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A sensitive and robust method for the determination of alkylphenol polyethoxylates and their carboxylic acids and their transformation in a trickling filter wastewater treatment plant

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Abstract

This paper presents a method for the determination of alkylphenol, alkylphenol polyethoxylates (APEO) and alkylphenol ethoxycarboxylates (APEC) in the aqueous and solid phase of wastewater samples. Quantification was by liquid chromatography tandem mass spectrometry. Method detection limits for the analytes in the dissolved phase were 1.2 to 9.6 ng l^{-1} for alkylphenol, short chain (n = 1–3) APEO and APEC, and 0.1 – 4.1 ng l^{-1} for long chain alkylphenol polyethoxylates. The method detection limit for adsorbed phase samples ranged from 6 to 60 ng g^{-1} for AP, short chain APEO and APEC; and long chain APEO ranged from 0.5 – 20 ng g^{-1}. The method was utilised to evaluate the removal of these compounds over a trickling filter wastewater treatment plant. The major biotransformation products of nonylphenol polyethoxylate, nonylphenoxy acetic acid and nonylphenoxy monoethoxy acetic acid were present in the final effluent at concentrations ranging from 35 – 1797 ng l^{-1}. Short chain APEO were present in higher proportions in the suspended solids , due to their higher affinity to particulate matter compared to the long chain oligomers.

Keywords: Alkylphenol; alkylphenol polyethoxylates; alkylphenoxy carboxylates; trickling filter; liquid chromatography- tandem mass spectrometry

1. Introduction

Alkylphenol polyethoxylates (APEO) are non-ionic surfactants widely used in commercial and domestic applications with a worldwide production of approximately 500 kilotons (Petrovic and Barcelo, 2001a; Langford and Lester, 2002). Although a voluntary ban on APEO use in household cleaning products was introduced in 1995 in northern Europe (including the UK), followed by restrictions on industrial cleaning applications in 2000 (Renner, 1997); these surfactants are still used in substantial amounts in institutional and industrial applications. However, octylphenol ethoxylates (OPEO) and nonylphenol ethoxylates (NPEO) remain two of the most common surfactants in commercial use. APEO are continuously discharged into sewage treatment works (STW) or directly released into the aquatic environment (Langford et al., 2005).

Environmental concern in relation to these compounds results from their estrogenic activity (Jobling and Sumpter, 1993; Jobling et al., 1996; Routledge and Sumpter, 1996; Sonnenschein and Soto, 1998; Petrovic and Barcelo, 2002). Biodegradation of APEO during biological wastewater treatment can occur under both aerobic and anaerobic conditions and results in the production of more persistent and estrogenic metabolites; short chain APEO and alkylphenols (AP) including nonylphenol (NP), octylphenol (OP) and AP mono- to triethoxylates (NP_1EO, NP_2EO and NP_3EO) (Giger et al., 1984). The biodegradation of APEO has been well documented (Ahel et al., 1994a; Di Corcia et al., 1998; Di Corcia et al., 2000; Jonkers et al., 2001; Staples et al., 2001; Petrovic and Barcelo, 2003; Langford et al., 2007). Information about the concentrations and mass balances of APEO and their degradation metabolites in the environment, especially STW, and sewage sludge treatment processes (Scrimshaw and Lester, 2002), is essential in assessing the environmental impact of these compounds.

Analysis of APEO and their metabolites is a complex process due to the ethoxylated oligomers and alkyl-chain isomers which can be present (Langford et al., 2004; Scrimshaw et al., 2004). Gas chromatography (GC) and liquid chromatography (LC), are now commonly used for the determination of APEO. The direct use of GC for analysis is limited to surfactants with lower numbers of ethoxy groups, while

metabolic products and long chain ethoxylates require derivatization to increase volatility. Traditionally, LC analysis with fluorescence (Kiewiet and De Voogt, 1996; Bennie et al., 1997; Ahel et al., 2000; Marcomini et al., 2000) or ultraviolet (Ahel and Giger, 1985a, b; Zhou et al., 1990) detection has been widely used for monitoring APEO. However, these techniques often lack the sensitivity and specificity required at low concentrations. Liquid chromatography coupled with tandem mass spectrometry (MS/MS) is now increasingly being used to determine these alkylphenolic compounds (Jonkers et al., 2001; Houde et al., 2002; Loyo-Rosales et al., 2003; Petrovic et al., 2003; Schmitz-Afonso et al., 2003; Jahnke et al., 2004). Most studies describe LC/MS/MS analysis of only a limited number of the analytes either NPEO and NPECs (Jonkers et al., 2001; Houde et al., 2002; Petrovic et al., 2003), $AP_{1-3}EOs$ and AP (Ferguson et al., 2001), or $AP_{1-5}EOs$, AP and APEC (Loyo-Rosales et al., 2003; Schmitz-Afonso et al., 2003; Jahnke et al., 2004; Loos et al., 2007).

