Phthalates and Phthalate Replacements Analysis Using Gas Chromatography Mass Spectrometry With Demonstration using Silicone Passive Samplers and Real-World Samples

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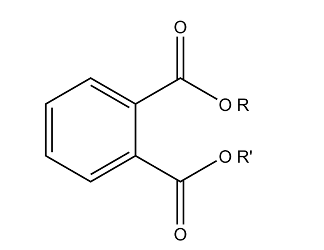
Link to the FSES website

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 replacement 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 replacement phthalates for passive samplers. See Table 1 for full analyte list.

Table 1. Full compound list with peak number, structure, physical and chemical properties, and limits of detection (LODs) and of quantitation (LOQs). Orange compounds are replacement plasticizers.

Peak #	Compounds	CAS	Structure	Mol Wt (g/mol)	Log Koa	Log Kow	Henry's Law	LOD (ppb)	LOQ (ppb)
1	Dimethyl Phthalate	131-11-3		194.2	6.69	1.6	1.97E-07	200	1000
2	Diethyl Phthalate	84-66-2		222.24	7.44	2.65	6.10E-07	150	750
3	Diisopropyl Phthalate	605-45-8		250.29	8.03	3.48	6.14E-07	50	250
4	Diallyl Phthalate	131-17-9		246.26	8.16	3.36	1.17E-07	50	250
5	Di-n-propyl Phthalate	131-16-8		250.3	8.18	3.63	4.03E-07	50	250
6	Diisobutyl Phthalate	84-69-5		278.35	8.76	4.46	6.43E-07	150	750
7	Di-n-butyl Phthalate	84-74-2		278.35	8.63	4.5	1.22E-06	200	1000
8	Bis(2-methoxyethyl) Phthalate	117-82-8		282.29	9.77	1.11	2.81E-13	50	250
9	Diisopentyl Phthalate	605-50-5		306.4	9.5	5.45	1.29E-06	50	250
10	Bis(2-ethoxyethyl) Phthalate	605-54-9		310.34	10.5	2.1	5.11E-13	50	250
11	Diamyl Phthalate	131-18-0		306.4	9.67	5.59	8.89E-07	50	250
12	Bis(4-methylpentyl) Phthalate	146-50-9		334.46	10.2	6.43	2.56E-06	50	250
13	Di-n-hexyl Phthalate	84-75-3		334.4	10.4	6.57	2.57E-05	150	750
14	Butyl Benzyl Phthalate	85-68-7		312.4	10.6	4.83	1.26E-06	150	750
15	Di(2-ethylhextl) Adipate	103-23-1		370.58	11.2	8.12	2.15E-05	150	750
16	Bis(2-butoxyethyl) Phthalate	117-83-7		366.4	12	4.06	2.03E-12	50	250
17	Bis(2-ethylhexyl) Phthalate	117-81-7		390.57	11.7	8.39	2.70E-07	200	1000
17	Diheptyl Phthalate	3648-21-3		362.5	11.1	7.56	3.54E-06	200	1000
18	Dicyclohexyl Phthalate	84-61-7		330.4	11.6	6.2	1.00E-07	200	1000
19	Diphenyl Phthalate	84-62-8		318.32	10	4.1	3.06E-08	50	250
20	Bis(2-ethylhexyl) Isophthalate	137-89-3		390.56	11.7	8.39	2.44E-07	50	250
21	Di-n-octyl Phthalate	117-84-0		390.6	11.9	8.54	2.57E-06	150	750
22	Bis(2-ethylhexyl) Terephthalate	744-45-6		318.3	10	4.1	3.06E-08	150	750
22	Diphenyl Isophthalate	6422-86-2		390.56	11.7	8.39	1.02E-05	50	250
23	Dibenzyl Phthalate	523-31-9		346.4	12.3	5.08	1.02E-11	50	250
24	Bis(2-propylheptyl) Phthalate	53306-54-0		446.66	13.2	10.4	1.55E-07	50	250
25	Di-n-nonyl Phthalate	84-76-4		418.6	####	9.52	1.41E-05	250	1250
27	Didecyl Phthalate	84-77-5		446.66	13.3	9.05	2.81E-06	250	1250
28	Diundecyl Phthalate	3648-20-2		474.7	14.1	11.5	5.60E-05	50	250
33	Tris(2-ethylhexyl) Trimellitate	3319-31-1		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
2	Diethyl Phthalate-d4	93952-12-6		225.26	--	--	--	--	--
7	Di-n-butyl Phthalate-d4	93952-11-5		282.37	--	--	--	--	--
11	Diamyl Phthalate-d4	358730-89-9		310.43	--	--	--	--	--
25	Di-n-nonyl Phthalate-d4	1202865-43-7		422.6	--	--	--	--	--
26	Diisodecyl Phthalate-d4	1346604-79-2		450.69	--	--	--	--	--
27	Di-n-decyl Phthalate-d4	1276197-18-2		450.66	--	--	--	--	--



