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Determination of nineteen 4-alkylphenol endocrine disrupters in Geneva municipal sewage wastewater

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Abstract

A method for the determination of 18 isomeric 4-nonylphenols and 4-*tert*.-octylphenol in wastewater using GC–MS and LC–MS has been developed. This procedure has been applied to the determination of the free alkylphenols and the analysis of these substances in the form of 4-alkylphenol polyethoxylates, their various hydrosoluble metabolites and other hydrosoluble 4-alkylphenol containing degradation products (''bonded'' alkylphenols) after their cleavage with hydroiodic acid. In the environment, the final degradation products of 4-alkylphenol polyethoxylates and their metabolites are the long-chain free 4-alkylphenols, which are responsible of endocrine disruption in various animal species. The average concentration of free alkylphenols in the wastewater of the sewage plant in Aïre, Geneva (Switzerland) ranges from 1.0 to 6.8 μg/l (average 2.5 μg/l). The concentration of ''bonded'' 4-alkylphenols can reach about 0.66 mg/l. The precision of the method and its accuracy are satisfactory with recovery rates for the free 4-alkylphenols and ''bonded'' 4-nonylphenols ranging from 74 to 79% and 80 to 87%, respectively. The relative standard deviation is lower than 6% for all analyzed compounds. The detection limits are in the range of 0.4 to 6 ng/l (typically 2 ng/l) and quantification limits are between 2 to 22 ng/l (typically 10 ng/l) for all individual isomeric alkylphenols.

Keywords: Water analysis; Environmental analysis; Alkylphenols; Nonylphenols; Endocrine disrupters; Alkylphenol polyethoxylates; Ethoxylates; Phenols

1. Introduction

In the last decade, endocrine disrupters have been an important topic of interest to the scientific community. There are a large amount of papers dealing with a broad amount of substances endowed with endocrine disruption properties including pesticides, polychlorinated biphenyls (PCBs), dioxins, plasticizers, pharmaceuticals and nonionic surfactants [1–3]. These last products are manufactured by a Friedel–Crafts acid catalyzed reaction of a technical mixture of nonenes with phenol resulting in various complex mixtures of isomeric 4-nonylphenols [4–6] (see Fig. 1). When these complex mixtures react with ethylene oxide, they give rise to still more complicated mixtures (e.g., more than 2000 compounds in the case of Triton X-100). Several works on the metabolism of nonionic surfactants have shown that their degradation liberate long-chain 4-alkylphenol derivatives which reportedly act as estrogen disrupters in many aquatic animal species (Fig. 2) [7,8]. The structures of the isomeric 4-nonylphenols were partially established by mass spectrometry (MS) and IR

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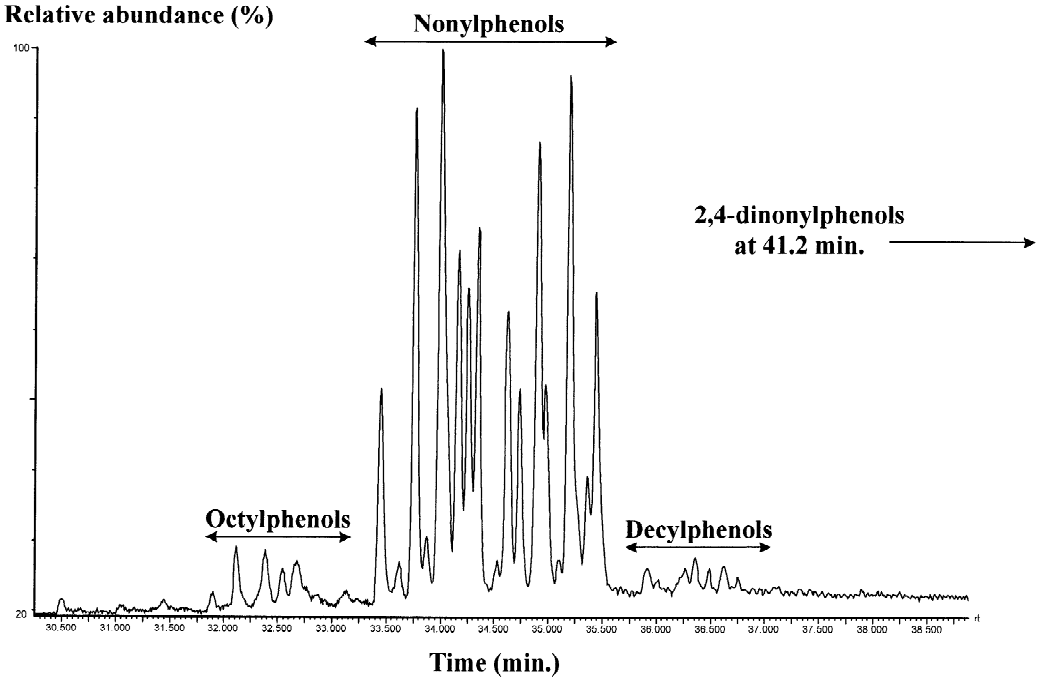

