

## **Determination of phthalates – effects of extraction parameters on recovery**

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### **SUMMARY**

Phthalates are ubiquitous contaminants in indoor environments, and are reported to cause adverse health effects. Due to their semi-volatile nature, phthalate samples require a multi-step preparation before quantification with GC-MS. Many different sampling media, extraction procedures, and solvents are utilized in phthalate analytics, while comparisons and justifications for each choice are lacking. In this study, phthalate recoveries were compared from glass fibre filter (GFF), XAD resin + GFF, and polyurethane foam (PUF) after ultrasonic treatment (US), and Soxhlet extraction (SX) with different solvents. The recoveries varied between phthalates, sampling media, solvents, and extraction methods. The overall best performance was obtained using US and toluene (TOL) for GFF; SX and TOL for XAD+GFF; and dichloromethane and US for PUF. The recoveries ranged between 76 and 131 %. Each of these combinations is suitable in terms of recovery, whereas selecting the optimum sampling medium to be used in the air samplings requires further research.

### **INTRODUCTION**

There is a growing concern over a wide variety of chemicals in our living environment and their possible adverse health effects. Among these compounds are phthalates, the worldwide consumption of which is in the range of millions of metric tonnes per year. The phthalates are used as plasticizers in various building materials, furnishings, and household products, such as PVC floor coverings, wallpapers, cosmetics, toys, rain coats, and footwear. Recently, environmental organizations have raised these compounds into people's awareness by detecting these compounds from blood samples of various study groups (politicians, and females in different age groups). Also in the scientific literature, these compounds / their metabolites have been reported in the environment, in indoor air and dust samples, and in humans. This is a consequence of escape from the materials the phthalates were added to by volatilization, leaching or abrasion. The major concern related to phthalates is their suspected toxicity to reproduction [1-2]. Also, asthma and allergies have been suggested to be associated with two phthalates; di(2-ethylhexyl)phthalate (DEHP) and butylbenzylphthalate (BBP), respectively [3].

Research groups around the world have made great efforts in setting up analytical methods for the determination of phthalates from air and dust samples. Due to their semi-volatile nature (Table 1), phthalate samples require a multi-step preparation before quantification with gas chromatography-mass spectrometry (GC-MS). Many different sampling media, extraction procedures, and solvents are utilized in phthalate analytics, while comparisons and justifications for each choice are lacking. Only after large databases on phthalate emissions and concentration levels in the (indoor) environment have been gathered, it is possible to

estimate the risks related to exposure via respiratory tract and skin. Eventually, this will also increase possibilities for material and product development.

The aim of this study was to address the problematic issues of phthalate analysis by comparing phthalate recoveries from glass fibre filter (GFF), XAD resin + GFF, and polyurethane foam (PUF) after ultrasonic treatment (US) and soxhlet extraction (SX) with different solvents.

## METHODS

### Reagent and material preparation

Clean glassware were either rinsed with ethanol, hexane (HX), and toluene (TOL) or baked at 450 °C for 4 h prior to use and wrapped in aluminium foil. Caps and metal equipment were rinsed with acetone or ethanol. Glass fiber filters (GFF,  $\phi$  25 mm, pore size 1  $\mu$ m, Gelman Sciences, Ann Arbor, MI, USA) and cotton wool for filtering liquids were sonicated with dichloromethane (2 $\times$ 20 min). Polyurethane foam (PUF,  $\phi$  18 mm $\times$ 5 cm, Klaus Ziemer GmbH, Langerwehe, Germany) was cleaned using Soxhlet extractor with hexane (16 h). XAD-2 resin (Amberlite XAD-2, Supelco, Bellefonte, PA, USA) was rinsed with hexane and the solvent residue was removed using purified nitrogen. The filter cassettes ( $\phi$  25 mm, Millipore) were washed with purified water and dried in nitrogen flow. The XAD-GFF sampler was prepared right before the experiments by packing 1.7 g of XAD-2 resin on filter cassettes covered with a nylon net filter ( $\phi$  25 mm, pore size 180  $\mu$ m, Millipore, Billerica, MA, USA), and a GFF was placed on the top to cover the XAD-2 resin.

