

Supporting Information to:

Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers

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Summary: this file contains eleven SI pages with seven SI Tables and four SI Figures.

Table S1. Characteristics of the WWTP sampled in the study.

Location	Mean flow rate (m ³ day ⁻¹)	Population served by the plant	Sampling mode ^c (volume and frequency of sampling)	Sampling date/period
Santiago de Compostela	53000	136500 ^a	Grab sampling	15 th March 2015 & 15 th April 2015
			24 h composite (120 mL every 10 min)	19 – 25 th April 2016
Ares	13620	24490 ^b	24 h composite (110 mL every 30 min)	2 nd June 2016
Baiona	4881	12072 ^b	24 h composite (480 mL every 60 min)	14 th June 2016
Cambados	12334	13895 ^b	24 h composite (500 mL every 60 min)	7 th June 2016
Gondomar	12743	14056 ^b	24 h composite (110 mL every 30 min)	1 st June 2016
Nigrán	8954	17619 ^b	24 h composite (100 mL every 30 min)	1 st June 2016

^a Population calculated as 2.5 times the number of servers connected to the sewage system, according to the recommendations of the WWTP manager

^b Census data at 01/01/2015

^c 24 h samples collected in time proportional mode, taking aliquots of the indicated volume at the indicated frequency

Table S2. Chemical formulae, retention time (RT), deuterated compound used as surrogate or internal standard (IS), transitions (precursor/product) used for quantification and confirmation and instrumental performance parameters for every analyte.

Compound	[M-H] ⁻ Formula	RT ^a min	Internal Standard	Precursor m/z	CV ^b	Quantifier Product		Qualifier Product		Linearity R ^{2d}	IQL ^e µg L ⁻¹	Repeatability %RSD ^f	Reproducibility %RSD ^g
						m/z	CE ^c	m/z	CE ^c				
MMP	C ₉ H ₇ O ₄	4.4	MMP-D4	179	40	107	95	77	175	0.9995	0.31	2.2	2.3
MEP	C ₁₀ H ₉ O ₄	5.1	MnBP-D4	193	44	77	165	121	105	0.9987	0.31	4.4	6.1
MiBP	C ₁₂ H ₁₃ O ₄	7.6	MnBP-D4	221	48	77	17	134	17	0.9991	0.23	2.8	4.9
MnBP	C ₁₂ H ₁₃ O ₄	7.9	MnBP-D4	221	48	77	17	177	9	0.9991	0.16	1.8	7.0
MBzP	C ₁₅ H ₁₁ O ₄	8.8	MnBP-D4	255	48	77	19	183	11	0.9988	0.01	1.9	5.9
MEHHP	C ₁₆ H ₂₁ O ₅	8.0	MEHHP-D4	293	60	145	13	121	18	0.9987	0.07	1.7	2.5
MEOHP	C ₁₆ H ₁₉ O ₅	8.2	MEHHP-D4	291	44	143	14	121	185	0.9986	0.11	3.9	4.2
MECPP	C ₁₆ H ₁₉ O ₆	8.0	MEHHP-D4	307	36	159	11	113	29	0.9991	0.09	3.0	4.7
MMP-D4	C ₉ H ₃ D ₄ O ₄	4.3	—	183	40	111	95	—	—	—	—	—	—
MnBP-D4	C ₁₂ H ₉ D ₄ O ₄	7.8	—	225	48	81	17	—	—	—	—	—	—
MEHHP-D4	C ₁₆ H ₁₇ D ₄ O ₅	7.9	—	297	60	125	18	—	—	—	—	—	—

^aRetention time

^bCapillary Voltage

^cCollision Energy

^dDetermination Coefficient (linearity evaluated between 1 and 500 µg L⁻¹)

^eInstrumental Quantification Limit

^fRelative Standard Deviation for consecutive injections of a 50 µg L⁻¹ standard (n=5)

^gRelative Standard Deviation for injections of 50 µg L⁻¹ standards in a nine-months period (n=5)

Table S3. Studies considered to calculate participant-weighted average excretion fractions for MnBP, MiBP, MBzP and DEHP metabolites (MEHHP, MEOHP and MCPP). To the best of authors' knowledge, human excretion fractions for MMP and MEP are currently not available. Therefore, MnBP average excretion fraction of 0.69 was used due to its similar structure with linear side alkyl chains (Saravanabhavan et al., 2014^a; Koch et al., 2003^b).

