.98	9.39	26.00
22.34	18.59	8.73	26.47
21.51	18.95	8.22	26.86
20.62	19.45	7.66	27.35
19.11	20.39	7.24	27.66
18.40	20.67	6.94	27.91
17.75	20.99	6.54	28.29
17.18	21.30	6.20	28.59
16.72	21.54	5.57	29.19

Table S12. Experimental weight fraction **(wt%)** data for the system composed of C_4Gu-Cl IL **(1)** + K_3PO_4 **(2)** + H_2O **(3)** at 25 °C and atmospheric pressure.

wt%$_1$	**wt%$_2$**	**wt%$_1$**	**wt%$_2$**
56.31	7.47	13.40	17.71
46.77	7.57	12.62	18.11
43.05	8.31	11.83	18.54
41.21	8.49	11.06	18.99
39.23	9.18	10.47	19.33
37.02	9.85	9.80	19.72
35.25	10.30	9.37	20.02
32.78	11.03	8.86	20.35
31.76	11.23	8.44	20.65
30.36	11.50	8.15	20.85
28.68	12.13	7.83	21.11
26.84	12.61	7.45	21.55
25.90	12.85	7.10	21.70
24.83	13.14	6.93	21.85
24.01	13.60	6.72	22.03
23.37	13.65	6.48	22.26
22.92	13.75	6.26	22.42
22.26	14.10	6.02	22.72
21.82	14.20	5.81	22.87
21.17	14.52	5.63	23.05
20.41	14.76	5.50	23.17
19.67	14.97	5.23	23.46
18.83	15.35	5.05	23.67
18.02	15.72	4.92	23.83
17.14	16.03	4.79	23.95
16.34	16.30	4.64	24.14
15.38	16.72	4.502	24.27
14.30	17.31		

Table S13. TLs, TLLs, and α of the developed ABSs composed of C_4Gu-Cl IL + salt + H_2O.

Salt	TL	Weight fraction composition (wt%)							
		$[IL]_{IL}$	$[salt]_{IL}$	$[IL]_{M}$	$[salt]_{M}$	$[IL]_{salt}$	$[salt]_{salt}$	TLL	α
Citrate buffer pH 6	*1*	59.12	7.053	29.64	24.59	13.08	34.44	53.57	0.36
	2	66.40	4.836	35.25	24.87	4.575	44.59	73.50	0.50
	3	71.85	3.540	39.50	25.36	1.955	50.69	84.31	0.54
Citrate buffer pH 7	*1*	66.03	5.278	29.64	24.90	4.935	38.23	69.41	0.40
	2	59.19	7.117	24.42	24.46	12.80	30.25	51.84	0.25
	3	79.78	2.666	35.15	25.04	3.242	41.03	85.62	0.42
Citrate buffer pH 8	*1*	79.40	1.824	31.08	25.44	3.300	39.02	84.71	0.37
	2	71.61	3.036	24.89	26.09	5.616	35.59	73.59	0.29
	3	81.72	1.540	33.31	25.28	2.638	40.33	88.08	0.39
$C_6H_5K_3O_7$	*1*	78.79	2.207	30.35	24.66	4.431	36.68	81.96	0.35
	2	70.16	3.623	25.72	24.47	7.468	33.03	69.25	0.29
	3	90.06	1.001	34.86	25.05	3.094	38.88	94.85	0.37
Phosphate buffer pH 7	*1*	80.13	1.813	29.41	24.95	2.707	37.12	85.10	0.34
	2	73.36	2.635	26.39	24.63	3.739	35.23	76.88	0.33
	3	60.22	4.991	19.67	25.50	5.604	32.61	61.20	0.26
K_2HPO_4	*1*	84.48	1.730	30.08	24.776	1.089	37.06	90.56	0.35
	2	78.16	2.395	24.75	24.86	2.033	34.41	82.59	0.30
	3	69.90	3.530	20.11	25.22	2.922	32.71	73.05	0.26
K_3PO_4	*1*	91.43	2.335	29.85	24.95	0.1253	35.86	97.26	0.33
	2	82.83	2.931	24.05	24.98	0.2636	33.90	88.18	0.29
	3	74.37	3.651	19.79	25.13	0.3935	32.76	79.50	0.26

TL1 (highlighted in the table) is the TL common for all the systems.

Table S14. Quality analytical parameters and precision of the chromatographic method (HPLC-FD), using standards of the target bisphenols in ACN/H_2O (20/80, v/v).