The solvents (HX, TOL, dichloromethane (DCM), acetone, ethanol) used were of p.a. or HPLC grade. Phthalates and the internal standard of analytical grade (Table 1) were supplied by Sigma-Aldrich and Fluka (Steinheim, Germany), and the recovery standard (deuterated phenanthrene) was obtained from Sigma-Aldrich. Stock solutions of the chemicals were prepared in TOL at concentrations from 0.6 to 1.9 mg/ml, and analytical standard solutions were made from appropriate dilutions of these stock solutions (target concentrations were 103-191  $\mu$ g/ml).

Table 1. Properties of phthalates.

Phthalate (abbr.); Cas RN	Formula; Molecular weight (g/mol)	Boiling point (C°); vapour pressure (mmHg) @ 20 °C	Phthalate (abbr.); Cas RN	Formula; Molecular weight (g/mol)	Boiling point (C°); vapour pressure (mmHg) @ 20 °C
dimethyl phthalate ( <b>DMP</b> ); 131-11-3	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub> ; 194,19	284; 0.01 (@ 25 °C)	butyl benzyl phthalate ( <b>BBP</b> ); 85-68-7	C <sub>19</sub> H <sub>20</sub> O <sub>4</sub> ; 312,39	370; 6*10 <sup>-7</sup>
diethyl phthalate ( <b>DEP</b> ); 84-66-2	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub> ; 222,24	298; 7,5*10 <sup>-3</sup>	dicyclohexyl phthalate ( <b>DCHP</b> ) **; 84-61-7	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub> ; 330,42	n.a.; 0,1 (@ 150 °C)
*dipropyl phthalate ( <b>DPP</b> ); 131-16-8	C <sub>14</sub> H <sub>18</sub> O <sub>4</sub> ; 250,29	305; n.a.	n-dioctyl phthalate ( <b>DNOP</b> ); 117-84-0	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub> ; 390,6	220 (390-420); 0.2 (@ 150 °C)
diisobutyl phthalate ( <b>DIBP</b> ); 84-69-5	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub> ; 278,35	320; 7,5*10 <sup>-5</sup>	bis(2-ethylhexyl) phthalate ( <b>DEHP</b> ); 117-81-7	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub> ; 390,6	385; 7,5*10 <sup>-6</sup>
dibutyl phthalate ( <b>DBP</b> ); 84-74-2	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub> ; 278,35	340; <7,5*10 <sup>-5</sup>			

\* = internal standard; \*\* = solid; n.a. = not available.

In preliminary tests of 3 replicate injections to the GC-MS system, the repeatability (relative standard deviation, RSD) of the response of 5 phthalates dissolved in TOL at 8 concentrations ranging between 0.01 and 10 µg/ml was better than that obtained when HX was used as a solvent. The RSDs were <12 % with TOL whereas the RSDs ranged between 17 and 46 % with HX. However, the instrumental detection limits were lower (0.01 µg/ml) with HX for all the 5 phthalates compared to the detection limits obtained with TOL (0.1 µg/ml for 4 phthalates and 0.5 µg/ml for BBP). Each compound showed linearity in the range between 0.1 and 5 µg/ml only with TOL. Thus, TOL was chosen as the solvent for GC-MS analysis in the following experiments.

### **Recovery tests**

For the recovery tests, GFF, XAD-GFF samplers, and PUF plugs were spiked with 2.1-3.8 µg of each phthalate and 2.9 µg of internal standard (DPP), and immediately extracted with one of the tested solvents (TOL, HX, or DCM) using ultrasonic (US) or soxhlet (SX) extraction. However, the PUF plugs were not extracted with TOL because the plugs were found to get deformed and loose their composition in the preliminary tests.

After ultrasonic extraction (2×5 ml×20 min), the samples were filtered through a clean cotton wool wad plugged in a Pasteur pipet. After filtration, samples were evaporated at room temperature (RT) using a gentle stream of nitrogen. In one experiment, when GFF was extracted with US-TOL, nonane was added to the sample to prevent sample losses during solvent evaporation with nitrogen. To the 1 ml volume of each sample in TOL, 0.57 µg of phenanthrene-d10 was added as a recovery standard before GC-MS analysis (Table 2) to measure the recovery of the internal standard.

