

Supplementary Material

Controlling Serum Protein Depletion in Aqueous Biphasic Systems: Interphase Precipitation versus Bottom Phase Retention for Bisphenol A Monitoring

Maria S. M. Mendes¹, Inês B. Santana¹, Mara G. Freire^{1*}, Francisca A. e Silva^{1*}

¹CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

*To whom correspondence may be addressed. E-mail: maragfreire@ua.pt; francisca.silva@ua.pt

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Figure S2. Comparison of BPA calibration curves obtained under the initial RP-HPLC mobile phase conditions and in the presence of PPG-rich top phase of an ABS comprising 30 wt% PPG 400, 30 wt% NaBr and 40 wt% PBS aqueous solution, including the slope ratio used to assess potential matrix effects.

Figure S3. Representative photographs of ABS illustrating the two protein depletion strategies investigated. *Interphase precipitation* (left) occurs in the system composed of 30 wt% PPG 400, 30 wt% NaBr, 38 wt% PBS aqueous solution and 2 wt% human serum, where high-abundance proteins accumulate at the interphase between the polymer-rich and salt-rich phases (highlighted by the dashed box). *Bottom phase retention* (right) occurs in the system containing 30 wt% PPG 400, 30 wt% [Ch][Ac], 38 wt% PBS aqueous solution and 2 wt% human serum, in which proteins preferentially remain in the salt-rich bottom phase and no precipitation is observed at the interphase.

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Figure S5. Comparative evaluation of literature-reported analytical strategies for BPA determination in biological matrices based on green and sustainability metrics: (A) AGREE, (B) AGREEprep, (C) SPMS, and (D) BAGI metrics. From top to bottom, the rows correspond to PP-LC-MS/MS (example 1) [1], SPE-GC-MS/MS (example 2) [2], LLE-LC-MS/MS (example 3) [3], IL-ABS-UV-Vis (example 4) [4], and HES-LLE-LC-MS/MS (example 5) [5]; the numbering of examples matches that in Table S5, enabling a direct comparison of the different approaches.

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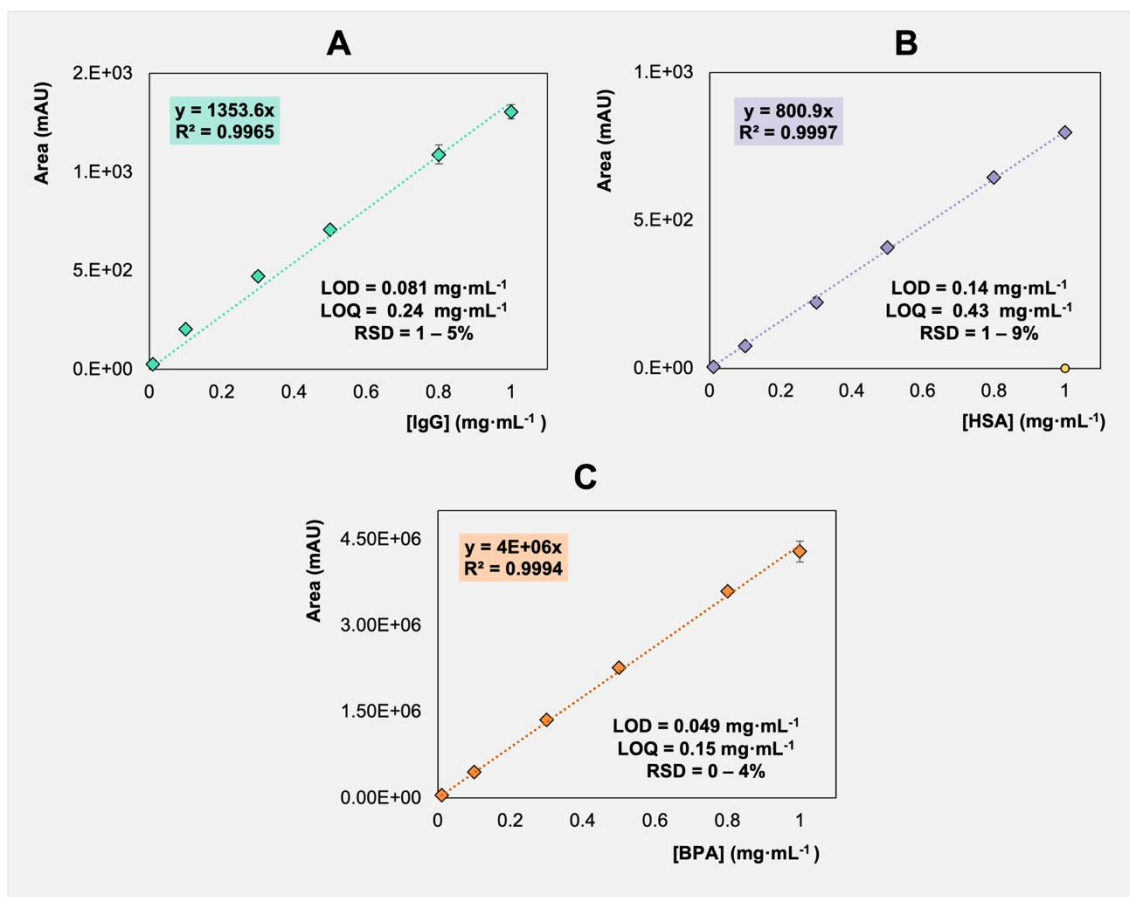


