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Utilisation of an enzyme-linked immunosorbent assay (ELISA) for determination of alkylphenols in various environmental matrices. Comparison with LC–MS/MS method

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ABSTRACT

Among the wide range of substances discharged continuously in the environment, alkylphenols became a major focus of environmental research in the last decades, as it was found that they possess endocrine disrupting properties. Knowledge about the occurrence and levels of alkylphenols in environment is critical for the risk assessment of these compounds on both ecosystem and human health. However, the analysis of traces of alkylphenols in environmental matrices is a very difficult task, and the suitable methods involve generally an extraction followed by an extensive sample clean-up before detection, steps often time-consuming and costly.

In order to reduce the analysis time, obtain a high throughput of analysis and thus improve work efficiency, the objective of the present study is to investigate the use of immunochemical technique (ELISA) for the determination of nonylphenol and octylphenol in soils and various kinds of water. To our knowledge, this is the first time that the determination of alkylphenols in soil using immunoassay technique is described. A methodology is developed, based on the combination of a single preparation step and the use of a simply ELISA kit. The performances of the method are compared with LC–MS/MS, considered as reference. The developed procedure offers the sensitivity and selectivity necessary for the detection of the target alkylphenols in the ng/g or ng/L range, and is successfully applied to the analysis of several samples. Results indicate that alkylphenols are quantified with concentrations in the same order than LC–MS/MS, meaning that ELISA may be useful not only in screening the samples and get a positive/negative response, but also it allows a good approximation of the concentrations.

1. Introduction

Alkylphenol ethoxylates are non-ionic surfactants widely employed in detergents, emulsifying agents, dispersing agents, and pesticide formulations. The presence of alkylphenol ethoxylates in environment has become a cause for increasing concern in recent years because these chemicals are degraded during activated sludge treatment in wastewater treatment plants in alkylphenols [1,2], known for their endocrine disrupting functions. Indeed it was found that alkylphenols possess the ability to mimic natural hormones by interacting with the estrogen receptor [3,4]. Since the early work describing the presence of alkylphenols in wastewater from the early 80s, many reports have dealt with the evaluation of occurrence and concentration of alkylphenols in effluents and receiving waters worldwide [2,5–8]. As octylphenol and nonylphenol ethoxylates are two of the most common surfactants used, octylphenol (OP) and nonylphenol (NP) were two alkylphenols which had attracted considerable attention. NP and OP are included in the priority list of the framework directive [9]. The other route for introducing alkylphenols into environment is sewage sludge. In Europe, the use of sewage sludge from wastewater treatment plants as organic amendment has become usual. This practice allows not only an enrichment of soils poor in organic matter, but it also represents an interesting way for disposal. It was expected that from the year 2005 on, about 50% of the sludge produced at wastewater treatment plants in EU would be used in agriculture [10], with proportions differing among countries, from 10% in Greece up to 65% in Denmark [11]. A transfer of alkylphenols from sludge to amended soil is possible. Part of alkylphenols in soil is then available to organisms and plants, so likely to have an impact on living organisms [12] or a further transport into aquatic compartments, both surface and groundwaters is also possible. In consequence, knowledge about the occurrence and levels of alkylphenols in soil is critical for the risk assessment of these compounds on both ecosystem and human health.

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Liquid chromatography (LC) coupled to mass spectrometry (MS) detection systems are now the commonly used methods to identify and quantify surfactants in environmental matrices [13–15]. Analysis of traces of alkylphenols in complex environmental matrices such as effluents of wastewater treatment plant or soils is a very difficult task, as the difficulty of the analysis is enhanced by the existence of matrix effect that reduces the sensitivity. Suitable methods involve efficient extraction followed by extensive sample clean-up before detection, steps often time-consuming and costly. As a consequence, the sample throughput is generally low and there is a relatively long period between sample collection and analysis result. Recently, the application of ultra-high-pressure liquid chromatography (UPLC) coupled to tandem mass spectrometry to investigate alkylphenols pollution in soil increased the analysis speed which is very important for large-scale environmental survey [16].