Bisphenol	$(b \pm t \cdot S_b)^a \cdot 10^{-6}$	R^{2b}	$S_{y/x}{}^c \cdot 10^{-4}$	LOD[d] ($ng \cdot g^{-1}$)	LOQ[e] ($ng \cdot g^{-1}$)	Low conc. level (80 $ng \cdot g^{-1}$)		High conc. level (750 $ng \cdot g^{-1}$)	
						Intraday RSD range[f] (%)	Intermediate precision RSD[g] (%)	Intraday RSD range[f] (%)	Intermediate precision RSD[g] (%)
BPF	3.0 ± 0.1	0.9992	3.5	2.0	5.0	0.90–2.1	5.9	0.60–5.6	3.6
BPA	3.9 ± 0.2	0.9992	4.8	2.0	5.0	0.50–2.1	3.5	0.60–4.8	3.8
BPB	4.7 ± 0.2	0.9992	5.6	1.8	4.9	0.40–1.9	1.6	0.50–2.8	2.5
BPC	7.3 ± 0.3	0.9994	7.6	1.5	4.5	0.60–3.5	4.4	0.70–3.4	3.6
BPP	10.6 ± 0.4	0.9992	13	1.5	4.5	2.4–2.8	2.8	1.2–1.3	1.2

[a] Slope and uncertainty of the slope within the calibration range (5–1000 $ng \cdot g^{-1}$, n = 6) for a confidential level of 95%.

[b] Determination coefficient.

[c] Standard deviation of the residuals.

[d] Limit of detection, determined by decreasing the concentration of the standards in ACN/H_2O (20/80, v/v) until a S/N ratio of 3 was obtained.

[e] Limit of quantification, estimated as 10/3 times the LOD, and experimentally verified by injection of the standards in ACN/H_2O (20/80, v/v) at the concentrations predicted.

[f] Range (day 1 to day 2) of relative standard deviation for intra-day precision (n = 4), tested with an aqueous standard not included as calibration point.

[g] Relative standard deviation for intermediate precision (n = 8, 2 non-consecutive days), tested with an aqueous standard not included as calibration point.

Table S15. Comparison of several parameters of the developed ABS/TPP-HPLC-FD method with other methods reported in the literature for the determination of BPs in human saliva.

Analytes in common / other analytes[a]	Saliva pretreatment	Saliva amount	Extraction material[b] / amount	Extraction method[c]	Post-extraction steps	Analytical technique[d]	Overall time[e] (min)	LOD[f] ($ng \cdot mL^{-1}$)	Ref.
BPA, BPB, BPC, BPF, & BPP	non required	1.1 g	C_4Gu-Cl IL / 0.35 g	ABS/TPP-based microextraction	dilution	LC-FD	0 + 21 + 30	0.40–1.4	This work
BPA, BPB, BPC, BPF, & BPP / 6 PBs + 7 BPs	deproteinization + lyophilization	1.0 g	acetone + ethanol / 1.5 mL + 1.5 mL	UAE	evaporation + reconstitution	LC-MS/MS	545 + 11 + 21	0.10–0.40	[9]
BPA & BPF / TCS + 4 PBs + 2 BPs + 6 PTs	centrifugation + enzymatic digestion	2.0 mL	acetonitrile / 200 µL	DLLME	derivatization	GC-MS	215 + 15 + 31	0.015–3.0	[10]
BPA & BPP / 4 BPs + 5 PBs + 5 BzPs + TCC	non required	0.50 mL	trichloromethane / 500 µL	DLLME	evaporation + reconstitution	LC-MS/MS	0 + 15 + 10	0.010–0.10	[8]
BPA, BPB, BPF & BPP / 10 BPs	centrifugation	1.0 mL	SUPRA (hexanol + THF) / (45 µL + 450 µL)	SUPRAS microextraction	evaporation + reconstitution	LC-MS/MS	30 + 35 + 32	0.012–0.049	[38]

[a] Abbreviations: PBs for parabens, BPs for bisphenols, TCS for triclosan, PTs for phthalates, BzPs for benzophenones, TCC for triclocarban.
[b] Abbreviations: C_4Gu-Cl for butylguanidinium chloride, IL for ionic liquid, SUPRA for supramolecular solvent, THF for tetrahydrofuran.
[c] Abbreviations: ABS/TPP for three-phase partitioning based on aqueous biphasic system, UAE for ultrasounds-assisted extraction, DLLME for dispersive liquid-liquid microextraction.
[d] Abbreviations: LC for liquid-chromatography, FD for fluorescence detection, MS for mass spectrometry, MS/MS for tandem mass spectrometry, GC for gas chromatography.
[e] Sample pretreatment time + extraction time + chromatographic analysis time.
[f] Limit of detection, expressed with respect to the saliva sample.

