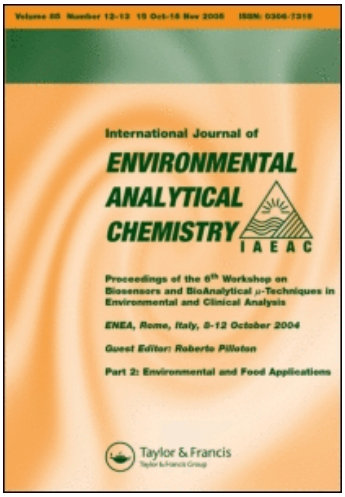


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# An investigation into possible sources of phthalate contamination in the environmental analytical laboratory

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# An investigation into possible sources of phthalate contamination in the environmental analytical laboratory

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A study of common laboratory equipment and components was performed in order to identify sources of contamination of phthalates prior to testing environmental samples for such compounds. A screening study revealed significant leaching from laboratory consumables, such as plastic syringes, pipette tips released maximum leachings of 0.36 $\mu g\,cm^{-2}$ diethylhexyl phthalate (DEHP) and 0.86 $\mu g\,cm^{-2}$ diisononyl phthalate (DINP), plastic filter holders produced maximum leachings of 2.49 $\mu g\,cm^{-2}$ dibutyl phthalate (DBP) from polytetrafluoroethylene (PTFE); specifically 0.61 $\mu g\,cm^{-2}$ DBP from regenerated cellulose and 5.85 $\mu g\,cm^{-2}$ dimethyl phthalate (DMP) from cellulose acetate and Parafilm® leached levels up to 0.50 $\mu g\,cm^{-2}$ DEHP. In addition, a high-temperature bake-out process was found necessary to eliminate quite high levels of two phthalates present in a commercial bulking agent for pressurized liquid extraction.

*Keywords:* Phthalates; Contamination; High-performance liquid chromatography

## 1. Introduction

According to Harris *et al.* [1], Europe produces an estimated 500,000 tonnes of phthalates per annum, with diethylhexyl phthalate (DEHP) being the most commonly used. Dimethyl and dibutyl phthalates (DMP and DBP) have lower molecular weights than DEHP, will partition more from the polymer matrix, and consequently are used to a lesser extent. These lower members of the phthalate series, however, tend to be more soluble in water, so we would still expect to see significant environmental concentrations of these compounds. An estimated 90% of plasticizers manufactured are used in polyvinyl chloride (PVC), and a flexible PVC product will contain between 20 and 50% plasticizer. DEHP accounts for 90% of all plasticizer usage in Europe [2]. Martinnen *et al.* [3] found DEHP to be the most abundant occurring pollutant in

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landfill leachate, with levels of up to $122\,\mu g\,L^{-1}$ DEHP being found and concentrations of other phthalates below $17\,\mu g\,L^{-1}$. Some phthalates have been identified as possible xeno-oestrogens, making them potentially harmful to human reproductive health and possibly playing a role in the development of breast cancer in humans [4].

Exposure to these agents is not confined to landfill runoffs; for instance, Kambia *et al.* [5], demonstrated that a DEHP quantity of $122.95 \pm 33.94$ mg was leached from PVC tubing during a single dialysis session. In a later study, 0.8–2 mg of DEHP was found to leach from PVC bags containing medical fluids [6]. This lead to the suspicion that phthalates could also be leaching from laboratory equipment typically used in environmental analysis. Detections of phthalates have also been reported in diverse media such as reagent-bottle liners [7] and air [8]. Such occurrences have led to the questioning of historical data on phthalate levels by industry trade representatives [9], leading some toxicology researchers to confining their determinations to the metabolites of phthalates [10].