In order to reduce the analysis time, obtain a high throughput of analysis and thus improve work efficiency, the use of immunochemical techniques could be interesting. Indeed extensive clean-up are typically excluded [17] and this utilisation offers a number of advantages for environmental analyses such as low limits of detection with sensitivity in many cases comparable to chromatographic techniques and cost-effective detection [18]. Among immunochemical techniques, ELISA (enzyme-linked immunosorbent assay) is particularly selective and simple to handle, and a wide range of organic pollutants, mainly pesticides have been successfully analysed in waters [18] and less frequently in soils [19]. Moreover, ELISA allows reaching low limits of detection with sensitivity in many cases comparable to chromatographic techniques [18]. Concerning the analysis of alkylphenols, several authors have published original reports dealing with the determination of alkylphenol carboxylates, alkylphenols polyethoxylates, and/or alkylphenols, based on the development of monoclonal [20] or polyclonal antibodies [21,22]. ELISA was presented as a convenient tool for screening alkylphenols in water samples.

To our knowledge, the determination of OP and NP in soil using immunoassay technique had not been previously described. The objective of the present study was to investigate the use of ELISA with a monoclonal antibody for the determination of NP and OP in soils and in various aqueous environmental matrices including sewage, surface and groundwaters. To limit the time of sample preparation, simple extraction methods have been implemented. The performance of the method was compared with LC–MS/MS. Due to its sensitivity and especially its selectivity, LC–MS/MS method using a triple quadrupole mass spectrometer was developed for this study and considered as a technique of reference, for the analyses of alkylphenols.

2. Materials and methods

2.1. Chemicals

Nonylphenol (NP) and octylphenol (OP) were purchased from Sigma–Aldrich. Their degree of purity was at least 99% and they were used without any further purification. Individual stock solutions at 250 mg/L were prepared in methanol and stored at −23 °C. Composite working solutions were prepared by diluting suitable aliquots of each individual solution.

Methanol, acetonitrile, acetone, dichloromethane, ethyl acetate and isopropanol were HPLC–MS grade and were obtained from Sigma–Aldrich as well as ammonium acetate. Pure water was obtained from a MilliQ device from Millipore.

2.2. Soil and water samples collection

The soil used in the methods development and validation was collected in an agricultural field from the upper 0–20 cm of the horizon. Three points were sampled, collected and homogenized in plastic containers, a few hours after collection. After homogenization, the soil was not kept in contact with plastic matter, to avoid any additional contamination. Soil was air dried and sieved through a 4 mm sieve before use.

The 6 soil samples analysed were collected from the surface of a garden located in urban area (soil 1), from the surface of untreated agricultural field (soil 2), from an agricultural soil fertilized with municipal sewage sludge 3 weeks before, at 10 cm depth (soil 3) and 40 cm depth (soil 4) and soil located in an industrial area at 10 cm depth (soil 5) and 40 cm depth (soil 6). Each soil sample is the mixture of three sampling points, collected in amber glass vessels from March to July 2010 in several areas of the Rhône-Alpes region (France) and stored at −23 °C until use.

The 4 grab water samples (groundwater, canal, lake and effluents of wastewater treatment plant) were collected in amber glass bottles. Suspended particle matter of the effluents was removed by filtration using a Millipore YT30 142 HW device, successively through a glass fiber filter and 8 μm and 0.45 μm nitrocellulose filters from Millipore. Extraction was performed within 4 h after sampling.

2.3. Soil and water samples extraction

For the development of the extraction method, 2 g of soil were spiked with 4 mL of a standard mixture (25 μg/L in methanol) of the analytes, and air dried 24 h before the extraction.

The soil was extracted in a Branson 2210 Ultrasonic bath. The extraction consisted in placing the soil with 8 mL of dichloromethane in ultrasonic bath for 20 min. The tube was then centrifuged at 5000 × *g* for 4 min and the supernatant was recovered. These steps were repeated twice, then the supernatants were collected and evaporated to dryness. Dry extracts were reconstituted in 400 μL of the ELISA buffer solution (phosphate buffer 0.3%, sodium chloride 0.8%, stabilizer 1%, pH 7.2) or in 400 μL MeOH/isopropanol (90/10, v/v), respectively for ELISA and LC–MS/MS analyses.