For comparison, the spiked samples were extracted with 150 ml of one of the tested solvents for 16 h with Soxhlet extractor. Nonane was added onto the samples after Soxhlet extraction, and the volume of the extracts was reduced to <1 ml using rotary evaporator at RT under reduced pressure. The samples were then transferred to test tubes with TOL and the excess solvent was evaporated at RT with nitrogen. The samples (V=1 ml) were transferred to GC vials, and recovery standard added as with the US extraction. Quantification was carried out using GC-MS (Table 2).

The measured phthalate concentrations were compared to the spiked quantities to determine the recoveries (ARR, relative analyte recovery rate). For DPP, the recovery was calculated by comparing the ratio of the peak areas of internal and recovery standards in a sample with the corresponding ratio obtained in the analysis of the analyte standard solution. The criteria for the best extraction method and solution for each sampling material included the uniform distribution of all the determined phthalates around the 100 % optimum recovery.

Table 2. GC-MS conditions for the determination of phthalates.

Gas chromatograph - mass spectrometer	HP 5890 GC + HP 5970 MSD
Column	HP-5MS (28 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, J.W Scientific, USA)
Injection volume	2 µL
Injector temperature program	275 °C (constant flow)
Carrier gas	Helium, 1 ml/min
Purge gas	Helium, 40 ml/min
Oven temperature program	70 °C (2 min), 25 °C/min to 200 °C, 5 °C/min to 280 °C (held 10 min)
Ionization source	Electron impact ionization (70 eV)
Interface temperature	280 °C
Selected ions (at <i>m/z</i> )	DMP:163,194, DEP:149,177,178,222, DPP:149,191,209:, DIBP:149,167,205, DBP:149,205,223, BBP:149,178,206, DCHP:149,167,191,249, DNOP:149, 161,167,279, DEHP:149,167,191, FENd10:188,180

## RESULTS

The extraction recoveries varied with respect to the phthalate, sampling material, solvent, and extraction method (Table 3). The loss of internal standard (ARR<sub>DPP</sub> 12-45 %) was pronounced when samples were extracted with HX, and also in the experiment with PUF-SX-DCM. Thus, the quantitation of the analytes could not be reliably performed in those experiments.

Table 3. The extraction recoveries of phthalates from diverse sampling materials following different extraction methods and extraction solvents.

Sampling material	Extraction		Extraction recovery, %								
	method	solvent	DMP	DEP	DIBP	DBP	BBP	DCHP	DEHP	DNOP	DPP*
<i>Added amount (ng) on each sample</i>			<i>3,41</i>	<i>2,84</i>	<i>2,26</i>	<i>2,71</i>	<i>3,82</i>	<i>2,06</i>	<i>2,80</i>	<i>2,29</i>	<i>2,99</i>
<b>GFF</b>	SX	DCM	55	84	113	132	105	104	368	107	72
	SX	TOL	157	187	127	199	221	208	201	261	57
	US	TOL+n	84	120	101	98	93	106	136	74	79
	US	TOL	57	22	82	104	97	114	156	142	67
	US	DCM	25	63	115	126	151	162	317	225	80
<b>XAD+GFF</b>	SX	TOL	92	97	102	90	107	94	4860	108	69
	SX	DCM	46	75	124	214	97	92	9370	194	72
	US	TOL	72	90	111	119	131	129	187	136	55
	US	DCM	61	82	109	121	132	139	513	179	59
<b>PUF</b>	US	HX	25	63	115	126	151	162	317	225	60
	US	DCM	84	88	94	108	76	131	744	198	67

\*= internal standard; GFF=glass fiber filter; XAD=XAD-resin; PUF=polyurethane foam; US=ultrasonic extraction; SX=Soxhlet extraction; TOL=toluene; DCM=dichloromethane; HX=hexane; n=nonane.

Another problem arose with the obvious contamination caused by DNOP and DEHP indicated as extremely high recoveries in several samples (Table 3). Therefore, the results on DNOP and DEHP were excluded when the best extraction method for each sampler was determined.