Reference	Number of participants	Phth. diester dose	Phth. diester	Phth. monoester	Molar excretion fraction (in 24 h)
Anderson et al. 2001 ^c	8	Low	DnBP	MnBP	0.64
	8	High	DnBP	MnBP	0.73
	8	Low	BzBP	MBzP	0.67
	8	High	BzBP	MBzP	0.78
Koch et al. 2012 ^d	1	–	DnBP	MnBP	0.84
	1	–	DiBP	MiBP	0.71
Anderson et al. 2011 ^e	20	Low	DEHP	MEHHP	0.16
	20	High	DEHP	MEHHP	0.15
	20	Low	DEHP	MEOHP	0.12
	20	High	DEHP	MEOHP	0.10
	20	Low	DEHP	MECPP	0.16
	20	High	DEHP	MECPP	0.12
Koch et al. 2005 ^f	1	–	DEHP	MEHHP	0.23
	1	–	DEHP	MEOHP	0.15
	1	–	DEHP	MECPP	0.18

^a Saravanabhavan, G.; Walker, M.; Guayb, M.; Aylward, L.; Urinary excretion and daily intake rates of diethyl phthalate in the general Canadian population. *Sci. Total Environ.* **2014**, 500–501, 191–198.

^b Koch, H.M.; Gonzalez-Reche, L. M.; Angerer, J. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. *J. Chromatogr. B* **2003**, 784, 169–182.

^c Anderson, W.; Castle, L.; Scotter, M. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Add. Contam.* **2001**, 18, 1068–1074.

^d Koch, H.M.; Christensen, K.L.Y.; Harth, V.; Lorber, M.; Bruning, T. Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. *Arch. Toxicol.* **2012**, 86, 1829–1839.

^e Anderson, W.A.C.; Castle, L.; Hird, S., Jeffery, J., Scotter, M. J. A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. *Food Chem. Toxicol.* **2011**, 49, 2022–2029.

^f Koch, H.M.; Bolt, H.M.; Preuss, R.; Angerer, J. New metabolites of di(2 ethylhexyl) phthalate (DEHP) in human urine and serum after single oral doses of deuterium labeled DEHP. *Arch. Toxicol.* **2005**, 79, 367–376.

Table S4. Correction factors applied to estimate exposure to phthalate diesters from phthalate monoester mass loads in wastewater. Average excretion fractions were obtained by weighting molar excretion fractions provided in every study (Table S3) by the number of participants involved in every case.

Phth. diester	Molecular weight	Phth. monoester	Molecular weight	Weighted average excretion fraction (in 24h)	Correction Factor (CF)
DnBP	278.344	MnBP	222.237	0.69	1.80
DiBP	278.344	MiBP	222.237	0.71	1.76
BzBP	312.36	MBzP	256.253	0.73	1.68
		MnBP	222.24	0.06	23.43
DEHP	390.556	MEHHP	294.343	0.16	8.40
		MEOHP	292.327	0.11	11.78
		MECPP	308.33	0.14	9.01
DEP	222.237	MEP	194.184	0.69	1.65
DMP	194.184	MMP	180.157	0.69	1.55

Table S5. Method quantification limits (MQL), percentages of recovery (%R) and relative standard deviations (%RSD) for the whole method.

Compound	MQL (ng L ⁻¹)		Internal standard corrected %R (%RSD)		
	Effluent	Influent	Ultrapure water (0.1 µg L ⁻¹)	Ultrapure water (1 µg L ⁻¹)	Influent (2 µg L ⁻¹)
MMP	31	32	105 (13)	124 (9)	97 (4)
MEP	7.5	8.1	100 (15)	115 (13)	76 (4)
MiBP	2.5	3.2	108 (9)	120 (9)	95 (4)
MnBP	2.8	3.9	107 (10)	122 (12)	93 (4)
MBzP	0.5	0.5	101 (9)	116 (12)	100 (4)
MEHHP	4.2	3.2	108 (11)	106 (5)	85 (2)
MEOHP	4.5	2.2	102 (13)	109 (8)	97 (1)
MECPP	4.0	1.7	107 (11)	100 (6)	94 (2)

Table S6. Regression coefficients (R) and p-values (p) for the pairwise correlation study between phthalate monoester excretion loads in 24 h influent samples. For Santiago, we used the average values of the seven days.