This study aims to develop a highly sensitive and specific analytical method to detect an increased number of alkylphenolic compounds ($NP_{1-12}EO$, $OP_{1-12}EO$, NP and OP), including carboxylated metabolites in sewage matrices (in both dissolved and adsorbed samples) and attempts to achieve lower detection limits. This method is successfully applied to a trickling filter works (TF) over which a mass balance has been attempted. The trickling filter work was chosen since many of the STWs in the south eastern of U.K. still employ these processes and there are not many studies reported in the literature on the fate and occurrence of APEO in such processes.

2. Experimental section

2.1. Reagents and chemicals

The technical 4-nonylphenol mixture of chain isomers and 4-*tert*-octylphenol were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). The long-chain OPEO and NPEO were available in technical mixtures (Igepal CO210, CO520, CO720) and (Igepal CA210, CA520, CA720) respectively containing a range of oligomers (Sigma-Aldrich). Nonyl- and octyl-phenoxy acetic acid (NP_1EC, OP_1EC), 4-nonyl- and octylphenolmono- and diethoxylate ($NP_{1-2}EO$, $OP_{1-2}EO$) were obtained from QMX Laboratories (Thaxted, Essex, UK). Standards for NP_2EC, NP_3EC, OP_2EC, OP_3EC were not available commercially. Due to the absence of commercially available standards, NP_2EC and NP_3Ec were quantified with NP_1EC standard, assuming similar response factors. Similarly OP_2EC and OP_3EC were determined with OP_1EC standard assuming similar response factors.

Acetone, ethylacetate (EtOAc), acetonitrile (ACN), methanol (MeOH) and dichloromethane (DCM) were obtained from Rathburn (Walkerburn, Scotland, UK) and acetic acid from Sigma-Aldrich. Single standard stock solutions were prepared in acetonitrile. Reagent grade MilliQ water (18.2 MΩ) (Millipore, Watford, UK) was used for spikes and preparation of solutions. The working standard solutions were prepared by further diluting the stock standard solutions with acetonitrile/MilliQ-water (50:50 v/v).

2.2. Analytical procedure

For determination of dissolved concentrations, settled sewage (100 ml) or final effluent (250 ml) was filtered through a Whatman GF/C filter (0.45 – 1 µm) (Whatman, Maidstone, UK.). The 100ml/250ml aqueous phase was then utilised for solid phase extraction (SPE) as described below. Solid phase extraction was performed using a syringe barrel tC18 (500 mg, 3cc) cartridge. The appropriate volume of sample was loaded onto the cartridges which were preconditioned with 5ml methanol followed by 5ml MilliQ water. The flow rate for sample extraction was kept constant between 5-10 ml min^{-1} under vacuum using Waters Sep-Pak Vacuum Manifold (Waters Ltd, Watford, UK.). When the sample had passed through, 4 ml of reagent grade water was used to rinse the solid phase; the cartridge was then dried by drawing air through it for half an hour. The analytes were eluted using 10 ml EtOAc, 10 ml DCM followed by 5 ml 0.1% acetic acid in methanol. A rotary evaporator (Heidolph Instruments, Schwabach, Germany) was employed to concentrate the extracts to 1 ml which was then evaporated to complete dryness under a gentle stream of nitrogen. The extract was reconstituted with 0.25 ml ACN/MQ-H_2O (50:50 v/v) and transferred to an autosampler vial prior to analysis using LC/MS/MS.