Representative phthalate structure

Method Validation and Optimization

Oven profile

- Started with from Takeuchi et al (2014)
- Broad peaks for high molecular weight (HMW) compounds
- Series of oven profile experiments
- Reduced hold on 3rd ramp to improve HMW compounds
- 4th ramp was added to improve ditridecyl phthalate
- 5 minute post-run added to reduce carry over
- Final oven profile achieved shape and resolution (15 scans/peak) for all compounds (Table 1)

Table 2. Full GC/MS settings for method

Description	Conditions
Instrument	Agilent GC/MSD 8890/5977, EI mode 70eV
Column	J&W Sicentific DB 5MS 30m x 250µm x 0.25µm, Helium 99.9% at 1.157 mL/min
Temperature	Injection 290 °C MSD 300 °C MS Source 300 °C MS quadripole 180 °C Column 40 °C (2 min hold) to 100 °C at 10 °C/min, to 200 °C at 25 °C/min, to 280 °C at 25 °C/min (3 min hold), to 335 °C at 20 °C/min (7 min hold). Than a 5 min post run at 340 °C. Total run time of 31.75 min
Inlet	Draw Speed 300 µL/min, Eject Speed 6000 µL/min, Pulsed Splitless mode 25psi until 0.5 min, Setpum Purge 3 mL/min, Purge to Split Vent 20 mL/min at 0.45 min
Scan Mass Range	50-600
Injection Volume	1 µL

Calibration curve

- Multi-level calibration curve
  - 250 to 10000 ng/mL & 2000 to 25000 ng/mL
- Average r^2 of 0.995
  - Only 5 quadratic fits needed.