Table S16. Statistical tests for the RRs to assess the matrix effect obtained with the ABS/TPP-HPLC-FD method when analyzing saliva samples different from that used for the matrix-matched calibration approach.

Bisphenol	Healthy male saliva						Healthy female saliva					
	F test[a]		Student's *t* test[c]				*F* test[a]		Student's *t* test[c]			
	Result[b]	Variances comparison	t_{cal}[d]	t_{crit}[e]	Result[f]	Matrix effect	Result[b]	Variances comparison	t_{cal}[d]	t_{crit}[e]	Result[f]	Matrix effect
BPF	$F_{cal} < F_{crit}$	Homogeneous	0.84	3.2	$t_{cal} < t_{crit}$	**No**	$F_{cal} < F_{crit}$	Homogeneous	0.37	2.8	$t_{cal} < t_{crit}$	**No**
BPA	$F_{cal} > F_{crit}$	Heterogeneous	3.7	3.2	$t_{cal} > t_{crit}$	**Yes**	$F_{cal} > F_{crit}$	Heterogeneous	1.9	3.2	$t_{cal} < t_{crit}$	**No**
BPB	$F_{cal} < F_{crit}$	Homogeneous	3.0	3.2	$t_{cal} < t_{crit}$	**No**	$F_{cal} < F_{crit}$	Homogeneous	0.43	2.8	$t_{cal} < t_{crit}$	**No**
BPC	$F_{cal} < F_{crit}$	Homogeneous	1.7	3.2	$t_{cal} < t_{crit}$	**No**	$F_{cal} < F_{crit}$	Homogeneous	0.37	2.8	$t_{cal} < t_{crit}$	**No**
BPP	$F_{cal} < F_{crit}$	Homogeneous	2.2	3.2	$t_{cal} < t_{crit}$	**No**	$F_{cal} > F_{crit}$	Heterogeneous	2.13	2.8	$t_{cal} < t_{crit}$	**No**

[a] *F* statistical test for variances (comparing the RRs obtained in each sample with those obtained in the matrix of the calibration).

[b] Result of the *F* test, considering n – 1 degrees of freedom (n = 3) and a confidential level of 95%.

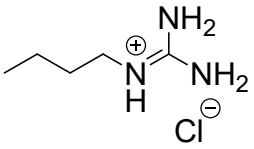
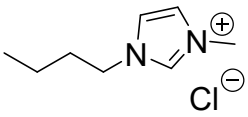
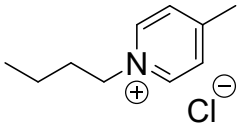
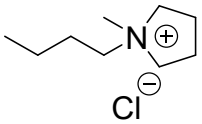
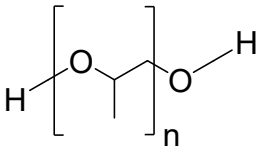
[c] Student's *t* statistical test for means comparison (considering equal variances or different variances, depending on the result of the *F* test).

[d] Calculated *t* value.

[e] Critical *t* value.

[f] Result of the *t* test, considering $n_1 + n_2 - 2$ degrees of freedom and a confidential level of 95%, and being n_1 and n_2 the number of RR data of each of the compared saliva samples (n = 3 for both analyzed matrices and for the calibration matrix).

Table S17. Comparison between the costs related to the use of the butylguanidinium chloride IL and those associated to the use of other ILs or other conventional benchmark materials commonly employed as ABSs phase-forming components.

Solvent	Chemical structure	€ per g (supplier)	€ per extraction[b]
Butylguanidinium chloride		11.37 (synthesized in this work[a])	3.98
1-butyl-3-methylimidazolium chloride		21.76 (Sigma-Aldrich)	7.62
1-butyl-4-methylpyridinium chloride		20.47 (Fisher Scientific)	7.16
1-butyl-1-methylpyrrolidinium chloride		6.82 (Sigma-Aldrich)	2.39
Polypropylene glycol		8.98 (Sigma-Aldrich)	3.14

[a] Cost calculated by considering the price of the commercial reagents involved in the synthesis of the IL, as well as the amounts used of these reagents and the amount of IL obtained per batch.

[b] Cost associated to the use of 0.35 g of the solvent (the amount of IL used in the ABS/TPP microextraction procedure proposed in this work). This amount not necessarily will work with other ILs or polymers, and therefore, perhaps higher costs for these other materials would be needed. The ideal scenario for these other materials is included in the Table.

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