For water samples, extraction was based on a slightly modified method previously developed for the analysis of priority substances in water samples [23]. SPE were conducted with an AutoTrace automated SPE system from Caliper. The various tests were performed from surface water spiked with 250 ng/L of a standard mixture of alkylphenols. The cartridges were the Strata C18-E 500 mg from Phenomenex. The extraction consisted in using the sorbent previously conditioned with 5 mL methanol then 5 mL water. A volume of 300 mL of acidified sample (pH = 3) was passed through the cartridge with a 4 mL/min flow rate. The cartridge was dried 15 min by a stream of nitrogen and then eluted with 7 mL of acetone followed by 7 mL of ethyl acetate. The eluate was evaporated to dryness under a gentle stream of nitrogen. The dry residue was dissolved in 500 μL of MeOH. This extract was separated into two aliquots. The first 450 μL was added to 50 μL of isopropanol for LC–MS/MS analyses. The remaining 50 μL was added to 5 μL of DMSO and 450 μL of pure water for ELISA analyses.

2.4. LC–MS/MS analysis

Liquid chromatography was performed on a HP1100 HPLC system (Agilent Technologies) equipped with a degasser, a binary pump, an autosampler and a column oven. The chromatographic separation was performed on a C18 Isis Nucleodur EC column (120 mm × 2.1 mm, 3 μm) from Macherey-Nagel. The column oven

temperature was set to 40 °C; injection volume was 20 μL. The mobile phase consisted in: (A) 10 mM ammonium acetate and (B) methanol, with a 0.25 mL/min flow-rate. The following gradient elution program was applied: 70% (B) during 14 min, from 70% to 100% (B) in 2 min and 100% (B) during 16 min.

The LC system was coupled to a triple-stage quadrupole mass spectrometer (Applied Biosystem/3200 QTrap) with electrospray ion source (TurboV, Applied Biosystems). Optimisation of the ion source and MS/MS settings was performed by the automatic optimisation function of the MS software assisted by manual infusion with a syringe-pump and flow injection of standard solutions of alkylphenols. Precursor and product ion masses as well as the individual declustering potential and collision energy voltages of both alkylphenols are shown in Table 1. Samples were analysed in negative mode. Nitrogen was used as unique gas, at 50 L/min for the nebulisation and 20 L/min as curtain gas. The dwell time was 100 ms and the temperature of the source 600 °C.

The analytes were identified by both their chromatographic characteristics and their multiple reaction monitoring (MRM) specific fragmentation. Indeed, we compared their specific retention times and their characteristic ion transitions with the standards. Data processing was achieved with the software Analyst (1.4.2 version).

2.5. Immunoassay

Alkylphenols were analysed with ELISA kits from Tokiwa Chemicals Industries Co. Each kit consisted of eight separate 12-well immunoreader strips precoated with specific antibodies. The assay was performed following the guidelines from manufacturer. Briefly 100 μL of antigen-enzyme conjugate solution and 100 μL of standard or sample prepared in water/methanol/DMSO (89/10/1) were mixed and transferred into each coated well. After an incubation time of 60 min at room temperature, the reaction liquid was tap off and each well was rinsed 3 times with 300 μL of washing solution, using a Wellwash 4Mk2 from Thermo Labsystems. After addition of 100 μL of color solution, the wells were incubated 30 min at room temperature. Then the reaction was stopped and the absorbance was read at 450 nm with a Multiskan EX detector from Thermo Labsystems. Data were processed with Ascent software from Thermo Labsystems.

2.6. Validation of the methods

The calibration curve of ELISA was generated using alkylphenols concentrations in the range 0.001–5000 μg/L. It was based on duplicate samples prepared in water/methanol/DMSO (89/10/1). The linearity of the LC–MS/MS instrument was studied by injecting 20 μL of seven concentrations of the standard solution of the target compounds in the range 0.25–1000 μg/L.

The limit of detection of ELISA was evaluated from 7 replicates of the blank control (water/methanol/DMSO in the proportions 89/10/1), and corresponded to three times the standard error of the control. The upper limit of the dynamic range was deduced from the calibration curve and corresponded to approximately 10% B/B_0, where B is the absorbance at a given concentration and B_0 the absorbance of the blank control. The instrumental detection limit of LC–MS/MS (IDL) was determined as the concentration with a signal-to-noise ratio of 3 when injecting 20 μL of decreasing analytical standard concentrations (from 100 to 1 μg/L). Concerning the determination of the limits of detection (LOD) and of quantification (LOQ) of the method, including extraction step, groundwater was spiked at 0.5; 2.5; 10 and 50 ng/L, wastewater effluents at 1; 10 and 100 ng/L, and soil was spiked at 0.5; 2.5; 10; 50 and 100 ng/g. The LOD and LOQ of LC–MS/MS were determined as the concentration with a signal-to-noise ratio of 3 and 10, respectively, when injecting the extracts, according to widely published and generally accepted definitions in LC–MS/MS. The alkylphenol signals in the unfortified samples were subtracted if necessary.