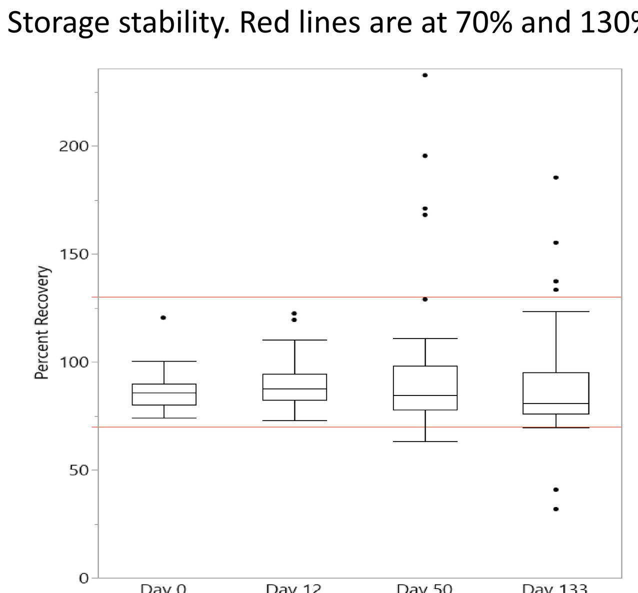
LODs and LOQs

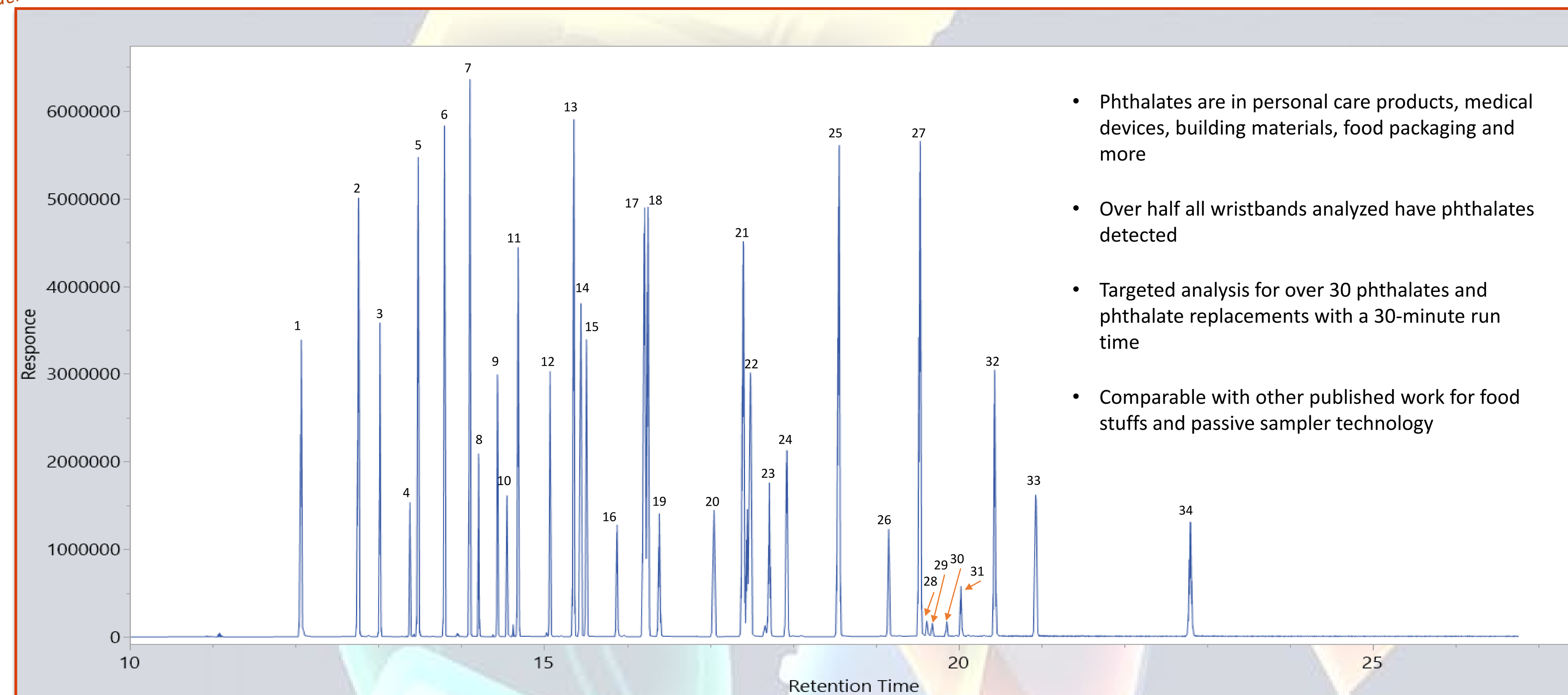
- Ran 15 repetitions over 3 days.
- From interday repetitions, limits of detection (LODs) calculated by standard deviation * t-value (99% confidence interval).
  - Average LOD is 83 ng/mL, from 1.6-231 ng/mL
- Limits of quantitation (LOQs) =LOD *5
  - Average LOQ is 535 ng/µL, from 250-1250 ng/mL
- Average percent recoveries for the targets: 111%
- LODs and LOQs values are shown in Table 1