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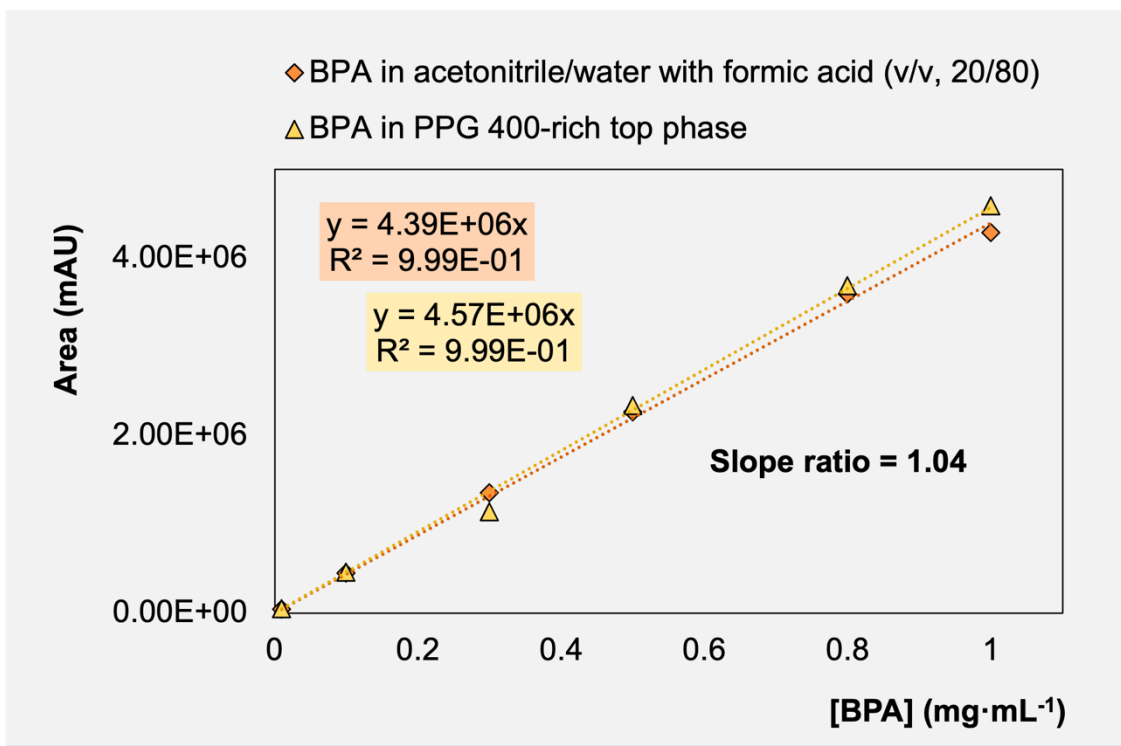
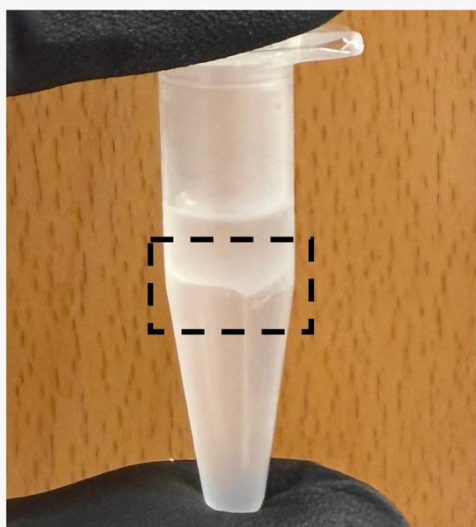