Fig. 1. GC–MS chromatogram of a technical nonylphenol mixture in full scan mode.

studies indicating that these substances can be separated into five groups according to the substitution of the α-carbon on the nonyl chain [6]. In this respect, the multiplicity of these compounds and the complexity of their structures bear some resemblance to those of PCBs [15]. Their synthesis was reported by a German group in a short communication [9] without detailed experimental data. Although the technical nonylphenol mixture shows powerful endocrine disrupter activity, the disruption potential of individual isomeric alkylphenols remains practically unknown.

There are a number of papers on the determination of 4-alkylphenols and 4-alkylphenol polyethoxylates in wastewater which indicate levels ranging from 0.5 ng/l to 11 mg/l [1–3,10]. Recently, a very sensitive analytical method has been reported which allows the determination of 4-alkylphenols and some hormonal substances in water at the pg/l level [11]. On the other hand, there is a report [12] claiming that an efficient biological treatment of wastewater completely eliminates all alkylphenol polyethoxylates which makes their monitoring practically useless.

Some analytical and biological studies [13,14] have dealt with 4-*n*-nonylphenol which could not be found in wastewater because it is not present in industrial nonylphenol products [6].

Until today, most publications have focused on the determination of 4-alkylphenols or 4-alkylphenol polyethoxylates. In our view, both classes of compounds should be considered for assessment of their impact on the environment because of the rapid degradation of 4-alkylphenol polyethoxylates already mentioned above. Therefore, the method was developed for the simultaneous determination of 4-alkylphenols in their free and ''bonded'' forms because the nonionic surfactants based on 4-alkylphenol polyethoxylates are still in limited industrial use.

2. Experimental

2.1. Materials

Acetone, *n*-hexane, and dichloromethane (all solvents for ultra-trace analysis) were purchased at EGT (Tägerig, Switzerland).