Storage Stability

- Three aliquots of the same full curve mix were taken four times at 0, 12, 50, 133 days.
- Percent recovery (Figure 2)
  - Average at 0 days: 84%
  - Average at 12 days: 87%
  - Average at 50 days: 94%
  - Average at 133 days: 86%
- All compounds are stable for at least 133 days

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





- Phthalates are in personal care products, medical devices, building materials, food packaging and more
- Over half all wristbands analyzed have phthalates detected
- Targeted analysis for over 30 phthalates and phthalate replacements with a 30-minute run time
- Comparable with other published work for food stuffs and passive sampler technology

Figure 1. Full chromatogram. Target peaks are numbered. See Table 1 for targets names and structures

Method Validation and Optimization (Continued)

SPE

- Sample clean-up used several types of solid phase extraction (SPE).
- C18, florisil, and primary secondary amine (PSA) columns were tested with a 15 pg/µL matrix spike. (Figure 3)
- C18 was effective for smaller MW, however the HMW compounds were not recovered.
  - Florisil percent recovery 90%
  - PSA percent recovery 96%
- PSA had 91% compounds within data quality objectives (DQO's)
  - florisil (77%)
  - C18 (68%)

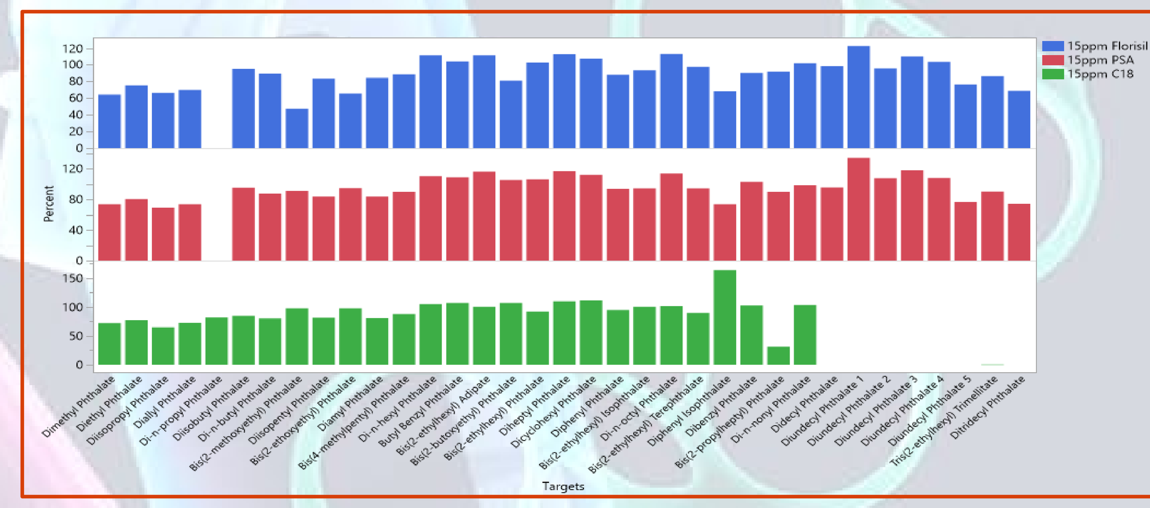


Figure 3: Comparison of the percent recoveries of C18, Florisil, and PSA SPE cartridges. Di-n-propyl phthalate was detected for both Florisil and PSA but 15 ng/µL is over its calibration range of 0.25 ng/µL -10 ng/µL. It is a quadratic fit, and the ratio of compound to ISTD was 5.3 which is over the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