		MMP	MEP	MiBP	MnBP	MBzP	MEHHP	MEOHP	MECPP
MMP	R		-0.213	-0.185	-0.3027	0.9527	-0.2248	-0.3607	-0.341
	p		0.6847	0.7262	0.5598	0.0123	0.6685	0.4824	0.5084
MEP	R	-0.2134		0.9975	0.9935	0.9532	0.4844	0.3867	0.1868
	p	0.6847		0	0.0001	0.0121	0.3302	0.4489	0.723
MiBP	R	-0.1846	0.9975		0.99	0.9342	0.494	0.3984	0.1959
	p	0.7262	0		0.0001	0.0201	0.3193	0.434	0.7099
MnBP	R	-0.3027	0.9935	0.99		0.945	0.45	0.3713	0.17
	p	0.5598	0.0001	0.0001		0.0154	0.3705	0.4686	0.7474
MBzP	R	0.9527	0.9532	0.9342	0.945		0.3348	0.1558	-0.0347
	p	0.0123	0.0121	0.0201	0.0154		0.5818	0.8025	0.9558
MEHHP	R	-0.2248	0.4844	0.494	0.45	0.3348		0.9766	0.9354
	p	0.6685	0.3302	0.3193	0.3705	0.5818		0.0008	0.0061
MEOHP	R	-0.3607	0.3867	0.3984	0.3713	0.1558	0.9766		0.9762
	p	0.4824	0.4489	0.434	0.4686	0.8025	0.0008		0.0008
MECPP	R	-0.341	0.1868	0.1959	0.17	-0.035	0.9354	0.9762	
	p	0.5084	0.723	0.7099	0.7474	0.9558	0.0061	0.0008	

Table S7. Phthalate monoester estimated concentrations in urine (this study) versus median values of the concentrations found in 21 urine samples from the Spanish population (Herrero et al. 2015) and geometric mean of the concentrations measured in urine from 120 Spanish mothers (Cutanda et al. 2015).

Average concentration ($\mu\text{g L}^{-1}$)	MMP	MEP	MiBP	MnBP	MBzP	MEHHP	MEOHP	MECPP
Ares	32.7	358.5	65.7	66.0	4.2	12.7	7.1	7.2
Baiona	37.6	396.4	71.6	70.6	2.6	8.0	5.4	3.7
Cambados	150.0	906.5	143.0	138.7	10.6	35.2	15.6	14.9
Gondomar	27.7	173.8	38.9	31.7	0.8	35.9	19.6	25.0
Nigrán	611.7	130.2	34.2	19.0	<0.04 ^c	8.2	2.5	2.5
Santiago	34.3	216.3	38.5	40.1	3.4	6.4	2.9	3.4
Weighted average	87.8	276.4	49.7	48.7	3.4	11.1	5.3	5.9
Herrero et al. 2015^a	7.0	68.6	23.3	19.3	2.6	5.3	6.2	22.5
Cutanda et al. 2015^b	–	161.1	37.4	32.7	8.5	21.4	13.8	–

^a Herrero, L.; Calvarro, S.; Fernández, M.A.; Quintanilla-López, J.E.; González, M.J.; Gómara, B. Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples. *Anal. Chim. Acta* **2015**, *853*, 625–636

^b Cutanda, F.; Koch, H.M.; Esteban, M.; Sánchez, J.; Angerer, J.; Castaño, A. Urinary levels of eight phthalate metabolites and bisphenol A in mother–child pairs from two Spanish locations. *Int. J. Hyg. Environ. Health* **2015**, *218*, 47–57

^c In Nigrán, the estimated concentration in urine of MBzP was calculated considering a concentration in wastewater equal to LOD/2

Figure S1. MEHP chromatogram of a procedural blank (green line) overlapped to a 10 ng mL⁻¹ standard (red line).