At the ultra-trace level typical of modern environmental analysis, the potential for corruption of sample matrices is exacerbated. Furtmann [11] reported a solid-phase extraction (SPE) method prior to analysis by gas-chromatography mass-selective (GC/MS) detection. In our case, we wished to avoid concerns about the blockade of the $C_{18}$ reverse-phase (RP) pores in the extraction material, so we used an organic modifier with the twin objective of desorbing phthalates from particulate or suspended matter and also ensuring sample preservation. Subsequent to removal of particulates using HPLC filters, we then carried out an analysis on the narrow-bore system described below. EPA Method 8061A [12] recommends GC with electron capture detection (GC/ECD) for analysis of phthalates, but our objective was to develop and validate a sensitive yet simple isocratic HPLC method for determination of trace levels of phthalates without using any sophisticated or expensive equipment such as a mass spectrometer. To date, most studies have been carried out using GC/MS, although there have been a few gradient methods examined. Using an RP gradient HPLC method combined with SPE for environmental analysis, Jen *et al.* [13], developed a method for the separation of dimethyl-, diethyl-, and dibutyl phthalate with respective limits of detection of 12.2, 7.0, and $15.7\,\mu g\,L^{-1}$. Another method developed by De Orsi *et al.* [14], for analysis of phthalates in cosmetics, showed limits of detection (LOD) values for DBP and DEHP and diisonoyl phthalate (DINP) of 0.4, 0.5, and $0.6\,\mu g\,mL^{-1}$, respectively. However, we have developed a method which delivers detection limits following a pre-concentration protocol (magnification factor equal to 2500) of 19.2, 22.0, 28.4, 52.8, and $51.2\,ng\,L^{-1}$ for DMP, DBP, DEHP, DINP, and diisodecyl phthalate (DIDP), respectively.

As no prior testing of waterways had been carried out in the Irish Midlands Shannon Catchment preceding this study, a method was developed to quantify levels of phthalates in the nanogram per litre range. Before testing environmental samples, however, a battery of tests were run to identify the possible sources of contamination in the analytical laboratory so that these sources could be avoided when carrying out sampling, enrichment techniques, and analytical detection. There is a lack of quality control data available on this type of work to date, despite increasing interest in oestrogenic-type contaminants in the environment, and so this article aims to make the reader aware of some potential pitfalls in analysing for certain types of organic substances.

## 2. Experimental

Five phthalates—1,2-benzenedicarboxylic acid dimethyl ester CASRN 131-11-3 (dimethyl phthalate; DMP); 1,2-benzenedicarboxylic acid dibutyl ester CASRN 84-74-2 (dibutyl phthalate; DBP); 1,2-benzenedicarboxylic acid *bis*(2-ethylhexyl) ester CASRN 117-81-7 (diethylhexyl phthalate; DEHP); 1,2-benzenedicarboxylic acid *bis*(3,5,5-trimethylhexyl) ester CASRN 28553-12-0 (diisononyl phthalate; DINP); and 1,2-benzenedicarboxylic acid diisodecyl ester CASRN 26761-40-0 (diisodecyl phthalate; DIDP)—all >99%, were selected for analysis based on their extensive usage. All were analytical grade and were sourced from Sigma-Aldrich. Solubility studies were carried out to determine the respective solubilities according to the OECD [15] shake flask method, and the results over various temperatures showed solubilities in the following order; DMP > DBP > DEHP > DINP > DIDP.

All chromatographic measurements were performed on a modular liquid chromatographic system consisting of a Waters® Autosampler 717, Waters® Pump 510, and Shimadzu LC-6AD Detector. The column used was a Pinnacle™ II Phenyl ($150 \times 2.1$ mm, 5 μm) and an equivalent guard column was also purchased from Restek, Ireland. A Dionex 100 Accelerated Solvent Extractor (purchased from Dionex, UK) with 66-mL extraction cells is used to perform pressurized liquid extractions of solid samples in our laboratory, and its extraction process was also screened for leachables. An isocratic method of elution using an acetonitrile–water (70 : 30) mix pumped at 0.2 mL $min^{-1}$ and ultraviolet detection at 226 nm was devised. The main laboratory components tested are shown in table 1. SPE cartridges and syringe barrels, which were considered initially for the extraction of environmental samples, were also examined as possible sources of contamination.



