Introduction

Phthalates are common plasticizers found in everyday items from single use plastics, PVC pipes, vinyl flooring, medical devices, toys, and electronics. Phthalates increase the plastics flexibility, durability, and temperature range (1). Since they are not chemically bound to polymers, they are therefore able to leach out. Over the years, more phthalates have been synthesized. From 2000 to 2010, global production increased from 3.5 to 6 mil tons/yr (2). Phthalates have been found in the air, ocean, dust, wastewater, cosmetics, and food (3, 5-15). Exposure to phthalates is associated with allergies, asthma, rhinoconjunctivitis, reduced birth weight, and endocrine disruptions (3, 15, 16, 17, 18). Due to increasing awareness of the environmental and health impacts of phthalates within the last thirty years, regulations have been implemented across the globe for particular phthalates. In the US, for example, 8 phthalates are regulated. This has caused a shift to alterative phthalates in the global market (3). We developed a selective ion monitoring (SIM) gas chromatography mass spectrometry (GCMS) method on an Agilent 8890 5977B GCMS for quantitation of 27 phthalates and 3 alternative phthalates for passive samplers and biological matrices. See table 2 for full analyte list.

Method Validation and Optimization

Oven profile

- Started with oven profile from Takeuchi et al (2014) (Table 1)
- Broad peaks for high molecular weight (MW) compounds
- Series of oven profile experiments
- Reduced hold on 3rd ramp to improve high MW compounds except ditridecyl phthalate
- Fourth ramp was added to improve ditridecyl phthalate
- 5 minute post-run added to reduce silicone passive samplers matrix carry over
- Final oven profile achieved Gaussian peak shape with a minimum of 15 scans across a peak for all compounds (Table 1 and Table 3) Table 3. Full GC/MS settings for method

Table 2. Full compound list with peak number, structure, physical and chemical properties, and limits of detection (LODs) and of guantitation (LOQs). Orange compounds are alternative plasticizers.

