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# Determination of nonylphenol and octylphenol in paper by microwave-assisted extraction coupled to headspace solid-phase microextraction and gas chromatography–mass spectrometry

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**Abstract**

A novel and simple method for the determination of active endocrine disrupter compounds (octylphenol OP, and nonylphenol NP) in paper using microwave-assisted extraction (MAE) and headspace solid-phase microextraction, coupled with gas chromatography–mass spectrometry has been developed. Parameters affecting the efficiency in the MAE process such as exposure time and extraction solvent were studied in order to determine operating conditions. The optimised method was linear over the range studied (1.25–125 $\mu g\,kg^{-1}$ for OP and 9.50–950 $\mu g\,kg^{-1}$ for NP) and showed good level of precision, with a RSD lower than 10% and detection limits at 0.10 and 4.56 $\mu g\,kg^{-1}$ for OP and NP, respectively. The results obtained from six different types of paper revealed the presence of the target compounds in all samples analysed, at levels ranging between 3 and 211 $\mu g\,kg^{-1}$.


*Keywords:* Extraction methods; Alkyl phenols; Phenols; Octylphenol; Nonylphenol; Paper

## 1. Introduction

Alkylphenol ethoxylates (APEs) are widely used non-ionic chemicals [1]. Because of their surfactant properties, APE-containing products have many industrial, commercial and household uses. Nevertheless, in the environment, there are two possible degradation ways for these compounds: (i) an aerobic degradation process, where the APE ethoxy chain is oxidised, leading to alkylphenol ethoxy carboxylates and (ii) an anaerobic degradation of APEs, where the ethoxy chain is cleaved, with the formation of octylphenol (OP) and nonylphenol (NP) that are persistent and ubiquitous environmental pollutants with endocrine disrupting properties [2,3]. So, in Europe, use of APEs has been restricted in industrial applications. Their replacement by other less toxic compounds, such as alcohol ethoxylates, has not been totally carried out because of the excellent technical performances of APEs and their low production costs [3].

In paper industries, APEs are used in many additives along the papermaking process. They are introduced into the paper machine as drainage aids, felt washing additives, anti-foaming agents and de-inking agents in the production of recycled paper [4]. As a result of these production processes, OP and NP have been identified in liquid effluents from paper industries [5]. Paper contributes to 48% of all packaging materials and chemical residues may be assumed as a pollution risk for the Environment. As an example, Triantafyllou et al. [6] recently reported the migration of a wide variety of compounds, such as acetophenone, naphthalene and benzophenone from the packaging into the food. In this way, the European Union has recently proposed limitations on the concentration of several potential migrants in recycled paper and board [7], taking into account the quantities of each possible contaminant that could endanger human health [8].

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Nowadays, extraction of migrants is one of the critical points in paper analysis. For example, ultrasonication allowed efficient extraction of benzophenone, phthalates or pentachlorophenol [6,9], whereas supercritical fluid extraction was used to increase the recovery [8]. Microwave-assisted extraction (MAE) is a rather new technique which has been applied to the extraction of organic compounds from different matrices (soil, seeds, sediments,. . .) including the extraction of pollutants from environmental samples [10–12]. In MAE, analytes are extracted from the matrix and transferred into a solution. Then, this solution can be sampled by solid-phase microextraction (SPME), another recent technique proposed by Pawliszyn in the early 1990's and developed by his group and many others in the world [13]. Such collected and concentrated samples are then analysed by gas chromatography–mass spectrometry (GC–MS) [14–16]. Recently, this method has been successfully applied to the determination of pesticide residues in strawberries [17] and appeared to be efficient for pyrethroid compounds, particularly resistant to common vegetal extraction methodologies [18]. In all these studies, the coupling of MAE and SPME proved to be more efficient compared to other traditional extraction methods such as Soxhlet and sonication [10], shortening sample preparation and reducing solvent use.

This paper aims to describe an analytical method using MAE followed by headspace (HS) SPME coupled to GC–MS for unequivocal determination of OP and NP in paper, and to exemplify its use with six types of common life paper samples.

## 2. Experimental

### 2.1. Chemicals and reagents

All standards and chemicals were of the best commercially available purity. Samples of pure 4-*tert*-octylphenol (OP) and a mixture of different isomers of 4-nonylphenol (NP), were obtained from Aldrich (Milwakee, WI, USA).

Sodium chloride (NaCl) was supplied by Riedel-de Haën (Seelze-Hanover, Germany) and hydrochloric acid 35% was supplied by Prolabo (Paris, France).