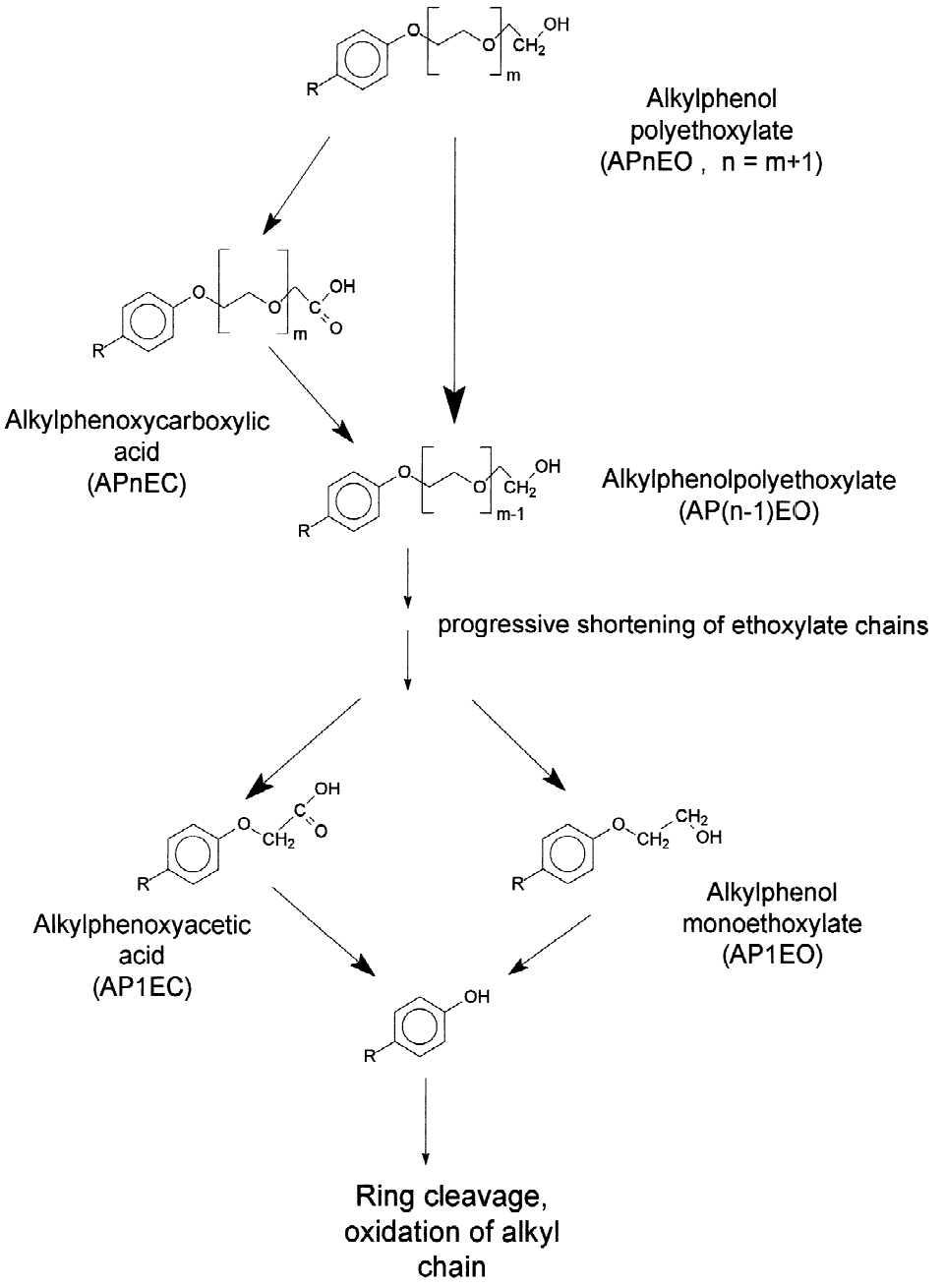

Fig. 2. Degradation process of alkylphenol polyethoxylates in the environment [16].

Standards of 4-alkylphenol polyethoxylates (Igepal CO 210, CO 520, CO 720, CO 890 and CO 990), methanol (HPLC Chromasolv gradient grade and for residue analysis), acetonitrile (HPLC Chromasolv gradient grade), and chemical standards of 4-*tert*.-octylphenol (>99%), 4-octylphenol (>99%), 4-*n*-nonylphenol (>99%) and the technical nonylphenol mixture (>85%) were purchased from Fluka (Buchs,

Switzerland). Sodium chloride, sodium hydroxide, sodium sulfate, potassium hypophosphite monohydrate, sulfuric acid, formic acid, hydrobromic acid, and hydroiodic acid were of the highest purity available (Fluka).

2.2. HPLC system

HPLC experiments were performed on an Agilent HP1100 LC–MSD 1946D system purchased from Agilent (Palo Alto, CA, USA).

Gradient elution was employed using a mixture of water and acetonitrile. At a flow of 300 μl/min, the gradient started at 40% of acetonitrile to reach 50% in 15 min, then the proportion of acetonitrile was gradually increased to 100% during the next 30 min. The column was washed with 100% acetonitrile for 10 min and reconditioned with the starting mobile phase for the next run.

Samples were injected and separated on a Supelco ABZ+, 5 μm particle size, 250×2.1 mm I.D. column at 40 °C. In diode array detection (DAD), the peaks were simultaneously monitored at 224, 254 and 278 nm. The MS system was operated in the negative electrospray ionisation (ESI) mode with the quadrupole temperature at 100 °C and the fragmentor voltage at 80 V. The spray nitrogen flow was adjusted to 12 l/min.

2.3. GC–MS systems

GC–MS experiments were performed on a Fisons gas chromatograph coupled to a Fisons MD 800 mass spectrometer equipped with an AS800 autosampler. A 60 m SGE BPX-5 fused-silica capillary (0.32 mm I.D., 0.25 μm film thickness) was programmed from 70 °C (maintained for 1 min) to 280 °C at a rate of 4 °C/min and then maintained for 10 min. The head pressure of helium carrier gas (99.9996%) was of 100 kPa. The injections were performed in ''on column'' mode with a 1 μl sample volume. Data acquisition and interpretation were carried out with MassLab 1.3 software. Full scan was performed within the mass range 25–550 u and the data acquisition was done in the selected ion recording (SIR) mode with ions m/z 107, 121, 135, and 149. In all cases, the electron impact ionization (70 eV at 180 °C) was employed.