## 3. Results and discussion

Triplicate blank injections of mobile phase in a vial with no lid and another with a lid in place were run, the purpose being to see if the plastic lid would cause any contamination. This experiment would also have demonstrated the purity and integrity

Table 1. Test materials.

| Test material | Packaging | Supplier |
|---|---|---|
| HPLC vial caps (polyethylene) | Plastic box | Waters® |
| HPLC autosampler | None | Waters® |
| Plastic syringes | Plastic bag | Omnifix® Braun (Germany) |
| Filter holder (three types made from various casings) | Plastic bag | Sartorious<br>Millex® SR Millipore<br>Schleicher & Schuell |
| Pipette tips (polypropylene) | Plastic bag | Plastibrand® |
| Stir bars | Plastic bag | VWR international |
| Winchester lids | Cardboard | Labscan Analytical Ltd |
| Parafilm® | Cardboard | Pechiney Plastic Packaging |
| Tinfoil | Cardboard | AGB |
| Chem tube-hydromatrix | High-density polyethylene | Varian |

of the selected solvent. No contamination was observed. Another set of injections of mobile phase, which had only been in contact with glass, was carried out, and no peaks were observed from this, thus eliminating the autosampler as a possible source of contamination. Further investigations of plastic syringes of the type commonly used for sample handling or transfer and indeed as holders for various phases in SPE work were carried out at room temperature for a period of 30 min. Mobile phase was transferred in triplicate from the same syringe into three separate vials. Another aliquot of mobile phase was transferred into a vial using a glass syringe for comparison. Results showed definite contamination arising from the plastic syringes with the phthalates DMP, DBP, and traces of DEHP being identified. Mobile phase held in glass syringes showed no contamination by comparison.

Three different types of filters were analysed: one brand made from polytetrafluoroethylene (PTFE), another from regenerated cellulose, and another from cellulose acetate. For each different filter, two types of comparisons were made; unfiltered mobile phase with filtered mobile phase and an unfiltered standard mix (5 $\mu g\,mL^{-1}$ of each phthalate) with a filtered standard mix. An unfiltered standard was run in triplicate, and this was used to compare with the filtered standards. This analysis illustrated two types of problems: the first problem was the presence of interferences in the filtered mobile phase, i.e. unwanted peaks probably due to leaching of unknown components in the filter casing; and the second problem was that the filters themselves appeared to be retaining some of the target analytes. The cellulose acetate filters showed the greatest amount of leaching from the filter casing, while the greatest retention (of the standard; data not shown here) occurred with those made from PTFE. The results in table 2 show that the PTFE casing leaches the most in terms of variety, while the cellulose acetate leaches the highest overall amount in terms of concentration. The regenerated cellulose appears to leach the least amount. The decision was thus made to avoid final sample clarification prior to injection and to rely on a guard column to prevent fouling of the analytical column.



Micropipette usage would be put to use during the reconstitution steps of SPE, and so an analysis of two types of micropipette tips (from the same supplier) of the type historically used in our laboratory was carried out. One type was size A (2–100 μL) and the other size B (50–1000 μL). Mobile phase was transferred into vials using a single pipette tip to pipette 200 μL five times into one vial. The analysis showed mild contamination from both pipette types, so their usage was avoided, and instead a 0.2-mL glass pipette was used. The levels of contaminant found (figure 1) were as follows: size A tips leached more into acetonitrile than methanol, but overall the size B pipette tips exhibited more leaching than the size A tips, and the amount of leaching was dependent on the surface area and the solvent with which it came into contact. Methanol leached more from the size B tips, and acetonitrile by comparison appeared to leach less. These two solvents were selected due to their prevalence in reverse-phase HPLC. These differences were slightly surprising; subjectively we found the larger tips to be somewhat more flexible to handle.