are al	ternative plasticizers	5.								Column	J&W Sicentific DB 5M
Peak #	Compounds	CAS	Structure	Mol Wt (g/mol)	Log Koa	Log Kow	Henry's Law	LOD (ppb)	LOQ (ppb)	Temperature	Helium 99.9% at 1.15 Injection 290 °C
1	Dimethyl Phthalate	131-11-3		194.2	6.69	1.6	1.97E-07	200	1000		MSD 300 °C MS Source 300 °C MS quadripole 180 °C
2	Diethyl Phthalate	84-66-2		222.24	7.44	2.65	6.10E-07	150	750		Column 40 °C (2 min 200 °C at 25 °C/min, t
3	Diisopropyl Phthalate	605-45-8		250.29	8.03	3.48	6.14E-07	50	250	Inlet	hold), to 335 °C at 20 min post run at 340 ° Draw Speed 300 μL/n
4	Diallyl Phthalate	131-17-9		246.26	8.16	3.36	1.17E-07	50	250		Pulsed Splitless mode Purge 3 mL/min, Purg
5	Di-n-propyl Phthalate	131-16-8		250.3	8.18	3.63	4.03E-07	50	250	Scan Mass Range Injection Volume	0.45 min 50-600 1 μL
6	Diisobutyl Phthalate	84-69-5		278.35	8.76	4.46	6.43E-07	150	750	Calibration cur	
7	Di-n-butyl Phthalate	84-74-2		278.35	8.63	4.5	1.22E-06	200	1000		alibration curve μL to 101 ng/μL
8	Bis(2-methoxyethyl) Phthalate	117-82-8		282.29	9.77	1.11	2.81E-13	50	250	ng/µL	
9	Diisopentyl Phthalate	605-50-5		306.4	9.5	5.45	1.29E-06	50	250	 Average r² o 30 comp 	f 0.995 ounds on a linea
10	Bis(2-ethoxyethyl) Phthalate	605-54-9		310.34	10.5	2.1	5.11E-13	50	250	•	ounds on a quad
11									250		
12	Bis(4-methylpentyl) Phthalate	146-50-9		334.46	10.2	6.43	2.56E-06	50	250	LODs and LOQsRan 7 aligud	s ots of the full cur
	Di-n-hexyl Phthalate		8							those were	run again on the
14	Butyl Benzyl Phthalate	85-68-7		312.4	10.6	4.83	1.26E-06	150	750	n=15 • Limits of det	ection (LODs) w
15	Di(2-ethylhextl) Adipate	103-23-1	m	370.58	11.2	8.12	2.15E-05	150	750	multiplying ⁻	the standard dev nt t value for the
16	Bis(2-butoxyethyl) Phthalate	117-83-7		366.4	12	4.06	2.03E-12	50	250	interval	
17	Bis(2-ethylhexyl) Phthalate	117-81-7	<u>~8-c</u>	390.57	11.7	8.39	2.70E-07	200	1000	Ŭ	LOD is 83 pg/µL n is 1.6 pg/µL
17	Diheptyl Phthalate	3648-21- 3		362.5	11.1	7.56	3.54E-06	200	1000	 Maximu 	m is 231 pg/μL
18	Dicyclohexyl Phthalate	84-61-7		330.4	11.6	6.2	1.00E-07	200	1000	•	antitation (LOQs the rounded LOI
19	Diphenyl Phthalate	84-62-8		318.32	10	4.1	3.06E-08	50	250	Average	LOQ is 535 pg/µ n is 250 pg/µL
20	Bis (2-ethylhexyl) Isophthalate	137-89-3	مكالموارب	390.56	11.7	8.39	2.44E-07	50	250	 Maximu 	m is 1250 pg/μL
21	Di-n-octyl Phthalate	117-84-0		390.6	11.9	8.54	2.57E-06	150	750	• •	cent recoveries Qs values are sl
22	Bis(2-ethylhexyl) Terephthalate	744-45-6	-Jor	318.3	10	4.1	3.06E-08	150	750	Storage Stabilit	
	Diphenyl Isophthalate		\sim							U	rate storage stat
23	Dibenzyl Phthalate	523-31-9		346.4	12.3	5.08	1.02E-11	50	250	same full cu 50, 133 days	rve mix were tal
24	Bis(2-propylheptyl) Phthalate	53306-54- 0		446.66	13.2	10.4	1.55E-07	50	250	Percent reco	overy (Figure 2)
25	Di-n-nonyl Phthalate	84-76-4		418.6	####	9.52	1.41E-05	250	1250	•	at 0 days: 84% at 12 days: 87%
27	Didecyl Phthalate	84-77-5		446.66	13.3	9.05	2.81E-06	250	1250	•	at 50 days: 94%
28	Diundecyl Phthalate	3648-20- 2		474.7	14.1	11.5	5.60E-05	50	250	–	at 133 days: 869 nds are stable fo
33	Tris(2-ethylhexyl) Trimellitate	3319-31- 1	monter	546.78	16.2	11.6	4.45E-07	200	1000		•
34	Ditridecyl Phthalate	119-06-2		530.8	15.5	13.5	2.23E-04	50	250	200-	•
2	Diethyl Phthalate-d4	93952-12- 6		225.26						150-	:
7	Di-n-butyl Phthalate- d4	93952-11- 5		282.37						Percent Recovery	• •
11	Diamyl Phthalate-d4	358730- 89-9		310.43							
25	Di-n-nonyl Phthalate- d4	1202865- 43-7		422.6						50-	
26	Diisodecyl Phthalate- d4	1346604- 79-2		450.69							
	Di-n-decyl Phthalate- d4		1								ay 0 Day 12 Day 50
		10 Z								Figure 2. Storage s	tability. Red lines are