The determination of alkylphenolic compounds in the adsorbed phase was also performed in this study. In order to prevent biotransformation and possible thermal decomposition of the long chain alkylphenolic compounds, samples were freeze-dried. A solvent mixture 10ml MeOH/Acetone (1:1 v/v) was used to extract the alkylphenolic compounds. A clean-up step involved using silica SPE cartridge and elution of all analytes from the SPE using 10ml of 10% acetic acid in MeOH. Subsequent to the elution of these compounds from the silica SPE, reconstituted with 0.25 ml ACN/MQ-H_2O (50:50 v/v) and transferred to an autosampler vial prior to analysis using LC/MS/MS,

2.3. LC/MS/MS analysis

Analytes were determined using LC/ESI/MS/MS consisting of an HPLC (Waters Alliance HPLC system 2695) coupled to a Waters Quattro Premier XE mass spectrometer with a Z-Spray ESI source (Micromass, Manchester, UK.). The AP, APEC and APEO were separated on a Gemini C18 column (3µm particle size, 100mm x 2mm i.d., Phenomenex, Macclesfield, UK.). The mass spectrometer was operated in the negative electrospray ionisation (ESI^-) (i.e. AP and APEC) or positive electrospray mode (ESI^+) (i.e. APEO) using multiple reaction monitoring (MRM). Instrument control, data acquisition and evaluation were performed with MassLynx software 4.1 (Waters Ltd, Hertfordshire, UK.). Nitrogen was used as the nebuliser gas and argon as the collision gas. The conditions for detection by the mass spectrometer were as follows, capillary voltage, 3.20kV in the positive mode and -2.3kV in the negative mode, extractor lens at 3.0V, RF lens at 0.5 V in the positive mode and 1.0V in the negative mode, multiplier voltage, 650V, desolvation gas flow, 1000 l h^{-1}, cone voltage as shown in Table 1, cone gas flow at 50 l h^{-1}, desolvation temperature at 350°C and source temperature at 120°C.

Two separate chromatographic runs were used, one for the separation of $AP_{1\text{-}12}EO$; and the other for $AP_{1\text{-}2}EO$, APEC and APs. In both cases, separation was achieved using MQ water containing 20mM NH_4OH (solvent A) and ACN containing 20mM NH_4OH (solvent B). The use of ammonia results in formation of the NH_4^+ adduct ions by the APEO, rather than more stable sodium adducts, which facilitates fragmentation of parent ions in the collision cell of the MS/MS (Table 1). The gradient conditions for $AP_{1\text{-}12}EO$ were; time zero, 45% solvent B (5 min) followed by a linear increased in gradient to 80% solvent B which was maintained for 40 min. Following this the gradient, the conditions were maintained at 90% solvent B for 5 min before the column was re-equilibrated to starting conditions at 20% solvent B. The total run time was 50 min and A sample volume of 10µl was injected at a flow rate of 0.2 ml min^{-1}.

Conditions for the $AP_{1\text{-}2}EO$, APEC and APs started with 20% solvent B increasing to 45% over 10 min. This was followed by a linear increased to 80% solvent B which was maintained for 20 minutes. Following the increase gradient from 80 to 90% solvent B, the conditions were maintained at 90% solvent B for 5 min before the

column was re-equilibrated to starting conditions at 20% solvent B for 10 min prior to next run. Similarly, the total run time for this analysis was 50 min with 10 µl sample volume injected and flow rate was kept at 0.2 ml min^{-1}.

3. Results

3.1 Optimization of LC and MS/MS conditions

The optimized LC/MS/MS conditions used for compound identification are presented in Tables 2. Analysis was performed in the MRM mode, parent ions were $[M - H]^-$ for AP, APEC, and $[M + NH_4]^+$ for APEO. The optimized characteristic MRM precursor to product ion pairs monitored for the quantification of the compounds together with cone voltage, collision energy and sensitivity of the MS/MS for the compounds investigated is presented in Table 1. The compound-dependent instrument detection limit (IDL) was calculated by a *S/N* ratio of 3 from the MRM chromatograms for the standard mixture with an injection volume of 10 µl. The sensitivity of the instrument for AP, short chain APEO and APEC ranged from 3 – 58 pg. The sensitivity for the NPEO ranged from 0.2 to 13.4 pg while OPEO ranged from 0.1 to 8.6 pg.

Please insert Table 1.