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Interphase Precipitation



30 wt% PPG 400
+
30 wt% NaBr

Bottom Phase Retention



30 wt% PPG 400
+
30 wt% [Ch][Ac]

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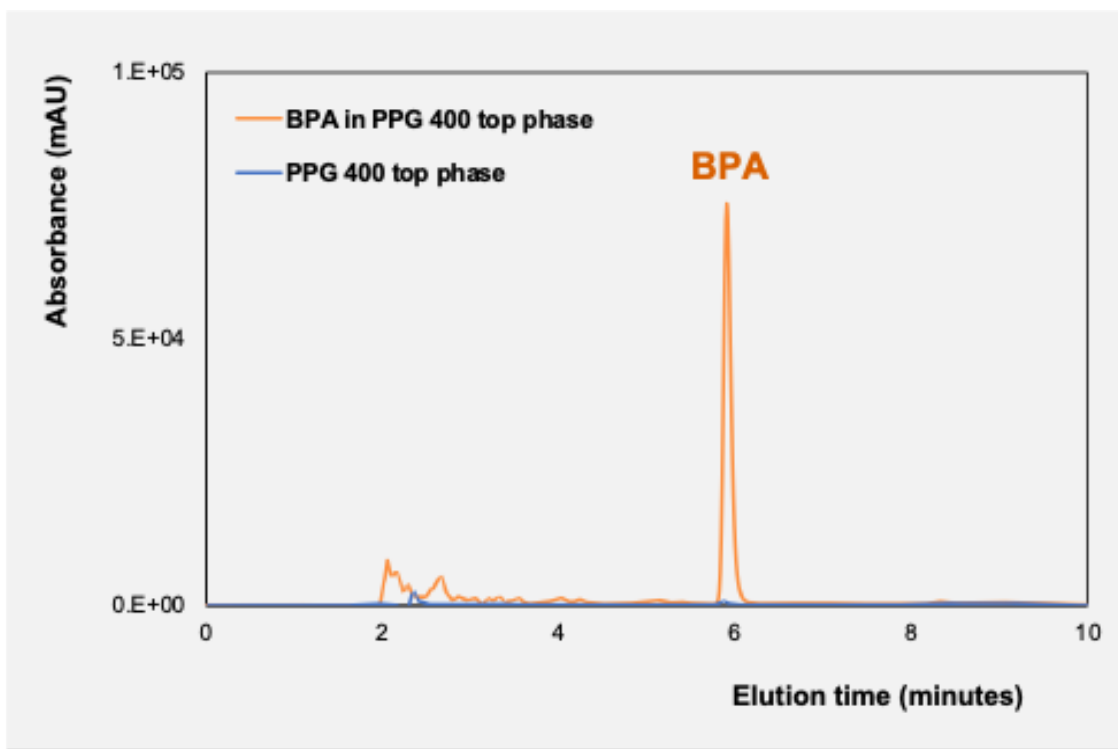