Method Comparison

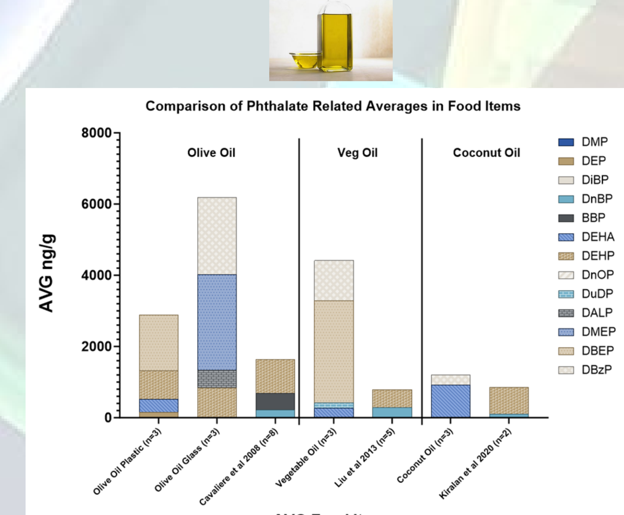
- Of the 11 phthalate method papers identified, the number of phthalates in the method ranged from 6 to 21 with run times of 16 minutes to 40 minutes. (Table 3)
- This method has at least 10 more phthalate compounds

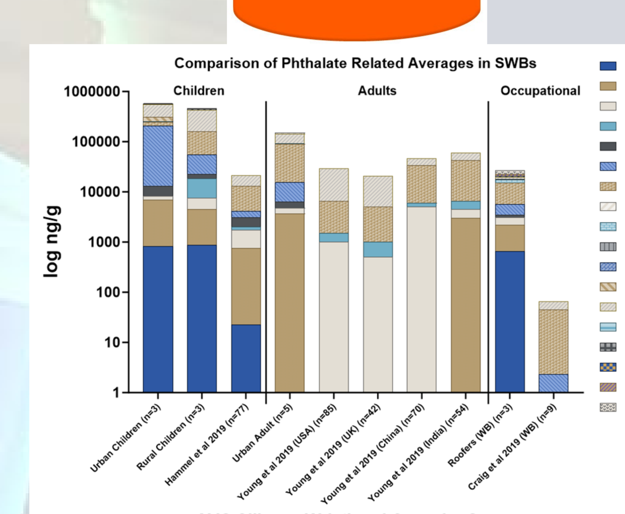
Table 3. Method comparison table comparing this method with 11 other phthalate method papers

Method Paper	This Method	Khan and Jahangir, 2020	Ye et al, 2014	Takeuchi et al, 2014	Raveane et al, 2013	Orecchio et al, 2015	Bradley et al, 2013	Gimeno et al, 2012	Sambolino et al, 2022
Phthalates	29	28	21	19	17	15	15	12	12
Matrixes	PSD (Silicone) and Edible Oil	Toys and Textiles	Meter Dose Inhalers	Indoor air	Wine	Perfumes	Food (Various)	Cosmetics	Fish and Squid
Replacements	2	-	-	15	-	-	-	-	1
Run time	31 min	20 min	30 min	28 min	-	30 min	32 min	27 min	20 min

Real World Samples

- To evaluate the method, we analyzed real world samples such as olive oil and phthalates in silicone wristbands from urban and rural children, pregnant people, and roofers.
- Roofers – detections for HMW compounds seen in building material.
  - Example: trimellitate, ditridecyl phthalate, didecyl phthalate, and diundecyl phthalate
- Wristbands worn for 48 hours by pregnant people had high hits for phthalates associated with personal care products.
  - High as 74860ng/g.
- Rural farm working children showed hits of 1000 – 200,000 ng/g.





Conclusions

- This method is a targeted analysis for many phthalates that can be used in conjunction with new exposure measure technology such as passive samplers.
- To our knowledge, this method quantifies at least four phthalates not commonly looked for in other studies and contains one of the biggest ranges of chemical diversity among the phthalates quantified.
- Together, this methodology and accompanying considerations for extraction provides a detailed framework and foundation for future phthalate methodology.
- As manufacturing patterns change for plasticizers, it will be increasingly important to expand phthalate analyses and especially phthalate replacement analyses in consumer goods or in personal monitoring. Future work should continue to expand along with trends in commerce.