The evaluation of intra-day precisions of the overall methods was conducted by the analysis of 6 replicates of groundwater (spiked with 50 ng/L), wastewater effluents (spiked at 500 ng/L) and soil at 5 and 50 ng/g.

Accuracy is usually described as the recovery obtained by adding known amounts of analytes. In environmental matrices, analyte recoveries were determined using 5 replicates of fortified samples, representative of real environmental scenario, by adding appropriate volumes of the working standard solution. Thus, groundwater and wastewater were spiked at 50 ng/L and 500 ng/L, respectively, while soil was spiked at 5 and 50 ng/g. As it was not possible to prepare a blank sample matrix without the presence of the analyte, recovery was evaluated by comparison of the spiked samples with samples that underwent the same protocol of extraction and spiking after the extraction.

3. Results and discussion

3.1. Development of LC–MS/MS method

The detection of trace levels of these chemicals requires MRM mode. The direct infusion of the compounds using electrospray interface indicated the deprotonated molecules $[M-H]^-$ to be the most abundant ion. Therefore $m/z = 219$ and $m/z = 205$ were selected as precursor ions of NP and OP, respectively. The product ion spectra of NP gave abundant fragments $m/z = 147$ and 133 consistent with alkyl cleavages and the losses of C_5H_{12} and C_6H_{14}, respectively [24]. OP produced a product ion at $m/z = 106$ corresponding to the loss of C_7H_{16} and a low signal at $m/z = 118$.

To achieve the most sensitivity during LC–MS/MS analyses, different mobile phases were tested, composed of methanol or acetonitrile as an organic phase with additive (ammonium acetate) at various concentrations in the aqueous phase. The results indicated that the best response was obtained in a methanol/water mobile phase with 10 mM ammonium acetate.

The elution gradient was chosen to allow a relatively rapid analysis of alkylphenols (tr 7 and 8 min, respectively for OP and NP) while leaving enough time for interfering substances, to be eluted.

3.2. Performance of both ELISA and LC–MS/MS methods

Validation of the developed methods was evaluated by estimation of the dynamic range, sensitivity, precision, recoveries and matrix effects.

3.2.1. Dose response of ELISA and linearity domain of LC–MS/MS

The calibration curve for the determination of alkylphenols by ELISA is shown in Fig. 1. The standard dose response is expressed as B/B_0, where B is the absorbance at a given concentration and B_0 the absorbance of the blank control. It was then transformed using the 4-parameter-logistic equation: $B/B_0 = d + (a-d)/(1 + \mathrm{conc}/c)^b$ where the constants a and d represent the upper and lower asymptotes, c represents the analyte concentration at the midpoint of the test (or concentration that produces a 50% response) and b represents the slope of the curve, at the concentration c. The four parameters were $a = 96.53238$, $b = -0.918156$, $c = 28.85956$ and $d = 14.60005$, with the correlation coefficient (r^2) for the standard curve corresponding to 0.9961128.

A good linearity was observed during the LC–MS/MS analysis of alkylphenols over the specified range (0.25–1000 μg/L) with correlation coefficients superior to 0.993.

Table 1
Mass spectrometry parameters of the target compounds: precursor and product ions, declustering potential (DP), collision energy (CE) and collision exit potential (CXP).

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP (V)	EP (V)	CE (V)	CXP (V)
NP	219	133, 147	−45, −45	−4.5, −4.5	−44, −34	−2, −2
OP	205	106, 118	−40, −40	−7.5, −7.5	−28, −48	−2, −2

Table 2
IDL of the LC–MS/MS and ELISA methods, RSD (%) of the whole ELISA and LC/MS–MS methods applied to groundwater (spiked with 50 ng/L), wastewater (spiked with 500 ng/L) and soil (spiked with 5 and 50 ng/g), as well as LOD and LOQ of the whole ELISA and LC–MS/MS methods.