### 2.2. Samples

The study involved the analysis of six different types of paper: white printing paper; laboratory tissue paper; newspaper (only the part without ink was analysed); laboratory filter paper; cardboard and recycled paper provided by a recycling paper mill.

### 2.3. Analytical procedure

The analytical protocol for the paper analysis consisted in three different steps: (i) microwave extraction of the paper in order to enhance the transfer of the analytes from the paper into water, (ii) extraction of the analytes from the aqueous solution by using HS-SPME and (iii) detection and quantification by GC–MS. A scheme of this analytical protocol is showed in Fig. 1.

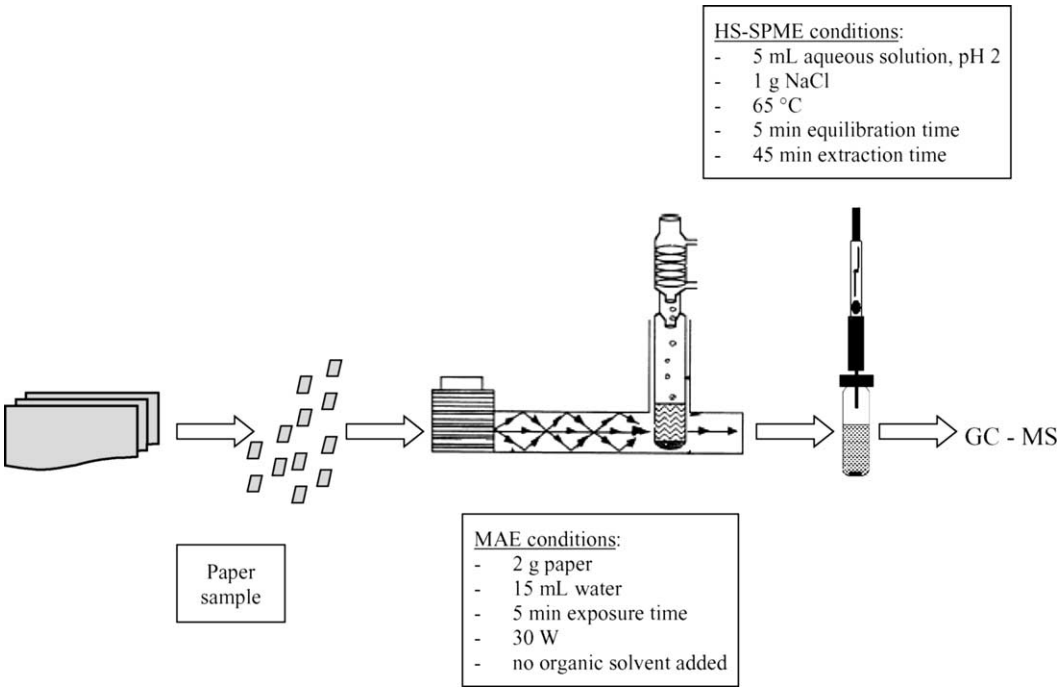


Fig. 1. Analytical procedure for the analysis of paper.

*2.3.1. Microwave-assisted extraction*

A MAE Soxwave Map, (Prolabo, France) was used. Samples (2 g) of cut paper (approximately 0.5 $cm^2$ squares), were placed in the quartz tube containing 15 mL of water. The tube was then put in the microwave oven and irradiated for different duration at 30 W (lowest available power). Under microwave effect, the sample temperature increased up to about 65 °C. Vapour losses were prevented by the presence of a reflux system on the top of the extraction vessel. The aqueous solution was then collected, cooled down to room temperature and decanted before being used in the SPME procedure. In order to obtain the highest signal for both target compounds, operating parameters like sample weight, solvent volume, exposure time and addition of a co-solvent (methanol), were optimised from cardboard samples. Cardboard was selected because it appeared to be an obviously contaminated matrix from preliminary experiments.

*2.3.2. Headspace-solid phase micro-extraction*

The automated extraction of NP and OP was performed using a StableFlex 2 cm–50/30 μm divinylbenzene–Carboxen–polydimethylsiloxane (DVB–CAR–PDMS) SPME fibre, from Supelco (St. Quentin Fallavier, France) and a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). Before use, the fibre was conditioned in a heated GC injection port under helium flow, according to the manufacturer's instruction. The extraction method is adapted from methods previously described in the literature [1,17–20]. Briefly, the analysis was performed in a 10 mL PTFE-lined screw-capped vial containing 5 mL of the MAE aqueous solution. NaCl was added to samples up to a concentration of 200 g $L^{-1}$, that is close to the saturated concentration of NaCl in water, and the pH was adjusted to 2 using HCl (19, 20). The equilibration and extraction time were set at 5 and 45 min, respectively and the extraction temperature was 65 °C, with constant stirring (800 rpm). Finally, the SPME fibre was withdrawn from the vial and directly introduced into the GC–MS injector for thermal desorption.