Table 1. Initial verses final oven profile **Initial Oven Profile** 40 °C, 2 min hold 25 °C/min, 200 °C, no hold 40 °C/min, 280 °C, no hold 10 °C/min, 320 °C, 7 min hold **Final Oven Profile:** 40 °C, 2 min hold 10 °C/min, 100 °C, no hold 25 °C/min, 200 °C, no hold 40 °C/min, 280 °C, 3 min hold 20 °C/min, 335 °C, 7 min hold 340 C/ 4 min hold Agilent GC/MSD 8890/5977, EI mode 70eV J&W Sicentific DB 5MS 30m x 250µm x 0.25µm, 57 mL/min n hold) to 100 °C at 10 °C/min, to to 280 °C at 25 °C/min (3 min

Conditions

Description

Instrument

) °C/min (7 min hold). Than a 5 C. Total run time of 31.75 min min, Eject Speed 6000 μL/min, e 25psi until 0.5 min, Setpum ge to Split Vent 20 mL/min at

 $L \text{ and } 2 \text{ ng/}\mu L \text{ to } 25$

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urve the first day, 4 of ne following 2 days for

were calculated by leviation by the onee 99% confidence

Qs) were calculated by OD value by 5

for the targets: 111% shown in Table 1

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for at least 133 days

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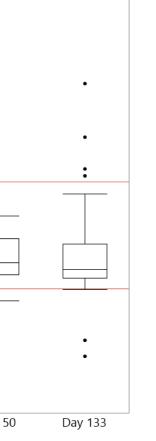
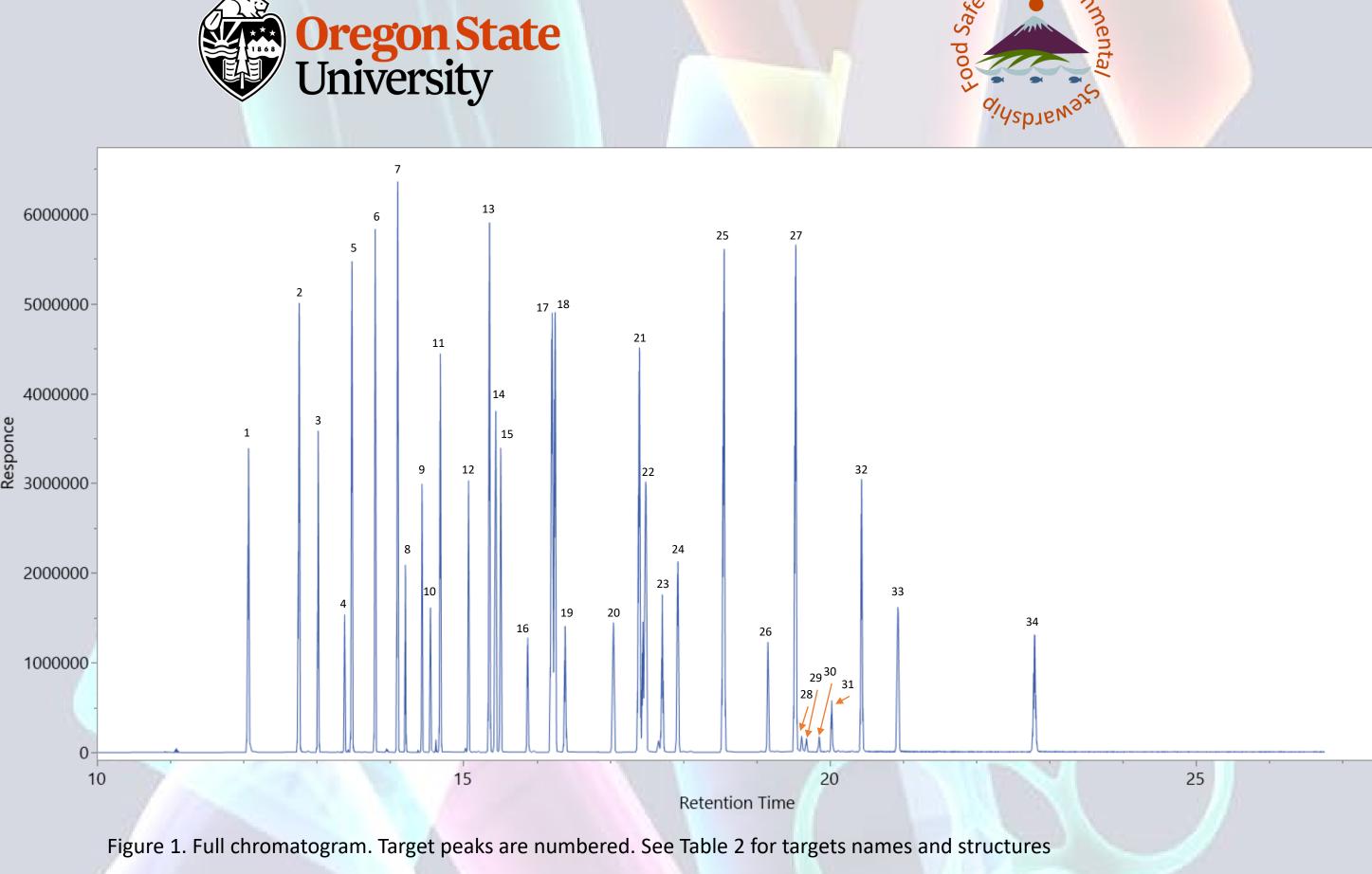