Parafilm® (a piece weighing 0.50 g), the SPE frit (8.36 g), solvent Winchester lids (20.75 g) and closures (0.93 g), rubber tubing sectioned from a nitrogen blow-down and drying apparatus (a piece weighing 5.00 g), and stir bars (2.37 g) were subsequently tested. Each component was sonicated in a beaker containing 15 mL of methanol for 30 min, and this 15 mL was then dried down under nitrogen at 37°C and reconstituted with 0.2 mL of solvent. The results were expressed as $\mu g\,cm^{-2}$ exposed to the 15 mL of

Table 2. Amount (μg) of phthalate leached per mL of solvent from the filter casings into mobile phase.

| Phthalate | SPE recovery quantified (%) | Detection limit (absolute) | Quantitation limit | PTFE | Regenerated cellulose | Cellulose acetate |
|---|---|---|---|---|---|---|
| DMP | 60.4 | 0.048 | ≤0.1 | 1.39 | 0.57 | 5.85 |
| DBP | 73.0 | 0.055 | ≤0.1 | 2.49 | 0.61 | 3.30 |
| DEHP | 83.6 | 0.071 | ≤0.1 | 0.12 | < LOD | < LOD |
| DINP | 114.8 | 0.132 | 1.00 | 0.28 | < LOD | < LOD |
| DIDP | 84.0 | 0.128 | 2.00 | 0.20 | < LOD | < LOD |

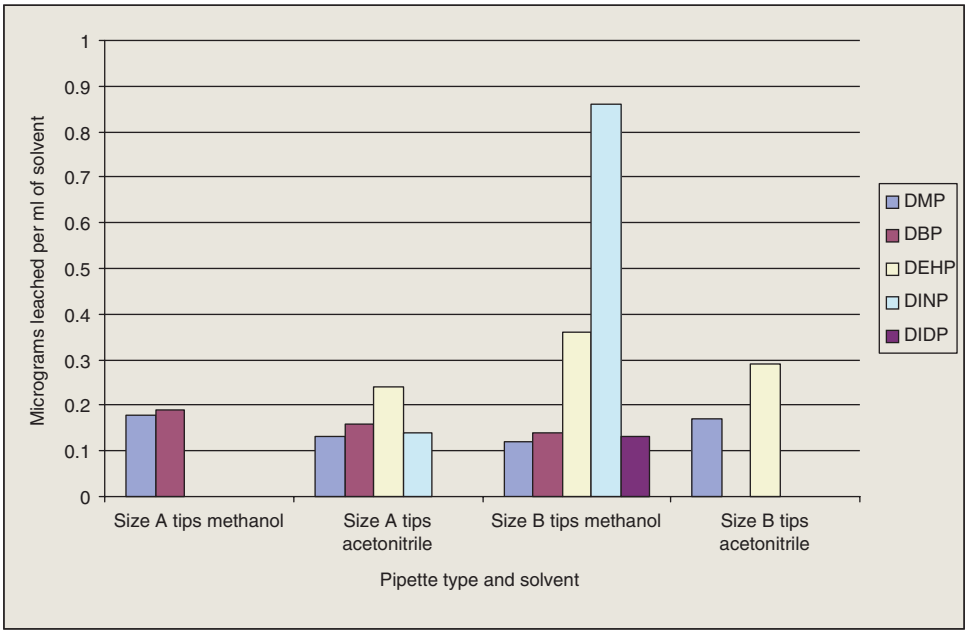


Figure 1. Levels and types of phthalate leaching from the two pipette tip types and the differences in amounts leached using different solvents.

solvent (figure 2), and we can see from the results that DEHP is by far the most omnipresent contaminant in all components.

Further investigations were carried out on the bulking agents used for packing the extraction cells for pressurized liquid extractions of solid environmental samples such as sediments and sludges. A dispersing agent is required in this scenario to prevent aggregation of sample particles and also acts as a filler to reduce the volume of solvent in the final extract. Initial studies were carried out using acid-purified analytical-grade sand purchased from Sigma-Aldrich. The use of sand proved to be unsuitable for trace analysis, as there was severe discoloration of extracts leading to poor chromatographic results. Further experimental work showed that there was contamination arising from Chem Tube-Hydromatrix, which was purchased from Varian through JVA Analytical (Ireland) as an alternative to the sand. It was not possible to obtain this material in a glass container, and so a 'bake-out process' was required to remove the detected contaminants, DEHP and DINP. This involved cremating portions of the hydromatrix in crucibles in the