*2.3.3. GC–MS*

GC–MS analysis was performed with a Trace 2000 gas chromatograph coupled with an ion trap mass spectrometer, (ThermoQuest, Les Ullis, France). A MDN-5S fused silica capillary column (30 m × 0.25 mm i.d. with 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA) was programmed from 60 °C (keeping this temperature for 1 min) to 90 °C at 12 °C $min^{-1}$ and from 90 to 280 °C at 6 °C $min^{-1}$ (5 min). The total analysis time was 40 min. Helium was used as the carrier gas at constant flow rate of 1.2 ml $min^{-1}$. The ion trap mass spectrometer was operated in the electron impact mode (EI), scanning masses from $m/z = 100$ to $m/z = 250$ at 1 scan $s^{-1}$. The ion source and transfer line temperatures were held at 250 and 275 °C, respectively.

OP was characterised by comparison with the retention time of the standard (16.6 min) and the corresponding mass spectrum. Mixture of NP isomers was characterised by peaks with retention times between 18.4 and 19.4 min and showing fragments ions at *m/z* 107, 135 and 149, as suggested by the analysis of the reference standard mixture and cited papers (1, 19, 24).

*2.3.4. Quantification*

The most abundant ions were used for quantification (*m/z* 135 for OP and *m/z* 107, 135 and 149 for NP). Calibration curves were performed from spiked laboratory filter paper samples and observed signals were corrected for signals found in unspiked samples of the same paper. Three spiking procedures were tested for optimising the extraction efficiency. In the first one, 100 μL of an OP and NP mixed solution at 10 μg $L^{-1}$ in ethyl acetate were dropped to the 2 g cut cardboard sample that was directly MAE–SPME treated 1 h latter. In the second and third procedures, the same type of cardboard samples were immersed into 1 mL of OP and NP solutions at 1 μg $L^{-1}$ in acetone and ethylacetate, respectively. In the two latter cases, samples were then left overnight at room temperature for complete solvent evaporation.

## 3. Results and discussion

### 3.1. MAE parameters

The effect of microwave exposure time was investigated. Results obtained by treating cardboard samples for 0, 1, 3, 5, 7 and 9 min are shown in Fig. 2A. Signals observed for both compounds increased to a relative maximum at 5 min (179% for OP and 239% for NP). A decrease was then noticed for longer exposure times, possibly due to degradation of the compounds resulting from higher temperature. This profile was already observed for other compounds such as pesticides [17,18,20] or chlorophenols [21].

Selection of a suitable solvent in the MAE process implies that a compromise has to be found between the microwave-absorbing properties of the solvent, the interaction of the solvent with the matrix, and the solubility of the analytes in the solvent. In addition, the compatibility between the MAE extraction solvent and the SPME feature remained the most important point to consider. So, for this study, water appeared as the most convenient solvent for the microwave extraction [11,22,23] although it could be mixed with less polar organic solvent in order to better dissolve the analytes and absorb microwave energy. In order to study the effect of such a solvent addition, different volumes (ranging from 0 to 1 mL) of methanol chosen as a typical organic co-solvent, were added to the cardboard samples and completed to 15 mL with water. Microwave extraction was then applied for 5 min and the temperature increased rapidly to the boiling point of the solution. Corresponding results are shown in Fig. 2B. In all the cases, addition of methanol caused a decrease in the signal. After adding 1 mL of methanol, the signal for OP was the half of this obtained without methanol. The effect was less important