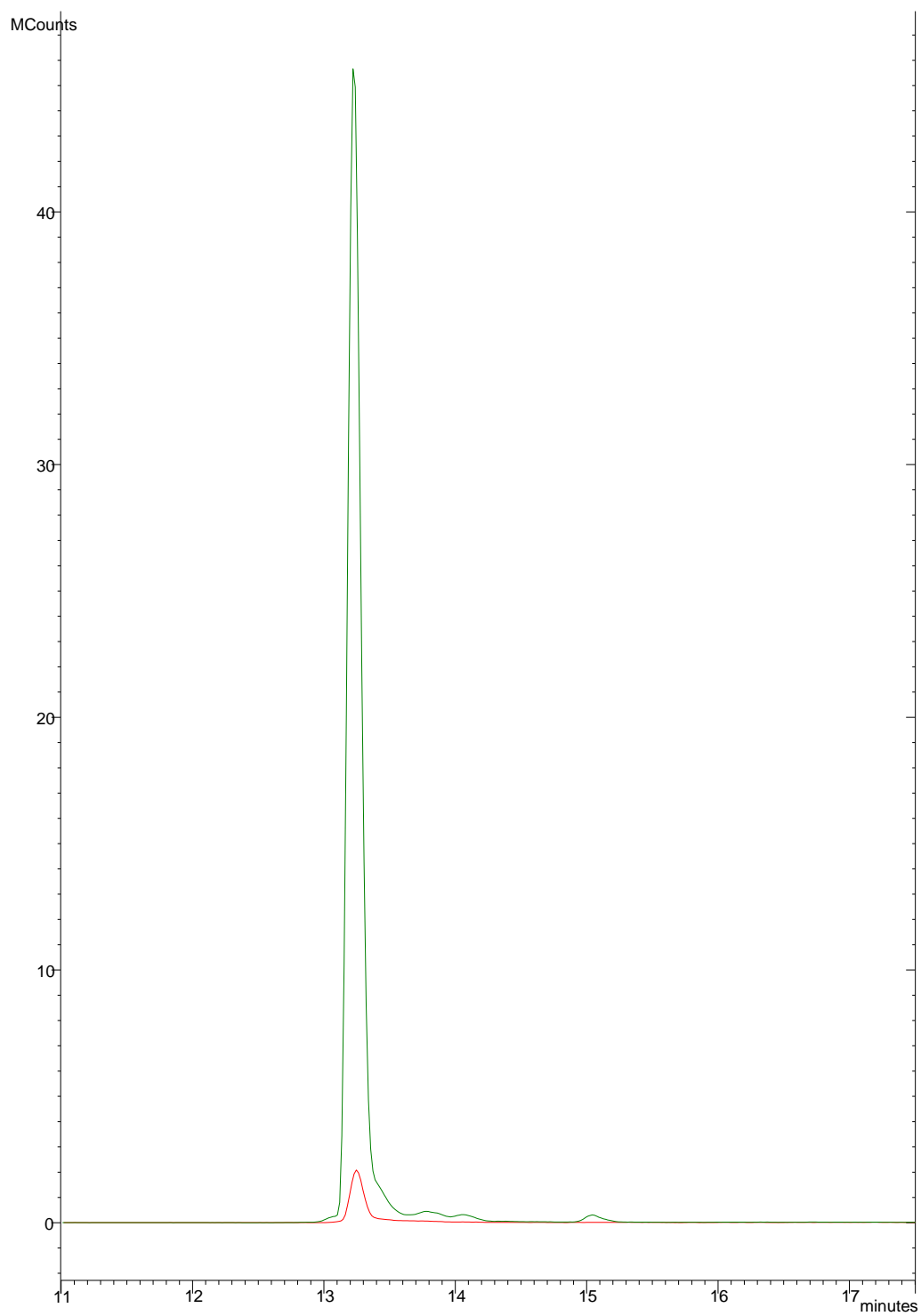


Figure S2. Isomer separation (MiBP and MnBP) on the C18 and the Phenyl-Hexyl column using water-MeOH and water-ACN as mobile phases.