The best average recoveries of 6 phthalates and DPP using US were obtained with TOL+nonane for GFF samples (ARR 84-120 %) and with DCM for PUF samples (76-131 %), whereas SX was the best method for XAD+GFF when extracted with TOL (ARR 90-107 %). In these samples, recovery of the internal standard ranged from 67 to 79 % (Table 3). Generally, the recoveries DPP and DMP were the lowest regardless of the extraction parameters. The loss of DPP was controlled, and the recoveries of DMP, DEP, and DIBP were improved when nonane was added to the US sample compared to the extraction without nonane (Table 3). The glass fiber filters should not be extracted with US+DCM, and the SX+DCM combination seems to be the worst choice for the extraction of all the tested phthalates on XAD+GFF. The HX+US was not the optimum extraction combination for PUF samples. However, each of the three last mentioned less-optimal extraction combinations may work satisfactory when only few phthalates are targeted, but with respect to detection of all 6 phthalates at the same time, the recoveries from the samplers can be optimised by choosing other extraction parameters.

## **DISCUSSION**

In the construction and furnishing products, such as floor and wall coverings, wire and cable insulation, and electrical conduits, vinyl plastics are widely used because of their durability, easy installation, and cost-effectiveness. The phthalates make possible the flexible, colour-fast, durable, and low-maintenance qualities of vinyl. Unfortunately phthalates migrate from the products, and have been recognized as ubiquitous contaminants in the environment. Due to their potential impact on human health, standardized procedures are warranted to monitor the exposure levels to these compounds in dwellings and workplaces. The large variety of sampling adsorbents and extraction procedures utilized in the indoor air phthalate analytics emphasises the need for their reciprocal comparison. This study was attempted to evaluate the capacity of two common extraction methods to recover phthalates from three frequently used sampling media.

In this study, three solvents, HX, DCM, and TOL, were compared. The loss of the internal standard in HX experiments indicated that HX may not be the best choice for an extraction solvent. This can also be deduced from the literature, as HX has been used as an extraction solvent only as a mixture with diethyl ether [4-6]. On the other hand, acetone that was not evaluated in the present study might be another suitable solvent and worth comparing [7,8]. The selection of DPP as the internal standard may be justified with the fact that it has not been extensively found in the environmental samples [6,9]. The recoveries of DPP ranging from 67 to 79 % in the best extraction combinations for each sampling material indicate some losses during sample handling, and this may also be the case for DMP and DEP having lower boiling points than DPP (Table 1). Thus, due to similarities in volatility, DPP is a suitable internal standard for at least DMP and DEP. Other researchers have described losses of DBP during evaporation of samples [10] but this was not evident in these experiments. However, it might be advisable to use another internal standard, e.g., a deuterated one, to detect possible losses during sample handling of those phthalates with boiling points in the range of 320-390 °C.

The contamination caused by DNOP and especially DEHP in this study was partly traced to plastic sample holders and a contaminated nonane reservoir. Xie et al. [8] suggest that all solvents be distilled for purification and stored thereafter in full glass bottles. These authors also used active carbon cartridges to filtrate the air entering the Soxhlet apparatus and rotary evaporator, and XAD-2 resin in connection with nitrogen evaporation to catch any impurities

from the nitrogen and the bleeding of tubings. In addition, in the field sampling, the adsorbent holders should be made of glass instead of plastic to avoid contamination thereof. These precautions and collection and analysis of laboratory and field blanks should be routinely performed in the phthalate analytics to control and identify potential contamination sources and to verify the authenticity of the results.

With respect to the recoveries of DMP, DEP, DIBP, DBP, BBP, and DCHP, the overall best performance was obtained using US and TOL for GFF; SX and TOL for XAD+GFF; and DCM and US for PUF. The recoveries ranged between 76 and 131 %. These conclusions are based on single experiments. The recoveries of DNOP and DEHP with the presented extraction procedures should be verified as well. Even though the sampling media and/or the extraction methods and solvents have varied in other studies, the reported recoveries for these compounds have ranged between 78 and 138 % (Table 4) suggesting that these are probably the reasonably obtainable and acceptable recoveries in the phthalate analytics. The recoveries exceeding 100 % may be due to matrix effects: residues of the sampling media that remain in the extract protect the analytes from adsorbing, and thus, allow the injection of a higher concentration into the GC column compared to the concentration injected from a “clean” analyte standard solution prepared by dissolving analytes in a solvent. It might therefore be advisable to prepare quantification standards following the same procedure as with the sample preparation.