3.2 Evaluation of method performance

The recoveries and relative standard deviations were determined in experiments where the analytes in the dissolved phase were in the concentration range of 0.1 µg l^{-1} (low spike) and 1 µg l^{-1} (high spike) using MQ water and wastewater samples (n = 3). Filtration of the wastewater samples was undertaken prior to SPE to remove particles. Recoveries in MQ water (low and high spikes) ranged from 71% to 98% for AP, APEO and APEC. Generally, the relative standard deviations (RSDs) were in the range of 2 to 7. Typically, recoveries for settled sewage (low and high spikes) ranged from 62% to 98% for AP, APEO and APEC. Relative standard deviation obtained were 2 to 8 for these alkylphenolic compounds. Recovery values and RSD were similar for spiked (low and high) final effluent samples for all alkylphenolic compounds.

The recoveries and relative standard deviations were also determined in experiments of adsorbed samples where the analytes were in the concentration range of 0.125 µg g^{-1} (low spike) and 1.25 µg g^{-1} (high spike) using suspended solids samples (n=3) and

sludge samples (*n*=3). Recoveries for adsorbed phased ranged from 51% to 105% for AP, APEO and APEC for low and high spikes and their corresponding RSDs were in the range of 1 to 14. Recoveries in spiked sludge ranged from 51% to 104% for AP, APEO and APEC and their RSDs ranged from 1 to 8. This methodology demonstrates that consistent recovery values and low RSD could be obtained from different matrices.

3.3 Fate and behaviour in a trickling filter works

The method was applied for the determination of nonyl- and octylphenol compounds in settled sewage and final effluent at a trickling filter treatment plant. Sampling was undertaken winter, with the sewage temperature at 10°C. The concentrations of the nonylphenol ethoxylates and associated carboxylates are shown in Fig. 1, demonstrating the change in distribution following the biological process and the affinity of the parent nonylphenol for the solid phase. In the settled sewage, the higher ethoxylates ($NP_{4\text{-}12}EO$) accounted for 91% of the nonylphenol compound flux (3908 mg d^{-1}) in the settled sewage, with a similar distribution observed for the octylphenol oligomers, with the $OP_{4\text{-}12}EO$ accounting for 58% of the flux (218 mg d^{-1}) in the settled sewage (Table 2). Following the biological treatment stage, the distribution of both nonyl- and octylphenol compounds was significantly altered, with carboxylated metabolites with one to three ethoxy units dominating the distribution of nonylphenol compounds (58%) and being significant for the octylphenols ($OP_{1\text{-}3}EC$, 20%). Overall mass balances for the compounds indicate that 64% of the nonylphenol compounds and 86% of the octylphenols passed through treatment to the final effluent and that there was a significant change in the distribution of oligomers due to cleavage of ethoxy units and carboxylation through biological treatment.

Please insert Fig. 1.

Please insert Table 2.

4. Discussion

4.1. Optimization of the conditions for LC/MS/MS

The APEO exhibit a high affinity for alkali metal ions resulting in the formation of sodium adducts $[M + Na]^+$ rather than the protonated molecules in unmodified mobile phases (Di Corcia et al., 2000; Jonkers et al., 2001; Petrovic and Barcelo, 2001b; Koh et al., 2005). However, the sodium adducts tend to be stable and do not fragment in the collision cell, and ammonium adducts have frequently been used (Takino et al.,

2000; Cohen et al., 2001; Houde et al., 2002; Jonkers et al., 2003; Loos et al., 2007). In this study, the use of ammonium adducts for MRM detection enhanced detection limits due to high fragmentation in the collision cell.

Identification of the compounds was ensured by monitoring two characteristic precursor-product ion transitions and obtaining an exact retention time match. For quantification, the most sensitive transition was used. However, some compounds produced only one sensitive product ion (refer to Table 1). The characterization and quantification of APEO mixtures in environmental matrices is a challenge because of the complexity of the mixtures available, there are no individual standards commercially available. Quantitative results for individual APEO were interpreted with caution because of the variations in the oligomer distributions. Peak integration was performed automatically using MassLynx 4.1 (Micromass). Quantification using external calibration with standard solution mixtures was used. Calibration standards obtained through linear regression was r^2 = 0.99 for AP, APEO and APEC. The instrument variability of the LC/MS/MS is usually <5%.