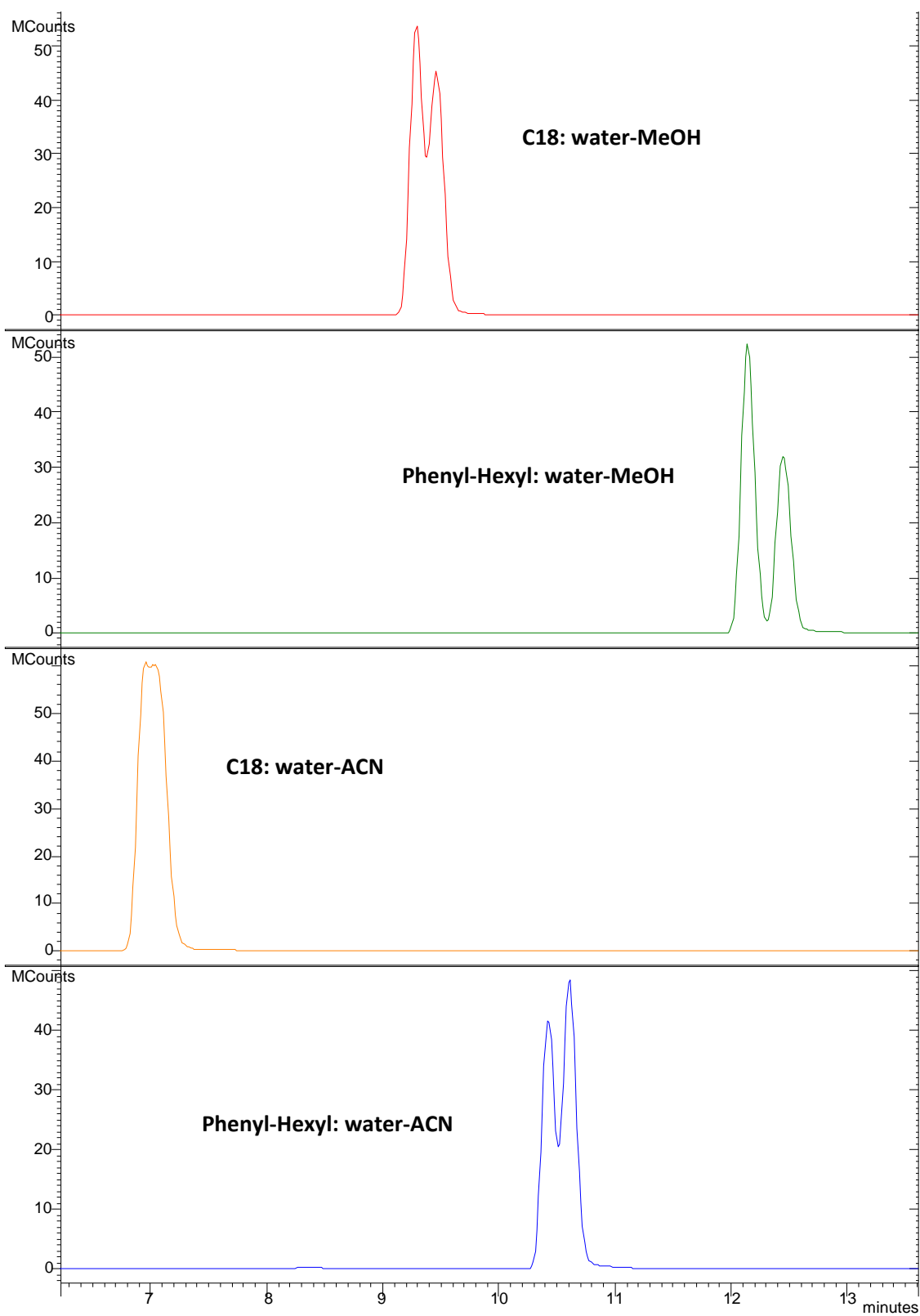


Figure S3. Percentage of molar formation of MnBP and MBzP relative to the parent concentration in ultrapure water, at different pH and different temperatures (n=3).