Figure 2. Storage stability. Red lines are at 70% and 130% FSES DQO's

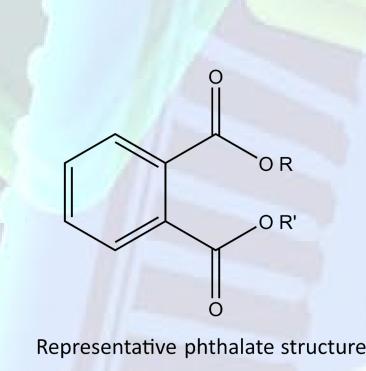
Phthalates and Phthalate Alternatives Analysis **Using Gas Chromatography Mass Spectrometry for** Wristband and Environmental Passive Sampling Matrices

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Phthalates are in PVC, personal care products, medical devises, vinyl flooring, perfumes, sanitary pads, diapers, insect repellent, plastic cling film, etc.

- Out of the 7766 wristbands analyzed by FSES, 5751 hits for phthalates.
- Exposure associated with asthma, allergies, and endocrine disruptions.
- This method is a targeted analysis for over 30 phthalates and phthalate alternatives to be used with passive sampler technology as well as with biological matrices.





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Method Validation and Optimization (Continued)

SPE

- This method was built for analysis of passive samplers, some of which can be deployed on humans. Removal of fatty acids and other interferences was explored using solid phase extraction (SPE)
- C18, florisil, and primary secondary amine (PSA) columns were tested with a 15 pg/ μ L matrix spike. (Figure 3)
- C18 was effective for smaller MW, however the higher MW compounds percent recovery was
- Florisil had average percent recovery of 90% and PSA had 96%
- compared to florisil (77%) and C18 (68%)
- PSA was selected for SPE

Method Comparison

- Of the 11 phthalate method papers identified, the number of phthalates in the method ranged from 6 to 21 with run times of 16 minutes to 40 minutes. (Table 4)
- This method has at least 10 more compounds and kept the total run time
- compo Altern Un comp Matr Run LOD (µ

- comparable with most other published phthalate methods.
- phthalate, ditridecyl phthalate.
- citrate.

Real World Samples

Table 5. Data for environmental samples to evaluate the method. Table is separated out by sample type and further delineated by sample or matrix spike.