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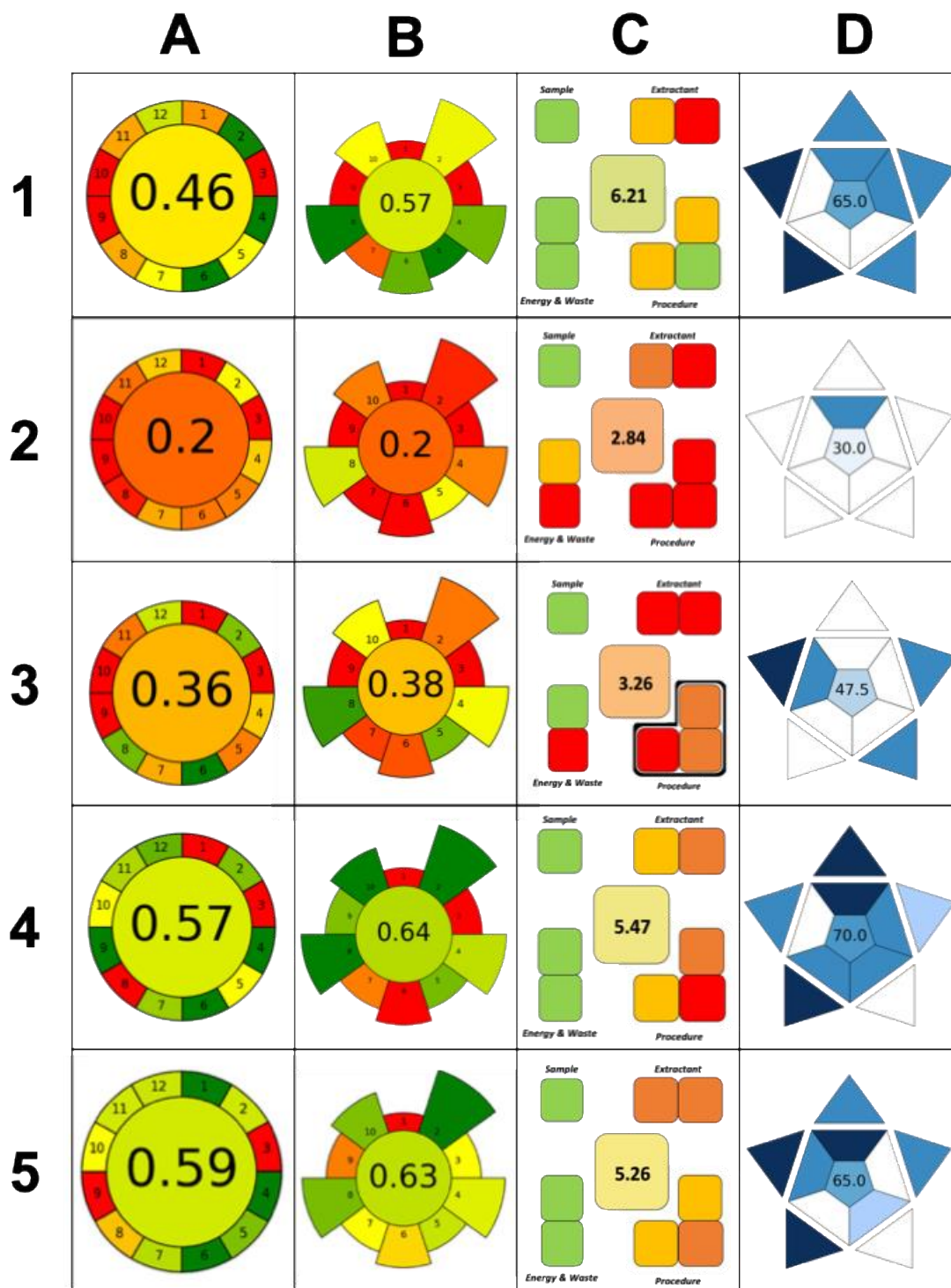