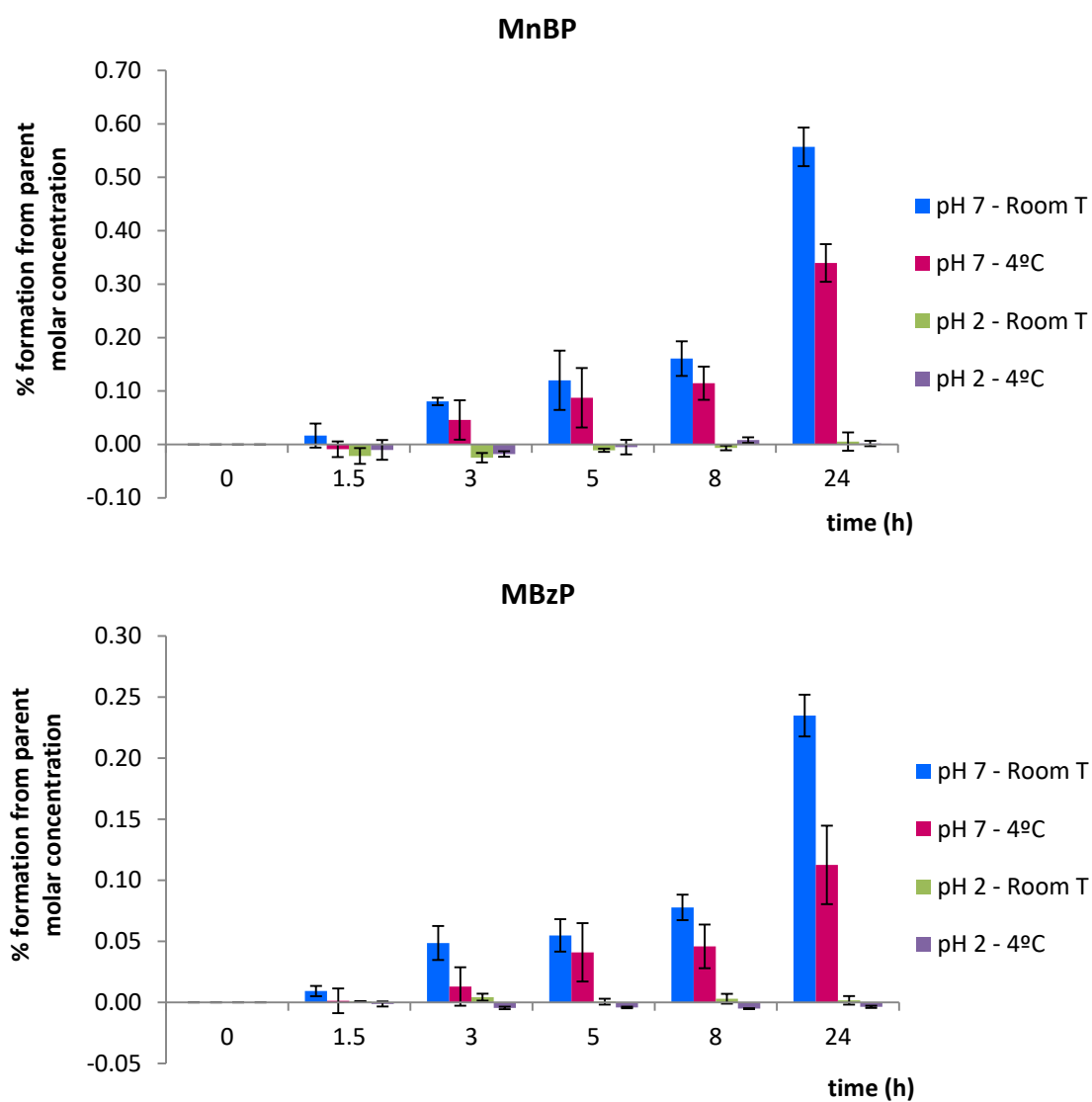


Figure S4. Recovery of the SPE process in wastewater extracted on Oasis HLB and Oasis MAX cartridges. Matrix effects for both sorbents, calculated by comparing the response of a spiked-after-SPE extract with that of a standard in MeOH (n=3).