Sample Matrix	Sample n		Dimethyl Phthalate	Diethyl Phthalate	Diisopropyl Phthala _{te}	Diallyl Phthalate	Di-n-propyl Phthalate	Diisobutyl Phthalate	Di-n-butyl Phthalate	Bis(2-methoxyethyl) Phthalate	Diisopentyl Phthala _{te}	Bis(2-ethoxyethyl) Phthalate	Diamyl Phthalate	Bis(4-methylpentyl) Phthalate	Di-n-hexyl Phthalate	Butyl Benzyl Phthalate	^{Bis} (2- ^{eth} ylhexyl) Adipate	Bis(2-butoxyethyl) Phthalate	Bis(2-ethylhexyl) Phthalate	Diheptyl Phthalate	Dicyclohexyl Phthalate	Diphenyl Phthalate	Bis(2-ethylhexyl) Isophthalaro	<i>Di-n-octyl Phthalate</i>	Bis(2-ethylhexyl) Terephthalse	Diphenyl Isophthalate	Dibenzyl Phthalate	Bis(2-propylheptyl) Phthalate	Di-n-nonyl Phthalate	Di-n-decyl Phthalate	Diundecyl Phthalate	Tris(2-ethylhexyl) Trimellitz+	Ditridecyl Phth.
Urban Children's Si Wristband	3	ppm	0	23.7	0	0	0	6.37	7.19	0	0	0	0	0	0	13.1	1230	0	117	0	0	0	581	0	0	2470	0	0	10.2	15.6	2.36	9.1	0
Urban Children's Si Wristband MS	2	% recovery	119	109	145	186	155	757	221	197	243	225	56.7	295	312	1040	412	1110	156	423	0	268	0	545	52	0	0	6290	149	167	977	171	478
48 hour Adult Si Wristband	5	ppm	0	14	0	0	0	3.12	7.52	0	0	0	0	0	0	1.63	21.7	0	177	0	0	0	0	0	0	160	0	0	0	0	1.66	12.4	0
48 hour Adult Si Wristband MS	1	% recovery	104	0	110	189	124	562	184	135	164	179	0	159	186	902	125	274	541	0	185	0	339	0	44.5	0	0	0	134	152	266	0	240
Rural Children Si Wristband Rural Children Si	3	ppm % recovery	9.05 169	73.6 310	0 191	0 193	0 159	143 1260	113 0	0 1640	0 417	0	0 19	0 1460	0 269	136 919	1150 393	0 258	3870 1580	0	0	0 273	0 300	0 471	0	309000 13600	0 260	846 5240	0 354	0 266	0 499	213 163	0 521
Wristband MS Silicone Brest	3	ppm	0	0.737	0	0	0	0	0.004	0	0	0.876	0.026	0	0	0.188			1.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Explants Silicone Brest Explants MS	1	% recovery	0	0	41.1	0	0	0	9.98	70.1	49.7	140	1.74	53.2	1.49	5.05	64.4	110	0	25.8	19.8	79.2	0	22.7	28.2	18.3	162	79.7	0	0	98.5	283	69.6
Packaged Smoked Salmon	8	ррт	0	0.529	0	0	0	0.026	0	0	0	0	0	0	0	0	0.189	0	0.194	0	0	0	0	0	0	2.52	0.077	0.132	0	0	0.043	0	0
Packaged Smoked Salmon MS	6	% recovery	40	39.6	53.9	74	50.4	199	45.6	64.3	59.1	65.3	9.15	68.4	64.5	217	47.4	82.7	45.9	53.2	51.4	67.6	108	76.6	14.8	199	41.3	95.3	30.4	35.1	84.6	356	88.9
Fresh Smoked Salmon	10	ppm	0.116	1.22	0.056	0.068	0.041	0.03	0.113	0.056	0.05	0.058	0.027	0.059	0.22	0.178	0.549	0.086	0.18	0.043	0.171	0.057	0.099	0.262	0.054	0.206	0	0.036	0.131	0.153	0.096	0.374	0.09
Fresh Trout	3	ppm	0	1.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Roofers	4	ppm	0.077	0.36	0	0	0	0.011	0	0	0	0	5	0	0	0.116	0.996	0	4.67	0	0	0	0	0	0	0.752	0	0.242	0	0.064	0.083	0.769	0.16
Roofers MS	1	% recovery	9.52	7.51	44.5	78.7	0	38.9	3.53	74	52.2	64.5	4.25	57.5	0	31.9	7.7	181	7.93	38.4	27.1	111	76.8	28.7	40.2	35.7	91.3	11.2	0	10.8	74.8	191	84.3
Fire Test Chamber (Si Wristband) Fire Test	4	ppm	0	0	0	0	0	5.31	0	0	0	5	0	0	0	1.26	0	1.83	0	0	0	0	0	0	0	0	0.636	4.58	0	0	0.52	0	0
Chamber (Si Wristband) MS	1	% recovery	31.8	21.1	93.2	155	9.25	12	0	146	106	132	12.6	114	6.77	32.6	23.6	174	0	49.4	45.9	187	154	43.1	73.4	51.7	179	75.9	0	0	76.4	556	141