	IDL (μg/L)	Groundwater			Wastewater			Soil			
		RSD 50 ng/L	LOD (ng/L)	LOQ (ng/L)	RSD 500 ng/L	LOD (ng/L)	LOQ (ng/L)	RSD 5 ng/g	RSD 50 ng/g	LOD (ng/g)	LOQ (ng/g)
ELISA	5.3	4.4	1.5		6.6	0.5		22.4	10.7	0.2	
LC–MS/MS											
NP	0.2	12.2	0.6	2.0	2.6	0.3	1.0	47.7	13.0	1	3
OP	0.6	3.4	1.1	3.6	3.1	0.4	1.3	50.9	21.9	3	9

3.2.2. Sensitivity

The limit of detection of ELISA can be expressed as the least detectable dose (IDL) and corresponds to three times the standard error of the control. The relative standard variation was evaluated at 4.8%, corresponding to IDL value of 5.3 μg/L. The dynamic range was between 5.3 μg/L and 1000 μg/L. The IDL value is comparable to the immunoenzymatic assay based on polyclonal antibodies, developed by Mart'ianov et al. [21] for the detection of NP and that allows the quantitative determination at 10 μg/L. On the other hand, the other known commercial ELISA kit for immunodetection of alkylphenols, based on monoclonal antibodies [25], is characterized by a worse sensitivity (70 μg/L for B/B_0 = 80%). The IDL of ELISA and LC–MS/MS is compiled in Table 2, as well as LOD and LOQ covering the preconcentration step. The values indicate that chromatography coupled with tandem mass spectrometry is more sensitive than ELISA. This observation remains true for the analysis of environmental samples of water after SPE concentration step, even if, for analysis of effluents, the sensitivities of both methods are comparable.

The limit of detection of ELISA method in groundwater is 1.5 ng/L, which is lower than that obtained by Cespedes et al. [22] (6 ng/L) in the same type of water. However the authors use a polyclonal anibody-based ELISA preceeding by a SPE step allowing a concentration factor more important than our method. On the other hand, for solid samples, the sensitivity of the ELISA method is better than that obtained by LC–MS/MS. This is probably due to matrix effects that are different with the two methods. In environmental samples of water or solids, alkylphenols are analysed by different techniques including GC–MS [26–29], LC–MS [26,30–32], LC–FLD [33] or LC–MS/MS [16,34–36]. These techniques are themselves preceded by various sample preparation steps, generally SPE for the liquid phases and PLE or ultrasound followed by purification by SPE, for the solid phases. The LOD reported thus vary according to the method used. Globally, the LODs of the methods based on ELISA in this paper are better than those using the GC–MS, LC–MS or LC–FLD, whether for water or soil. This LOD, however, are globally similar to those reported with LC–MS/MS. With such low detection limit, ELISA constitutes a suitable technique for the detection of alkylphenols in various kinds of waters and in soil.

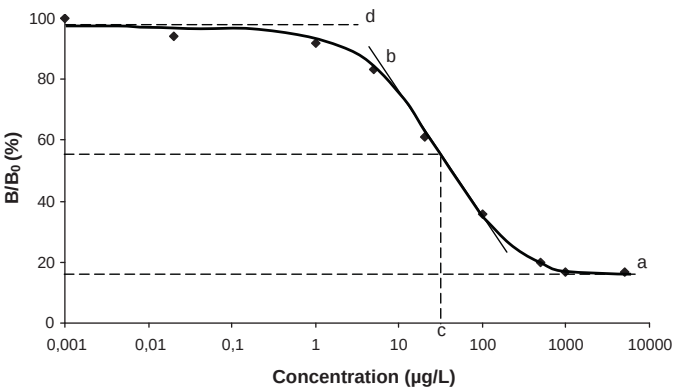

Fig. 1. Dose–response curve for alkylphenols calibration.

3.2.3. Precision

For the evaluation of intra-day precisions of the overall methods, spiked samples were extracted and analysed in parallel by LC–MS/MS and ELISA. The data, presented in Table 2, indicate that the precision is overall better with ELISA, whatever the matrix, and whatever the concentration of alkylphenols. It is worth noting that RSD are superior to 45% with the LC–MS/MS method when the soil is spiked with 5 ng/g.

3.2.4. Recoveries

In order to evaluate the accuracy of the methods, recovery experiments were performed in groundwater, wastewater and soil. The results are presented in Table 3. Mean recoveries lie in the range 74–117% with coefficient of variation ±18%, which mean that both methods were accurate and reproducible. Only coefficients of variation in soil spiked at very low concentration (5 ng/g) reach about ±18%. These values permit the quantitative determination of NP and OP in environmental samples. To our knowledge, no other work has been reported relating to immunoenzymatic detection of alkylphenols in the soil. However, our results in the groundwater are similar to those described by Cespedes et al. [22]. Indeed authors reported recoveries comprised between 87–73% and 117–51% by ELISA for OP and NP, respectively, in the case of groundwater spiked between 500 ng/L and 50 μg/L.