4.2 Evaluation of method performance

Method detection limit (MDL) was determined by subjecting the entire analytical extraction and detection procedure to the determination of spiked reagent water, settled sewage and final effluent samples in both aqueous and adsorbed phases. The MDL determined for the entire SPE/LC/MS/MS ranged from 1.2 to 9.6 ng l^{-1} for AP, APEO and APEC (Table 1). The MDL ranged from 0.1 – 4 ng l^{-1} for long chain APEO (Table 1). The comparison of MDL is restricted because of the different sample matrices, the spectrum of analytes and the reported information on LODs and how they were derived. In comparison to methods that allow the determination of selected APEO, APEC and AP in aqueous samples, MDLs of the presented LC/MS/MS method for individual analytes are mostly lower and in some cases in the same range as in other studies (Table 3). Method detection limit (MDL) was also determined for adsorbed phase samples in ~0.2 g TSS samples. The MDL determined for the entire SPE/LC/MS/MS ranged from 6 to 60 ng g^{-1} for AP, short chain APEO and APEC. The MDL ranged from 0.5 – 20 ng g^{-1} for long chain APEO.

Please insert Table 3.

4.3 Mass balance and removal of alkylphenolic compounds

The difference in concentrations between the NPEC and OPEC observed in this study is in agreement with previous studies in which NP_nEC dominated at 64% of the total in relation to the OP_nEC (Ahel et al., 1994b). The major biotransformation product of the NPEO, $NP_{1-3}EC$, formed during the wastewater treatment, were present in the final effluent at relatively high concentrations (35 – 1797 ng l^{-1}) as a result of biodegradation of higher-chain APEO. As demonstrated in this study progressive shortening of the ethoxylate chain occurs during the biological stage of wastewater treatment, although this is not efficient enough to remove all of the higher oligomers ($AP_{4-12}EO$) which were still observed in the final effluent. The presence of anaerobic zones within the stratified layers of trickling filters, may also contribute to the formation of parent alkylphenols. Under anaerobic conditions the alkylphenols are thought to be the final degradation products (Montgomery-Brown and Reinhard, 2003) and it is possible that the flux of these compounds, which may be adsorbed in the humus sludge, could account for the mass balance at the works. The final effluent concentration of nonylphenol found at this plant would meet the PNEC value set by the UK Environment Agency (330 ng l^{-1}), although it would fail for octylphenol (6 ng l^{-1}).

4.5 Comparison with other countries

Few studies have reported on the fate and behaviour of APEO during trickling filter treatment. However, two studies indicated removal of APEO (68 – 77%) from a domestic trickling filter works STW (Brown et al., 1987; Gerike, 1987). This efficient removal capacity was attributed to its high removal of COD which was comparable to that for an activated sludge process. This study corroborated with the findings of Gerike, 1987 in that APEO do degrade, however, estrogenic carboxylated metabolites are generated in the process. The concentrations of APEO and their degradation products reported here are slightly lower or within the concentration ranges reported for the same analytes in STW effluents of other countries (Ying et al., 2002).

5. Conclusions

An analytical method for alkylphenolic compounds has been developed, consisting of a SPE extraction (dissolved phase); and solvent extraction with silica clean-up (adsorbed phase) followed by LC/MS/MS detection. The use of a triple quadrupole instrument in the MRM mode demonstrated high selectivity and sensitivity. The MDL determined for the entire method ranged from 1.2 to 9.6 ng l^{-1} for AP, $AP_{1-3}EO$ and APEC and 0.1 – 4.1 ng l^{-1} for $AP_{4-12}EO$. The results demonstrate the importance of

determining not only the alkylphenols and ethoxylates, but also the APEC intermediates, as the highest concentrations found in the final effluent were for NP_1EC and NP_2EC (476 and 1797 ng l^{-1} respectively). Concentrations of NP (28 ng l^{-1} in dissolved phase sample) were not found above the predicted no-effect concentration (PNEC) of 0.33 µg l^{-1}, although this study demonstrated that in comparison to the activated sludge process, trickling filters are inefficient at removal of these compounds.

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Table 1. Characteristic LC/MS/MS parameters and detection limits of alkylphenol polyethoxylates (NPEO and OPEO).