• To evaluate the method, we analyzed breast explants, smoked salmon stored in plastic from 6 stores, and silicone wristbands from urban and rural children, pregnant people, and roofers.

• Roofers – hits for high MW compounds seen in building material.

- products.
- As high as 177 ng/μL.
- Rural farm children showed hits of $1000 30000 \text{ ng/}\mu\text{L}$

Acknowledgments

We would like to thank the FSES laboratory members, without which none of this work could have been performed. This work was funded by grants P30ES030287 from EHSC and P42ES016465 from SRP in partnership with the FSES lab and OSU.



Link to the FSES website

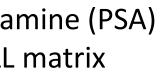


Figure 3: Comparison of the percent recoveries of C18, Florisil, and PSA SPE cartridges. Di-n-propyl phthalate was detected for both Florisil and PSA but 15 ng/ μ L is over its calibration range of 0.25 ng/ μ L -10 ng/ μ L. It is a quadratic fit, and the ratio of compound to ISTD was 5.3 which is over the vertex of 4.55 and therefore no concentration was given by MassHunter software.

• PSA had the highest number of compounds within FSES detection quantitation objectives (DQO's) (91%)

Table 4. Method comparison table comparing this method with 11 other phthalate method papers

Method Paper	This Method	EPA Method 8061A	Guo et al, 2010	Gimeno et al, 2012	Feng et al, 2013	Raveane et al, 2013	Gimeno et al, 2014	Ye et al, 2014	Orecchio et al, 2015	_	Kartalovic et al, 2021	Sambolin o et al, 2022
Number of compounds	31	7	6	12	6	17	14	21	15	10	6	13
Alternatives	TOTM, DEHA, DEHT	-	-	-	-	-	TOTM, DINCH, DEHA,	-	-	-	-	DEHA
Unique compounds	9	-	-	-	-	-	2	-	-	-	-	-
Matrixes	PSD (Silicone and LDPE)	-	Ham Sausages	Cosmetics	-	Wine	Medical devices	Meter Dose	Perfumes	Wastewa ter	Honey	Fish and Squid
Run time	31 min	40 min	19 min	27 min	16 min	-	31 min	30 min	30 min	34 min	31 min	20 min
Percent Recovery (%)	89-130	-	87-108	80-120	-	70-120	78-127	86-108	89-102	84-108	88-112	70-117
LOD (µg/mL)	50-250	42-640	0.31-0.61	100-10	16-2	0.06-10	70-250	0.1-4.2	>10	3-46	0.28-1.38	0.5-5
LOD Calculation method	SD * t value(n- 1,0.99)	SD * t value(n- 1,0.99)	3σ	y intercept + (3*SD of y intercepts)	Etune and Atune	3σ	y intercept + (3*SD of y intercepts)	response=si gnal+3 or 10*SD	3σ	Not discussed	3σ	LCL

 Our method is one of 3 methods which incorporates alternatives plasticizers. • This method had 9 unique compounds: diamyl phthalate, bis (4-methylpentyl) phthalate, diheptyl phthalate, diphenyl isophthalate, dibenzyl phthalate, bis (2-propylheptyl) phthalate, didecyl phthalate, diundecyl

The only other method to incorporate unique phthalates was Gimeno et al (2014): DINCH and acetyl tributyl

• bis (2-ethylhexyl) phthalate, trimellitate, ditridecyl phthalate, and diundecyl phthalate

• Wristbands worn for 48 hours by pregnant people had high hits for phthalates associated with personal care