3.2.5. Matrix effect

ELISA methods often have a potential for nonspecific binding between non target analytes and antibodies leading to enhancement of the response. On the other side, a diminution could be observed if interfering components tend to inhibit selective interactions between the target analyte and antibodies [37]. A method for quantitatively assess matrix interferences [38] consists in a normalization of the absorbance values for matrix blank with respect to the absorbance of the blank control matrix. However, this approach is suitable only if the matrix is completely free of contaminant, a situation that is difficult to obtain in the case of the ubiquitous alkylphenol pollutants.

In this case another approach is to fortify samples in dilution series with a known amount of each alkylphenol. Therefore, the

Table 3
Mean recoveries (%) of the target compounds in spiked water and soil samples.

	Groundwater 50 ng/L	Wastewater 500 ng/L	Soil 5 ng/g	Soil 50 ng/g
ELISA	74 ± 4	81 ± 5	82 ± 6	75 ± 8
LC–MS/MS				
NP	117 ± 18	99 ± 2	106 ± 32	85 ± 9
OP	94 ± 5	91 ± 5	87 ± 27	81 ± 14

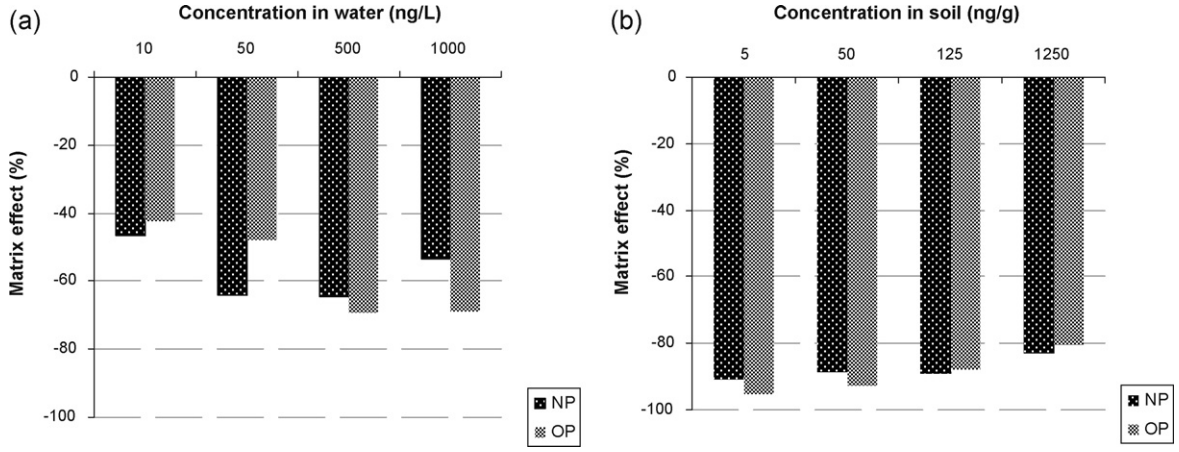

Fig. 2. Evaluation of matrix effect during the analysis of alkylphenols in wastewater effluents and soil by LC–MS/MS at different spiked concentrations.

matrix effect was evaluated by comparing results obtained by interpolation on the calibration curve and standard addition method.

In soil sample, the results indicate concentrations of 800 and 62.3 ng/g, respectively by direct measurement and standard addition method, highlighting the existence of a matrix effect.

In water samples, a significant difference between the measurements obtained with ELISA by a direct measurement or standard addition method is also observed, confirming the existence of a matrix effect. But this effect depends on the origin of the water. Indeed, in groundwater and canal, the direct measurements are less than the measurements by the standard additions (by a factor 21 and 8, respectively in groundwater and canal), meaning that interfering components tend to inhibit interactions between the target analyte and antibodies [37]. On the contrary, the response by the direct measurement is stronger in lake and effluents (by a factor 5 and 36, respectively). In this case, the high potential of ELISA for nonspecific binding between non target analytes and antibodies leads to enhancement of the response.

These data indicate that it is difficult to obtain a good estimate of the amount by direct measurement, and it is better to use the method of standard additions.