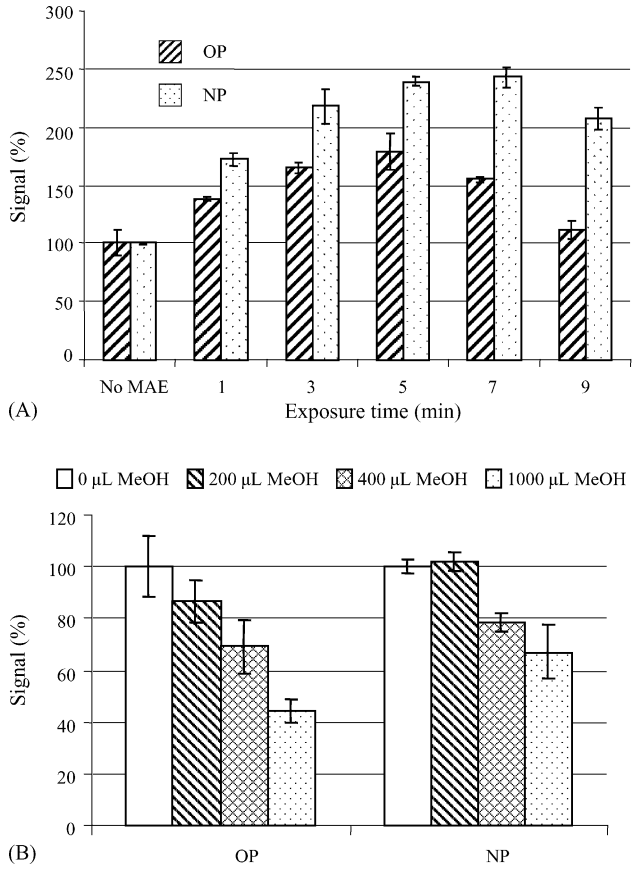


Fig. 2. (A) Effect of microwave irradiation time at 30 W on the signal obtained by SPME–GC–MS from cardboard in water (signals normalised to those obtained with no microwave). (B) Effect of adding different volumes of methanol to the water before microwave extraction.

for NP but the signal was approximately 30% reduced. In fact, this effect may be mainly explained by considering the HS-SPME procedure: the addition of an organic solvent induced a decrease in the polarity of the water solution and, as a result, an increase of the solubility of OP and NP in this solution. As a consequence, the transfer of these very compounds from paper to the aqueous solution may increase during MAE, meanwhile, it may decrease in the headspace for the same reason.

### 3.2. Spiking procedure and calibration curves

Results relative to the different spiking protocols indicated in the quantification section, have been reported in Fig. 3. Although the same amount of spiking materials was used in the three experiments, the signal obtained by using 1 mL of the diluted ethylacetate spiking solution was 20–25% higher for OP and slightly higher for NP, compared to those obtained with an acetone solution or by dropping directly the spiking standard solution to the paper sample. So, the calibration curve samples were prepared by adding 1 mL of the ethylacetate solution of OP and NP at different concentrations into a 25 mL beaker containing 2 g of cut paper (Fig. 4). Performances of

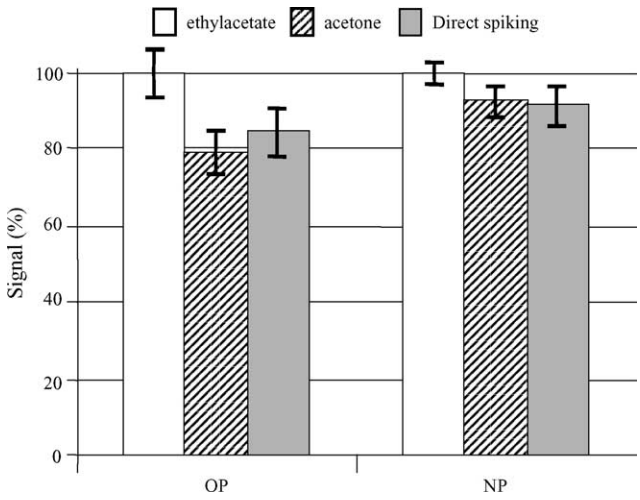


Fig. 3. Different conditions for spiking paper samples: (i) standards in diluted ethyl acetate solution, (ii) standards in diluted acetone solution, (iii) direct spiking with a concentrated standard solution in ethylacetate.

the method were indicated in Table 1 with correlation coefficients ($r^2$) higher than 0.99 for both compounds in the range 1.25–125 μg kg$^{-1}$ for OP and 9.5–950 125 μg kg$^{-1}$ for NP. Repeatability from five consecutive injections and within laboratory reproducibility from six injections in different days led to RSD values below 5 and 10%, respectively. Limits of detection (LODs) of the method, defined as the minimum amount of analyte which produced a peak with a signal to noise ratio of 3, was measured for each compound. Obtained values were 0.10 and 4.56 μg kg$^{-1}$ for both compounds, respectively.