Compounds	m/z precursor $[M + NH_4]^+$	Dwell time (msec)	Cone (V)	Product ions (Collision potential)	Retention time (min)	IDL (pg)	Recoveries	MDL (ng l^{-1}) (RSD)	MDL (ng g^{-1}) (RSD)
NP^3EO	370	100	40	[a]353(10), 226.5(20)	27.87	0.2	96(9)	0.1 (2)	0.5 (3)
NP_4EO	414	90	30	[a]397(14), 270(20)	27.71	4.7	89(3)	1.5 (2)	7.5 (2)
NP_5EO	458	90	30	[a]440(20), 315(25)	27.63	7.9	85(3)	2.1 (2)	10 (2)
NP_6EO	502	90	30	[a]485(20), 359(30)	27.47	13.4	93(2)	2.7 (2)	13 (3)
NP_7EO	546	90	30	[a]529(23), 403(25)	27.23	8.3	81(2)	0.8 (2)	4 (5)
NP_8EO	590	90	30	[a]573(25), 447(27)	27.07	3.5	85(2)	0.7 (2)	4 (6)
NP_9EO	634	90	30	[a]617(25), 335(30)	26.82	2.9	93(2)	2.2 (3)	11 (8)
$NP_{10}EO$	678	90	30	[a]661(25), 132.5(40)	26.66	3.0	97(4)	2.1 (3)	10 (2)
$NP_{11}EO$	722	90	30	[a]705(25), 291(40)	26.42	1.9	98(2)	0.9 (2)	4.5 (3)
$NP_{12}EO$	766	90	30	[a]749(30), 291(35)	26.26	1.0	95(2)	1.1 (4)	5.5 (4)
OP_3EO	356.5	100	20	[a]338.5(10), 227(10)	22.84	4.9	78(10)	1.8 (1)	9 (4)
OP_4EO	400.5	100	20	[a]382.5(15), 270.5(15)	22.84	5.9	83(5)	4.1 (2)	20 (3)
OP_5EO	444	100	20	[a]426.5(10),315(10)	22.76	0.2	85(7)	1.4 (3)	7 (2)
OP_6EO	488	90	30	[a]471(15), 359(20)	22.68	6.0	87(5)	3.0 (2)	15 (4)
OP_7EO	532	90	30	[a]515(20), 403(20)	22.60	8.6	87(3)	0.6 (2)	3 (5)
OP_8EO	576	90	30	[a]559(20), 447(25)	22.43	7.9	92(2)	1.3 (3)	6.5 (6)
OP_9EO	620	90	30	[a]603(20), 491(25)	22.27	5.5	85(2)	2.0 (5)	10 (2)
$OP_{10}EO$	664	90	30	[a]647(20), 535(30)	22.11	3.0	86(2)	0.8 (3)	4 (2)
$OP_{11}EO$	708	90	30	[a]690(20), 579(30)	22.03	0.1	93(2)	0.1 (2)	0.5 (3)
$OP_{12}EO$	752	90	30	[a]735(20), 623(30)	21.87	0.1	86(3)	0.5 (1)	2.5 (2)
[b]NP_1EO	282	120	25	[a]127(25), 265(25)	29.11	40	76(3)	1.2 (2)	6 (3)
[b]NP_2EO	326	100	40	[a]183(40), 121(40)	28.88	7	76(3)	2.5 (2)	12 (2)
[c]NP_1EC	263.5	100	20	[a]205(20), 106(30)	11.32	3	73(2)	2.5 (2)	12 (2)
[c]NP_2EC	307	100	20	205(20)	11.32	NA	-	NA	NA
[c]NP_3EC	351	100	20	205(20)	11.32	NA	-	NA	NA
[b]OP_1EO	268	120	15	[a]113(10), 250(20)	25.92	58	69(3)	9.6 (2)	48 (4)
[b]OP_2EO	312	100	20	[a]183(10), 295(10)	25.73	5	64(2)	5.1 (2)	25 (2)
[c]OP_1EC	277.5	100	20	[a]219(18), 133(40)	10.87	3	77(3)	12 (6)	60 (3)
[c]OP_2EC	321	100	20	219(18)	10.87	NA	-	NA	NA
[c]OP_3EC	365	100	20	219(18)	10.87	NA	-	NA	NA
[c]NP	219	100	30	[a]132.5(30), 147(35)	29.43	5	73(2)	2.3 (2)	11 (5)
[c]OP	205.5	100	30	[a]134(25), 106(20)	26.32	4	53(3)	6.9 (3)	34 (4)