During LC–MS/MS analysis, the presence of a complex matrix such as soil can also cause errors, leading to inaccurate results. Indeed the residual components of the matrix can promote either ion suppression or improvement of the analyte in the electrospray interface. In order to evaluate the influence of environmental matrix on the electrospray ionization efficiency, the following equation was used:

$$\text{matrix effect } (\%) = \left(\frac{S_{\text{matrix}} - S_0}{S_{\text{solvent}}}\right) \times 100$$

with S_{matrix} the signal of the sample spiked post-extraction, S_0 the signal of the non spiked sample and S_{solvent} the signal of standards prepared in solvent.

The diagram in Fig. 2(a) present the matrix effects evaluated during the analysis of alkylphenols in effluents by LC–MS/MS at four different spiked concentrations, namely 10, 50, 500 and 1000 ng/L. Both compounds are subjected to matrix effects, in proportions varying from 40 to 70%, depending on the compounds and the initial concentration. It may be noted that the effect of signal suppression is more pronounced for higher concentrations, which is unusual. As can be seen, NP is the most sensitive to the matrix effect for the lower concentrations (10 and 50 ng/L). On the contrary, OP exhibits more signal suppression than NP for the upper concentrations (500 and 1000 ng/L). These results demonstrate that, as in the case of ELISA, quantification can be severely compromised if an external calibration is employed.

The diagram in Fig. 2(b) present the matrix effects evaluated during the analysis of NP and OP in soil at four different spiked concentrations, namely 5, 50, 125 and 1250 ng/g. Both compounds were subjected to strong matrix effects, in proportions varying from 80 to 95%. As can be seen, the matrix effect tended to decrease when the initial concentration increased.

These results indicate that LC–MS/MS is much more susceptible to matrix effect than ELISA, and emphasise the need for an additional purification step to eliminate non targeted organic substances that interfere with the signal during mass spectrometry analysis.

3.3. *Analyses of various soils and waters by both ELISA and LC–MS/MS*

In order to investigate the applicability of the ELISA kit on soils and waters, various samples were extracted and analysed in parallel by both ELISA and LC–MS/MS, using standard addition methods. The values of LC–MS/MS represented the individual concentrations of NP and OP, while the values of ELISA corresponded to the total amount (NP + OP) in the samples. The results demonstrate that OP and/or NP were detected in all samples by both methods, which means the absence of false negative by ELISA, over the 10 samples analysed. The data obtained by both methods are broadly the same order of magnitude (Table 4). ELISA exhibited a tendency to overestimated levels in soil samples, and on the contrary, to underestimated levels in waters. Concentrations are very close in the case of groundwater and canal, while the gap is greater in the case of lake or wastewater effluent.

Table 4
Determination of alkylphenol concentrations in soil and water samples by both LC/MS–MS and ELISA.

	LC–MS/MS			ELISA
	NP	OP	NP + OP	NP + OP
Groundwater (ng/L)	97.6	6.7	104.3	76.6
Lake (ng/L)	132.1	1.2	133.3	35.7
Canal (ng/L)	62.3	1.7	64	59.0
Effluents (ng/L)	119.8	<LOD	119.8	24.4
Soil 1 (ng/g)	nd	8.1	8.1	19.1
Soil 2 (ng/g)	20.0	6.6	26.6	62.3
Soil 3 (ng/g)	26.3	nd	26.3	21.5
Soil 4 (ng/g)	4.8	4.2	9.0	21.2
Soil 5 (ng/g)	<LOQ	nd	<LOQ	0.8
Soil 6 (ng/g)	20.9	3.8	24.7	28.3

nd: not detected.

These results demonstrate that ELISA constitutes a good method which can be used as a screening tool for rapidly determining concentrations of NP and OP in various kinds of soils and waters.

4. Conclusion

The analytical procedure described provides a fast and simple method for the screening of OP and NP in soils and waters, based on ELISA technique. By the combination of a single preparation step and the use of a simple ELISA kit, the method offers the sensitivity and selectivity necessary for the detection of the target alkylphenols in the low ng/g range.

The method was successfully applied to the analysis of several waters, as well as soils contaminated by sewage sludge or atmospheric deposition. The analyses were performed by both ELISA and LC–MS/MS, considered as the method of reference. The comparison of these preliminary results indicates that method based on ELISA can be useful not only in screening the samples and get a positive/negative response, but also it allows an approximation of the concentrations. If necessary to obtain a precise concentration, the positive instances would be confirmed by the reference LC–MS-MS method.

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