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NaBr		Na[Ac]		NaCl		Na[DHP]		[Ch]Br	
[PPG] / wt%	[Salt] / wt%	[PPG] / wt%	[Salt] / wt%	[PPG] / wt%	[Salt] / wt%	[PPG] / wt%	[Salt] / wt%	[PPG] / wt%	[Salt] / wt%
57.29	7.23	55.72	1.79	76.33	2.28	74.23	0.11	13.80	24.90
53.71	7.61	53.72	2.19	69.66	2.55	65.10	0.30	13.10	26.10
51.15	7.90	49.73	2.49	64.29	2.82	62.18	0.56	12.20	27.70
48.41	8.12	46.24	2.72	60.77	3.12	58.50	0.79	11.40	29.70
46.03	8.41	44.07	2.98	56.71	3.27	57.46	0.88	10.80	31.30
43.97	8.68	40.79	3.12	53.86	3.52	55.39	0.99	9.90	33.60
41.33	8.65	39.07	3.34	51.52	3.76	52.46	1.29	9.10	36.40
40.30	8.98	36.92	3.47	47.62	3.83	51.59	1.39		
38.08	9.06	35.05	3.59	45.01	3.94	47.51	1.82		
36.60	9.22	33.77	3.76	43.35	4.10	46.68	1.87		
35.77	9.50	32.07	4.11	41.68	4.26	42.15	2.45		
34.95	9.75	30.31	4.37	40.13	4.42	41.82	2.49		
34.12	10.02	28.41	4.62	38.73	4.57	36.27	3.14		
32.68	10.46	26.78	5.05	37.40	4.70	30.89	3.88		
30.17	11.46			35.76	4.76	26.17	4.48		
29.59	11.64			33.94	5.00	21.45	5.07		
29.05	11.77			32.61	5.32	18.44	5.54		
28.44	12.01			31.02	5.56	16.67	5.96		
27.72	12.02			29.35	5.73	15.85	6.24		
26.79	12.28			28.11	5.93	13.60	7.01		
25.73	12.73			26.94	6.14	11.34	7.68		
24.96	13.29			26.10	6.38	9.58	8.51		
23.80	13.54			25.05	6.56	7.90	9.36		
22.76	13.81			24.33	6.75	6.35	10.29		
21.98	14.16			23.36	7.01	4.86	11.40		
21.21	14.51			22.44	7.29	3.51	13.20		
20.41	14.98					2.39	14.70		
						1.96	18.34		

Table S2. Recovery yield of IgG and HSA (RY , %) in the top, inter and bottom phase achieved with ABS comprising 30 wt% PPG 400, 15-30 wt% cholinium or sodium salt, 53-38 wt% PBS aqueous solution and 2 wt% human serum. Values are expressed as mean \pm standard deviation ($n = 3$). *Interphase precipitation* is shown in normal font, whereas *bottom phase retention* is indicated by grey shading.

Salt	IgG			HSA		
	RY_{Top} (%)	RY_{Inter} (%)	RY_{Bottom} (%)	RY_{Top} (%)	RY_{Inter} (%)	RY_{Bottom} (%)
30wt% [Ch][DHCit]	0.9 \pm 0.2	86 \pm 2	13 \pm 3	8 \pm 2	64 \pm 2	27 \pm 3
30wt% [Ch]Cl	12 \pm 3	88 \pm 3	0	11 \pm 6	84 \pm 1	0.8 \pm 0.7
30wt% [Ch]Br	0	80 \pm 2	20 \pm 3	0.2 \pm 0.1	70 \pm 2	30 \pm 2
30wt% NaBr	7 \pm 5	93 \pm 5	0	8 \pm 4	94 \pm 4	0
30wt% [Ch][DHP]	3 \pm 3	43 \pm 1	54 \pm 4	0.4 \pm 0.1	76 \pm 1	24 \pm 1
30wt% Na[DHP]	4 \pm 3	77 \pm 9	19 \pm 4	1.1 \pm 0.2	13 \pm 4	86 \pm 5
30wt% [Ch][Bit]	0	67 \pm 2	38 \pm 8	0.5 \pm 0.2	57 \pm 3	43 \pm 3
30wt% [Ch][Ac]	0.4 \pm 0.1	2 \pm 2	97 \pm 6	0.8 \pm 0.2	8 \pm 4	91 \pm 4
15 wt% NaBr	3.5 \pm 0.1	72 \pm 1	28 \pm 1	0	45 \pm 9	55 \pm 9
15 wt% Na[DHP]	2 \pm 1	62 \pm 8	36 \pm 9	1.2 \pm 0.4	82 \pm 6	16 \pm 6
15 wt% Na[Ac]	0	11 \pm 4	89 \pm 4	0	0	101 \pm 2
15 wt% NaCl	0.3 \pm 0.1	25 \pm 9	75 \pm 9	0.4 \pm 0.1	0	123 \pm 1