[a]MRM used for quantification; [b]$[M + NH_4]^+$; [c]$[M - H]^-$; IDL were calculated by a *S/N* of 3 from the MRM chromatograms of the standard solution mixture; MDL for the SPE-LC/MS/MS procedure were determined from spiked MQ (0.1 µg l^{-1}) in 250ml reagent water; NA: Not applicable; data for the adsorbed phase was based on spike at 0.125 µg g^{-1} (low spike) and 1.25 µg g^{-1} (high spike) in 0.2 g of sewage sludge. MDL for the SPE-LC/MS/MS procedure were determined from alkylphenolic compounds at 0.125 µg g^{-1} in 0.2 g sludge dwt; due to varying amounts of solids in the influent and effluent suspended solids (dry weight), a standardised weight of 0.2 g of suspended solids/sludge were used to derive the MDL; similar MDLs were obtained for sludge and suspended solids.

Table 2. Fluxes and change across the biological treatment stage at the trickling filter works.

Flux of nonylphenol compounds (mg d^{-1})				
Compounds	Settled sewage	Final effluent	Change (mg d^{-1})	Change (%)
NP	65	57	-8	-12
NP_1EC	48	309	+261	+544
NP_2EC	11	1168	+1157	+10518
NP_3EC	2	23	+21	+1050
NP_1EO	152	89	-63	-41
NP_2EO	46	75	+29	+63
NP_3EO	13	5	-8	-62
NP_4EO	207	170	-37	-18
NP_5EO	311	136	-175	-56
NP_6EO	674	165	-509	-76
NP_7EO	638	118	-520	-82
NP_8EO	533	88	-445	-83
NP_9EO	457	70	-387	-85
$NP_{10}EO$	375	42	-333	-89
$NP_{11}EO$	240	37	-203	-85
$NP_{12}EO$	134	20	-114	-85
Total flux	3906	2572	-1334	
Composition				
$\%NP_{1-3}EO$	5%	7%		
$\%NP_{4-12}EO$	91%	33%		
$\%NP_{1-3}EC$	2%	58%		

Flux of octylphenol compounds (mg d^{-1})				
Compounds	Settled sewage	Final effluent	Change (mg d^{-1})	Change (%)
OP	10	12	+2	+20
OP_1EC	4	4	0	0
OP_2EC	4	33	+29	+725
OP_3EC	0	1	+1	+900
OP_1EO	42	46	+4	+10
OP_2EO	28	38	+10	+36
OP_3EO	4	8	+4	+100
OP_4EO	9	6	-3	-33
OP_5EO	9	12	+3	33
OP_6EO	21	6	-15	-71
OP_7EO	34	8	-26	-76
OP_8EO	22	6	-16	-73
OP_9EO	16	3	-13	-81
$OP_{10}EO$	9	2	-7	-78
$OP_{11}EO$	3	1	-2	-67
$OP_{12}EO$	3	1	-2	-67
Total flux	218	187	31	
Composition				
$\%OP_{1-3}EO$	34%	49%		
$\%OP_{4-12}EO$	58%	24%		
$\%OP_{1-3}EC$	4%	20%		

Table 3. Comparison of method detection limit with other publications.

Analyte	MDL (this study)	MDL(Jahnke et al., 2004)[a]	MDL(Petrovic et al., 2002)[b]	MDL(Rudel et al., 1998)[c]	MDL(Loos et al., 2007)[d]	MDL(Houde et al., 2002)[e]	MDL(Jonkers et al., 2003)[f]
NP_1EO	1.2	10	100	14.4	100	50	15
NP_2EO	2.5	0.2	40	13	100	20	6
NP_1EC	2.5	0.1	20	86.7	1	10	13
OP_1EO	9.6	12	100	10	100	-	-
OP_2EO	5.1	0.1	40	3.3	100	-	-
OP_1EC	12	0.04	20	-	1	-	-
NP	2.3	2.0	20	8.4	10	-	11
OP	6.9	4.0	20	6.1	5	-	-

[a, d, e] $[APEO + NH_4]^+$ adducts and $[M - H]^-$ ions (AP and APEC) monitored using LC/ESI/MS/MS.
[b, f] $[APEO + NH_4]^+$ adducts and $[M - H]^-$ ions (AP and APEC) monitored using LC/ESI/MS.
[c] GC/MS after derivatization.

Fig. 1. Concentrations of NP, $NP_{1-3}EC$ and $NP_{1-12}EO$ showing partitioning between the dissolved and adsorbed fraction in settled sewage (A) and final effluent (B).