Table 4. Phthalate recoveries (%) reported in international studies.

Sampling material [ref]	Extraction method: solvent	Recovery (%) for							
		DMP	DEP	DIBP	DBP	BBP	DCHP	DEHP	DNOP
XAD [7]	elu:DCM	84	91	-	101	90	-	135	92
PUF+XAD [8]	SX: HX+DE (9:1)	138	131	123	117	78	-	99	108
GFF+Empore C18 filter [9]	sta+US: TOL+AC (3:7)	91	100	97	92	90	100	90	-
Quartz fiber filter+ Empore disk C18 filter [11]	US+shake:AC	97	98	98	101	105	102	106	-
Charcoal [12]	US:TOL	-	110	-	120	94	110	98	-
Silica gel [13]	elu:AC	88	91	95	-	99	94	106	-

- = not determined; AC=acetone; DCM=dichloromethane; DE=diethyl ether; ELU=elution; sta=static elution; SX=Soxhlet; TOL=toluene; US=ultrasonic extraction.

In conclusion, the occurrence of phthalates everywhere including laboratory environments causes high risk of contamination during the analysis. This and the lack of standardized methods for sampling and analysis of phthalates in indoor environments pose extra challenges in the phthalate analytics. However, this study showed that various sampling media can be utilized in conjunction with optimum extraction technique and solvent. The analytics enables quantification of phthalates in indoor environments and eventually also offers possibilities to develop products and materials with lower phthalate emissions. The suitability of the sampling media and analytics tested in this study for field sampling warrants further study.

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## **REFERENCES**

1. ATSDR, Agency for toxic substances and disease registry (2002). Toxicological profile for di(2-ethylhexyl)phthalate. Atlanta, USA. 291 p.
2. ATSDR, Agency for toxic substances and disease registry (2001). Toxicological profile for di-n-butyl phthalate. Atlanta, USA. 184 p.
3. Bornehag C-G, Sundell J, Weschler C J, et al. 2004. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. *Environ Health Perspect* 112:1393-1397.
4. Rudel, R A, Camann, D E, Spengler, J D, et al. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ Sci Technol* 37:4543-4552.
5. Adibi, J, Whyatt, R, Camann, D, et al. 2002. Phthalate diester levels in personal air samples during pregnancy in two urban populations, *Proceedings of Indoor Air 2002, Vol 4*, pp. 177-182.
6. Fromme, A, Lahrz, T, Piloty, M, et al. 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin. *Indoor Air* 14:188-195.
7. Zhu, J, Feng, Y, MacDonald, S, et al. 2003. Phthalates in indoor air of Canadian residences, *Proceedings of Healthy Buildings 2003*, pp. 542-547.
8. Xie, Z, Selzer, J, Ebinghaus, R, et al. 2006. Development and validation of a method for the determination of trace alkylphenols and phthalates in the atmosphere. *Anal Chim Acta* 565:198-207.
9. Matsumura, T, Hamada, M, Imanaka, T, Muramatsu, S. 2002. Development of a microanalysis method of phthalate esters in indoor air and its application to practical measurement, *Proceedings of Indoor Air 2002, Vol 4*, pp. 183-187.
10. Clausen P A, Bille R L L, Nilsson T, et al. 2003. Simultaneous extraction of di(2-ethylhexyl) phthalate and non-ionic surfactants from house dust. Concentrations in floor dust from 15 Danish schools. *J Chromatogr A* 986:179-190.
11. Yoshida, T, Matsunaga, I, Oda, H. 2004. Simultaneous determination of semivolatile organic compounds in indoor air by gas chromatography-mass spectrometry after solid-phase extraction. *J Chromatogr A* 1023:255-269.
12. Otake, T, Yoshinaga, J, Yanagisawa, Y. 2001. Analysis of organic esters of plasticizer in indoor air by GC-MS and GC-FPD. *Environ Sci Technol* 35:3099-3102.
13. Watanabe, T. 2001. Determination of dialkyl phthalates in high altitude atmosphere for validation of sampling method using a helicopter. *Bull Environ Contam Toxicol* 66:456-463.