References:

1. Rahman, Mustafizur, and Christopher S Brazel. "The Plasticizer Market: An Assessment of Traditional Plasticizers and Research Trends to Meet New Challenges." *Progress in polymer science* 29, no. 12 (2004): 1223-48.
2. Liang, Yirui, Olivier Caillot, Jianshun Zhang, Jiping Zhu, and Ying Xu. "Large-Scale Chamber Investigation and Simulation of Phthalate Emissions from Vinyl Flooring." *Building and Environment* 89 (2015): 141-49.
3. Bui, Thuy T, Georgios Giovanoulis, Anna Palm Cousins, Jörgen Magnér, Ian T Cousins, and Cynthia A de Wit. "Human Exposure, Hazard and Risk of Alternative Plasticizers to Phthalate Esters." *Science of the Total Environment* 541 (2016): 451-67.
4.*Method 8061a: Phthalate Esters by Gas Chromatography with Electron Capture Detection (Gc/Ecd)*, by Agency, U.S. Environmental Protection. 1996.
5. Guo, Zhiyong, Sui Wang, Danyi Wei, Meili Wang, Huina Zhang, Panpan Gai, and Jing Duan. "Development and Application of a Method for Analysis of Phthalates in Ham Sausages by Solid-Phase Extraction and Gas Chromatography–Mass Spectrometry." *Meat science* 84, no. 3 (2010): 484-90.
6. Gimeno, P., et al. (2012). "Analytical method for the identification and assay of 12 phthalates in cosmetic products: Application of the ISO 12787 international standard "Cosmetics–Analytical methods–Validation criteria for analytical results using chromatographic techniques"." Journal of Chromatography A 1253: 144-153.
7.Feng, S. H., Brian J (2013). Sensitive and Reproducible Phthalate Analysis Using the Agilent 5977A Series GC/MSD, Agilent Technologies, Inc.: 8.
8.Raveane, L., et al. (2013). Phthalates determination in wine and spirits using GC-MS and LC-MS/MS. 3rd MS Food day, Fondazione Edmund Mach.
9. Gimeno, P., et al. (2014). "Identification and quantification of 14 phthalates and 5 non-phthalate plasticizers in PVC medical devices by GC–MS." Journal of Chromatography B 949: 99-108.
10.Ye, X., et al. (2014). "Analysis of 21 phthalate leachables in metered dose inhalers by gas chromatography tandem mass spectrometry." Analytical Methods 6(12): 4083-4089.
11.Orecchio, S., et al. (2015). "Determination of selected phthalates by gas chromatography–mass spectrometry in personal perfumes." Journal of Toxicology and Environmental Health, Part A 78(15): 1008-1018.
12.Dong, C.-D., et al. (2020). "Detecting phthalate esters in sludge particulates from wastewater treatment plants." Journal of Environmental Science and Health, Part A 55(10): 1233-1240.
13.Kartalović, B., et al. (2021). "Detection of microplastic residues-developing a method for phthalates in honey."
14.Sambolino, A., et al. (2022). "Determination of phthalic acid esters and di (2-ethylhexyl) adipate in fish and squid using the ammonium formate version of the QuEChERS method combined with gas chromatography mass spectrometry." Food Chemistry 380: 132174.
15. Giovanoulis, G., et al. (2018). "Multi-pathway human exposure assessment of phthalate esters and DINCH." Environment International 112: 115-126.
16.Jaakkola, J. J. and T. L. Knight (2008). "The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis." Environmental health perspectives 116(7): 845-853.
17. Mariana, Melissa, et al. (2016) "The effects of phthalates in the cardiovascular and reproductive systems: A review." *Environment international* 94: 758-776
18. Engel, S. M. and M. S. Wolff (2013). "Causal Inference Considerations for Endocrine Disruptor Research in Children's Health." Annual Review of Public Health 34(1): 139-158.
19.Takeuchi, S., et al. (2014). "Detection of 34 plasticizers and 25 flame retardants in indoor air from houses in Sapporo, Japan." Science of the Total Environment 491: 28-33.

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