Table S3. Recovery yield of BPA (RY_{BPA} , %) in the top phase of ABS comprising 30 wt% PPG 400, 15-30 wt% cholinium or sodium salt, 53-38 wt% PBS aqueous solution and 2 wt% human serum. Values are expressed as mean \pm standard deviation ($n = 3$). *Interphase precipitation* is shown in normal font, whereas *bottom phase retention* is indicated by grey shading.

Salt	RY_{BPA} (%)
30wt% [Ch][DHCit]	76 \pm 1
30wt% [Ch]Cl	64 \pm 2
30wt% [Ch]Br	74 \pm 7
30wt% NaBr	86 \pm 8
30wt% [Ch][Ac]	42 \pm 5
15 wt% Na[Ac]	14 \pm 1
15 wt% NaCl	42 \pm 4

Table S4. Masses of the ABS phases, measured BPA concentrations in the PPG 400-rich phase, and calculated enrichment metrics for the *interphase precipitation* strategy. All systems contained 30 wt% PPG 400, 30 wt% salt, 38 wt% PBS aqueous solution and 2 wt% human serum, with the salt type varied for each system. Maximum enrichment factor (EF_{max}) is the ratio of total ABS mass (1 g) to PPG 400-rich phase mass, and concentration factor (CF) is the ratio of BPA concentration in the PPG 400-rich phase to the initial serum concentration (0.3 mg·mL⁻¹), indicating the effective preconcentration. Values are expressed as mean \pm standard deviation ($n = 3$).

Salt	PPG 400-rich phase (g)	Salt-rich phase (g)	BPA in the PPG-rich phase (mg·mL ⁻¹)	EF_{max}	CF
[Ch][DHCit]	0.23 \pm 0.02	0.65 \pm 0.01	0.023 \pm 0.002	4.3 \pm 0.4	0.08 \pm 0.01
[Ch]Cl	0.24 \pm 0.02	0.66 \pm 0.01	0.019 \pm 0.002	4.2 \pm 0.4	0.06 \pm 0.01
[Ch]Br	0.19 \pm 0.02	0.70 \pm 0.01	0.03 \pm 0.01	5.2 \pm 0.6	0.09 \pm 0.02
NaBr	0.30 \pm 0.01	0.60 \pm 0.01	0.020 \pm 0.001	3.4 \pm 0.1	0.07 \pm 0.01

Table S5. Comparison of literature-reported analytical strategies for BPA determination in biological matrices; the numbering of examples matches that in Figure S5, enabling a direct comparison of the different approaches. NR: not reported.

Example	Biological sample	Sample amount (mL)	Sample pretreatment strategy	Extraction solvent/material	Additional steps	Extraction time (min)	Analytical technique	LOD (ng·mL ⁻¹)	Ref.
This work	Human serum	0.02	ABS	PPG 400 + cholinium/sodium salt	Centrifugation	10	HPLC-DAD	49000	-
1	Human plasma	0.1	PP	Acetonitrile	Centrifugation; Filtration	5	LC-MS/MS	5	[1]
2	Urine	3	SPE	SPE cartridge with acetonitrile and water	Evaporation; Derivatization; Cooling	450	GC-MS/MS	0.05	[2]
3	Human serum	0.5	LLE	Acetonitrile	Centrifugation; Evaporation	40	LC-MS/MS	0.009	[3]
4	Urine	0.45-0.6	ABS	Ionic liquid and salts	Equilibration	720	UV-Vis	NR	[4]
5	Whole blood	0.1	LLE	Thymol:menthol eutectic solvent	Sonication; Centrifugation	30	LC-MS/MS	NR	[5]

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