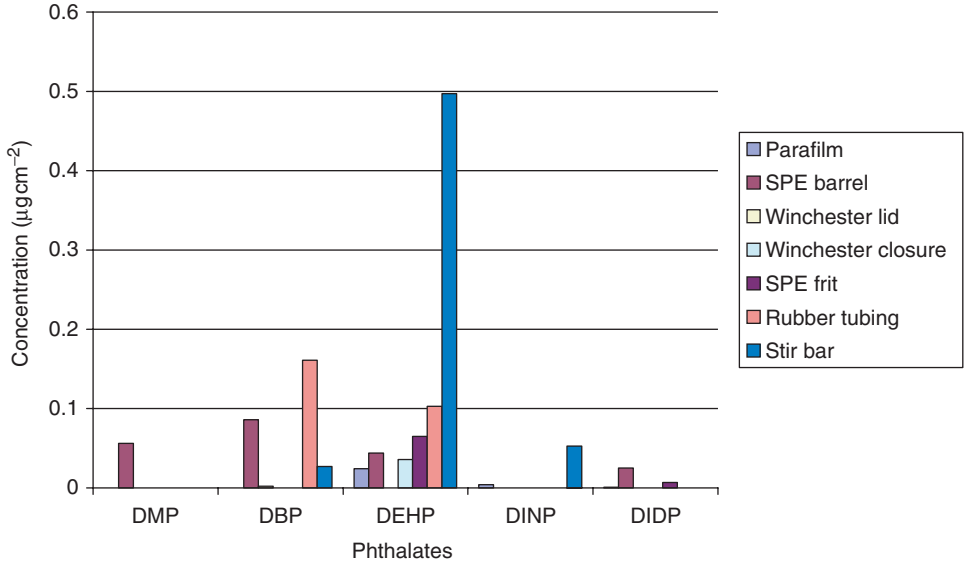


Figure 2. Amounts and type of phthalates leaching from various laboratory components.

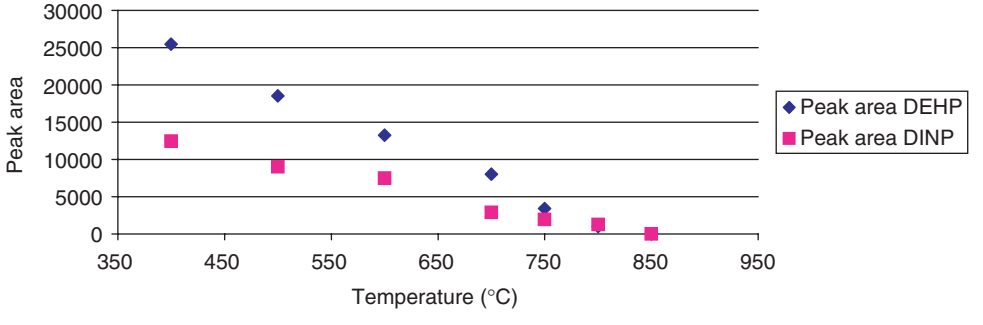


Figure 3. Decreasing levels of phthalate in Hydromatrix with increasing temperature in the bake-out.

furnace at a range of test temperatures to completely eradicate any interfering compounds. Bakings at 400, 500, 600, 700, 750, 800, and 850°C were carried out, and chromatographic results showed that a temperature of 850°C for 24 h was the optimum cremation temperature and time consistent with retaining accurate recoveries of analyte in excess of 85% (figure 3). This further increased the duration of experimental procedures but improved the chromatographic results dramatically.

As a final investigation, environmental samples were taken of river waters from the facing bank of an old, disused landfill facility adjacent to the river (Athlone Lock) and one at a downstream tributary location from a lined and managed facility (Ballydonagh). Samples from each location had contact either with glass only or solely with plastic. The following table (table 3) contrasts the handling of each. DBP, DEHP, and DIDP were identified in the sample matrices. The results (table 4) showed that the use of plastic laboratory components greatly augmented the levels observed.