### 3.3. Analysis of paper

The two studied compounds were detected in all the six samples from different origins. The full scan total ion chromatogram relative to filter paper spiked at the concentration of 37.5 μg kg$^{-1}$for OP and 285 μg kg$^{-1}$ for NP, was indicated in Fig. 5A whereas expansion fragmentograms (*m*/*z* 135 for OP and NP) corresponding to cardboard, unspiked filter paper, newspaper, white printing paper, recycled paper,

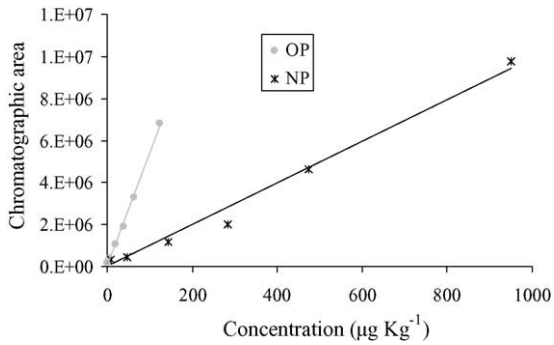


Fig. 4. Calibration curves relative to OP and NP concentrations from filter paper spiked samples. Observed signals were corrected for values found from unspiked samples.

Table 1
Results of method performance: linear range, calibration curve, $r^2$, repeatability, within laboratory reproducibility, RSD (%), and limits of detection (LOD) and quantification (LOQ)

| Compound | Linear range ($\mu g\,kg^{-1}$) | Calibration curve | $r^2$ | Repeatability (RSD%) | Laboratory reproduction (RSD%) | LOD ($\mu g\,kg^{-1}$) | LOQ ($\mu g\,kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| OP | 1.25–123 | $y = 54524x$ | 0.998 | 5 | 7 | 0.10 | 0.33 |
| NP | 9.5–950 | $y = 9953x$ | 0.991 | 6 | 10 | 4.56 | 15.2 |