Table 3. Comparison and contrast of the handling of the environmental sample.

| Sample handling | |
|---|---|
| 500 mL aliquots taken at aforementioned location | |
| 5% methanol organic modifier/preservative added to both | |
| Samples stored on ice and transported immediately to the lab for analysis | |
| Primary filtration followed by SPE carried out using 47 mm $C_{18}$ Empore™ disks (JVA Analytical Ltd, Ireland) | |
| Extracts were dried under a stream of nitrogen at 37°C, and then reconstituted into 200 µL of acetonitrile for subsequent chromatographic analysis | |
| **Sample in contact with glass only** | **Sample in contact with plastic** |
| Pre-cleaned (with acetone) amber glass bottle, rinsed with sample water from the site before filling | Pre-cleaned (with acetone) plastic bottle, rinsed with sample water from the site before filling |
| Taken using inert, stainless steel telescopic sampling pole | Taken using a plastic bottle attachment on the telescopic rod |
| Filled into glass sample bottle | Filled into a plastic sample bottle |
| Glass pipettes used | Plastic pipettes used |
| No syringes used | Syringes used |
| No filters used | Filters used |
| Tinfoil used | Parafilm® used |
| Teflon tubing used | Rubber tubing used |
| Glass rod used | Stir bar used |
| Glass sample collection tube | Plastic sample collection tube |

Table 4. Results from environmental samples ($\mu g\,L^{-1}$) where $n = 3$.

| | Without plastic | | | With plastic | | |
|---|---|---|---|---|---|---|
| *Ballydonagh* | | | | | | |
| Leachate | DBP | DEHP | DINP | DBP | DEHP | DINP |
| Amount found | 3.84 | 6.24 | 0.49 | 4.32 | 7.97 | 0.75 |
| RSD (%) | 3 | 3 | 16 | 13 | 5 | 22 |
| *Athlone Lock* | | | | | | |
| River water | DBP | DEHP | DINP | DBP | DEHP | DINP |
| Amount found | 1.02 | 4.28 | 0.25 | 1.22 | 6.22 | 0.59 |
| RSD (%) | 13 | 2 | 29 | 8 | 11 | 23 |

These results illustrate the extreme importance in eliminating potential contamination sources from experimental work as we see up to 1.94 $(6.22 - 4.28)$ $\mu g\,L^{-1}$ DEHP contributed from contact of the sample with plastic during analysis.

One must consider at this point, however, previous studies that have been carried out. EPA method 8061A4 [12] used GC/ECD analysis, and similar to this method, we also used an octadecyl- silica-bonded membrane disk, as we found contamination when using cartridges or syringe barrels, and although cartridge/barrel decontamination may be carried out, it was not plausible for our analysis, as it would have significantly increased the extraction and analysis times and is found to be only moderately successful [16]. Furthermore, a much greater volume of sample liquid may be analysed using disks, thereby improving analyte detection limits. We also found ethyl acetate to be a better elution solvent with improved recoveries, since the commonly used alternative, acetonitrile, eluted excess humic material and caused discoloration of the extracts. Blount *et al.* [17] analysed for the monoester metabolites of phthalates.

In our case, we wished to monitor and quantify the levels of phthalates entering aquatic environments from landfill leachate and sewage effluents at source before their subsequent ingestion by aquatic organisms as opposed to a human reference where metabolism occurred. Furthermore, Blount *et al.* [17], while analysing for the metabolite, avoided contamination from the parent compound but found difficulty when analysing for DINP, which is a technical mixture containing a mix of isomers. In this scenario, they were only able to choose a monoester metabolite of a single isomer, hence presenting a result which was likely to be an underestimate of DINP exposure.

Another study, carried out by Tienpont *et al.* [16], utilized a liquid–liquid extraction and an automated large volume injection GC/MS analysis in the $\mu g\,L^{-1}$ range. Similar to our study, they carefully selected tools, glassware, and reagents, and carried out frequent blank checks. The isocratic HPLC method in this study had very similar LOD values to those found by Tienpont *et al.* [16], who had method detection limits of 6.0, 80.0, 30.0, 45.0, and 45.0 $ng\,L^{-1}$ for DMP, DBP, DEHP, DINP, and DIDP, respectively, but did not require the use of any technologically advanced and expensive equipment. Furthermore, in the aforementioned study, filtration of wastewater samples to remove particulate matter was carried out, and they then applied thermal desorption GC/MS to isolated particulate which had been removed. In our study, we added an organic modifier to wastewater samples, which was compatible with the SPE procedure and had a double function of desorbing analytes from particulate matter and inhibited microbial activity in the sample, thus preventing metabolism of our analytes. Consequently, our overall aim, which was to develop a simple isocratic method of HPLC detection for ultra-trace levels, is consequently vindicated.



## 4. Conclusions

From the study carried out, significant quantities of phthalates were found to leach from various components commonly found in the environmental analytical laboratory. Items such as plastic syringes, pipette tips, plastic filters, and Parafilm® were thus completely avoided, and glass was used instead. Although the micropipette tips are made from polypropylene, which is supposed to be phthalate-free, phthalates were identified possibly from the plastic packaging or from the plastic pipette box. Nylon filters used in the filtration of mobile phase were shown to be contamination-free. Tinfoil was shown to have no contamination and was used instead of Parafilm®. For drying down samples under nitrogen, rubber tubing was eliminated due to significant levels of DBP and DEHP leaching. Glass pipettes replaced plastic pipettes, and bulking agents for accelerated solvent extraction required intensive pre-treatment prior to sample extraction; consequently, all contaminants were eliminated in subsequent experimental work, which was carried out on real environmental samples.

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## References

[1] C. Harris, P. Henttu, M. Parker, J. Sumpter. *Environ. Health Perspect*., **105**, 1 (1997).
[2] A. Turner, M. Rawling. *Mar. Chem*., **68**, 203 (2000).
[3] S. Marttinen, R. Kettunen, J. Rintala. *Sci. Total Environ*., **301**, 1 (2003).
[4] J. Vikelsoe, M. Thomsen, L. Carlsen. *Sci. Total Environ*., **296**, 105 (2002).
[5] K. Kambia, T. Dine, B. Gressier, S. Bah, A. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand. *Int. J. Pharmaceut*., **262**, 83 (2003).
[6] K. Kambia, T. Dine, B. Gressier, S. Bah, A. Germe, M. Luyckx, C. Brunet, L. Michaud, F. Gottrand. *Int. J. Pharmaceut*., **229**, 139 (2001).
[7] W. Kessler, W. Phokha, G. Csanady, J. Filser. *Arch. Toxicol*., **75**, 62 (2001).
[8] S. Hill, B. Shaw, A. Wu. *Biomed. Chromatogr*., **17**, 250 (2003).
[9] R. McKee. *Environ. Health Perspect*., **112**, A541 (2004).
[10] W. Kessler, W. Numtip, K. Grote, G. Csanady, I. Chahoud, J. Filser. *Toxicol. Appl. Pharmacol*., **195**, 142 (2004).
[11] K. Furtmann. *Anal. Bioanal. Chem*., **384**, 291 (1994).
[12] EPA Method 8061A. December (1996).
[13] J. Jen, T. Lui. *J. Chromatogr. A*, **1130**, 28 (2006).
[14] D. De Orsi, L. Gagliardi, R. Porra, S. Berri, P. Chimenti, A. Granese, I. Carpani, D. Tonelli. *Anal. Chim. Acta*, **555**, 241 (2006).
[15] Organization for Economic Cooperation and Development. *Guidelines for Testing Chemicals. Guideline 105, Water Solubility*. Organization for Economic Cooperation and Development, Paris, 1981.
[16] B. Tienpont, F. David, E. Dewulf, P. Sandra. *Chromatographia*, **61**, 365 (2005).
[17] B. Blount, M. Silvia, S. Caudill, L. Needham, J. Pirkie, E. Sampson, G. Lucier, R. Jackson, J. Brock. *Environ. Health Perspect*., **108**, 10 (2000).