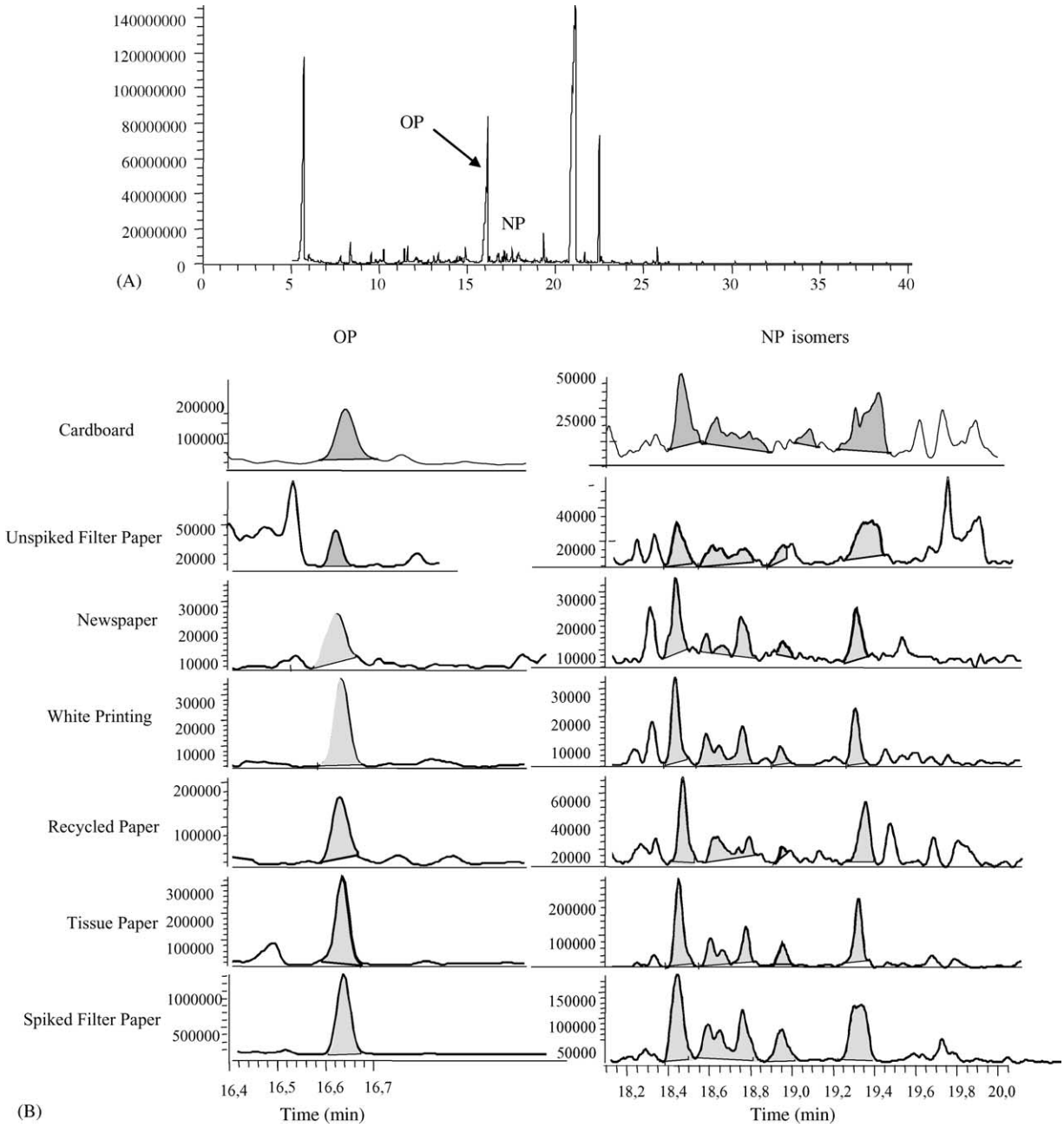


Fig. 5. (A) Full scan total ion chromatogram from a filter paper sample spiked at the concentration of 37.5 $\mu g\,kg^{-1}$ for OP and 285 $\mu g\,kg^{-1}$ for NP. Time scale in min. (B). Expansions of fragmentograms (*m/z* 135) obtained by GC–MS for OP and NP from unspiked filter paper, newspaper, printing paper, recycled paper, tissue paper and spiked filter paper (37.5 $\mu g\,kg^{-1}$ for OP and 285 $\mu g\,kg^{-1}$ for NP).

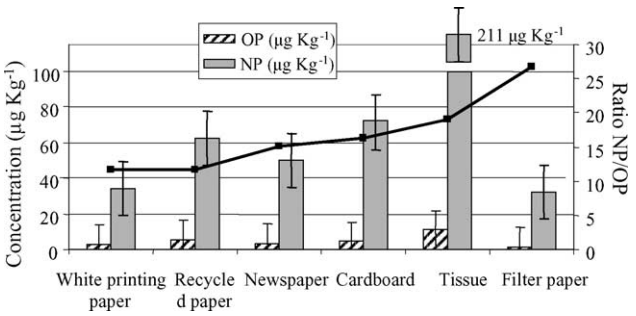


Fig. 6. Levels (in $\mu g\,kg^{-1}$) of target compounds in analysed papers samples. Corresponding observed NP/OP ratios scaled on the right hand axis.

tissue paper and spiked filter paper were given in Fig. 5B. The concentrations found in the different types of paper are shown in Fig. 6. Levels of OP and NP oscillated between 3 and 211 $\mu g\,kg^{-1}$, being found the highest levels in laboratory tissue paper. Similar levels of both compounds were found in recycled paper, cardboard and newspaper. In printing paper, made from fresh cut wood and chlorinated water, the levels found were 50% lower compared with those in recycled paper and newspaper. The sample with the lowest levels was this of filter paper, even if the NP level was similar to the level found in printing paper.

Corresponding ratios between NP and OP concentrations are also represented in Fig. 6 (black plots and right axis). Observed values are ranging from 10 to 25, at a much higher level than those considered as characteristic of environmental water samples that are generally lower than 5 [24,25]. This must be due to the high concentration of NP found in all samples, indicating that NP is probably better adsorbed than OP to the paper matrices. In fact, the comparison of the log $K_{ow}$ values ($K_{ow}$ = octanol – water partition coefficient) relative to both compounds (4.1 for OP and 4.5 for NP, [26]), is in agreement with this last result as long as NP, the more polar substrate, must adsorb more strongly to the relatively polar matrix that is the cellulose fibre.

## 4. Conclusions

The method proposed in this study includes a microwave assisted extraction using only water, coupled with a HS-SPME and GC–MS determination. The procedure is simple and allows a rapid assessment of OP and NP concentrations directly from paper samples. Compared to classical methods, this approach provides an efficient analytical tool, with a high level of sensitivity and detection limits at the low $\mu g\,kg^{-1}$ level. Compared to traditional methods, it also reduces and simplifies the extraction procedure and cancels the use of organic solvents. The contamination levels found for both compounds in six different types of papers, clearly demonstrate the toxicological risk represented by these two compounds for the environment.

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