2.4. Sampling and sample preparation

The 15 wastewater samples were collected between October and December 2001 after complete primary treatment (the average flow being 130 000 m^3/day) at the Aïre sewage treatment plant.

The samples were analyzed within 2 days of storage at 0 °C in amber glass bottles prior to extraction.

2.5. Determination of the free 4-alkylphenols

In contrast to the most publications, liquid–liquid extraction was preferred to solid-phase extraction (SPE) in our study. The collected wastewater formed a very fine emulsion which is very tedious to filter and easily clogs the SPE cartridge. The free 4-alkylphenol fraction was then isolated by an extraction of the organic phase, and the extract subjected to a multistage clean-up. Briefly, after acidification (1 ml of sulfuric acid) of wastewater (1 l) and addition of 5% sodium chloride, these compounds were extracted with *n*-hexane (3×30 ml). The residual wastewater was used for the determination of 4-alkylphenols in dissolved 4-alkylphenol polyethoxylates (described in Section 2.6). The organic phase was washed by a mixture of phosphate buffer (0.4 *M* at pH 7)–methanol (1:1) (3×30 ml). All free 4-alkylphenols were extracted with a mixture of methanol–0.25 *M* solution of sodium hydroxide (1:1) (3×25 ml). The aqueous phase was concentrated under reduced pressure to remove methanol, acidified with sulfuric acid to pH 2 and extracted with hexane (3×25 ml). The hexane was evaporated under reduced pressure, the residue was dissolved in a small amount of dichloromethane and transferred to a 1-ml volumetric flask. The solvent was evaporated under a gentle stream of nitrogen to dryness. For GC–MS analysis, the sample was dissolved in 1 ml of dichloromethane containing 25 ppm of an internal standard (4-*n*-nonylphenol, which is not present in the nonylphenol technical mixture). For LC–MS analysis a solution of 40% methanol in water was used instead of dichloromethane. The GC–MS and LC–MS systems were calibrated using the technical mixture containing 85% of 4-nonylphenol isomers and the reference standard of 4-*tert*.-octylphenol. The percentage composition of the

determined isomeric 4-nonylphenols was established by GC–flame ionisation detection (FID) [6].

2.6. Determination of the "bonded" 4-alkylphenols

The residual wastewater containing 4-alkylphenol polyethoxylates and their metabolites mentioned above (100 ml) was evaporated under reduced pressure to dryness. The residue (approximately 6 g) was extracted for 12 h in a Soxhlet apparatus with 100 ml of a mixture of acetone–toluene (1:1). The obtained solution was evaporated under reduced pressure to dryness. The residue was dissolved in hydroiodic acid (20 ml) and sodium hypophosphite (4 g; to prevent iodination of alkylphenols) and heated for 24 h at 100 °C. The reaction mixture was diluted with water (100 ml) and extracted with hexane (3× 30 ml). The organic phase was washed with water (3×20 ml), dried with sodium sulfate (1 g), filtered, and evaporated under reduced pressure. The residue was prepared for analysis as described earlier.

The flow-chart of the complete method is illustrated in Fig. 3.

2.7. Method precision and accuracy

The precision and accuracy of the complete method were determined by repeated GC–MS analysis in the selected ion monitoring (SIM) mode of the same sample (five times, five samples) and by spiking the sample of the wastewater having the lowest available content of alkylphenols (1 μg/l of free and 3.2 μg/l of "bonded" alkylphenols) with nonylphenol the technical mixture and the 4-alkylphenol polyethoxylates (in the form of a commercial product, containing, in average, 12 ethoxylate units). The wastewater sample was spiked at 1, 2, 4, 8 and 16 μg/l with the technical nonylphenol mixture for study of recovery rates of free nonylphenols. The same sample spiked with alkylphenol polyethoxylates contained 5, 10, 20, 40 and 80 μg/l of total "bonded" alkylphenols. The GC–MS system was calibrated in the range of 0.5, 1, 2.5, 5, and 10 μg/ml with the nonylphenol technical mixture. Taking into account the isomeric composition of the technical mixture

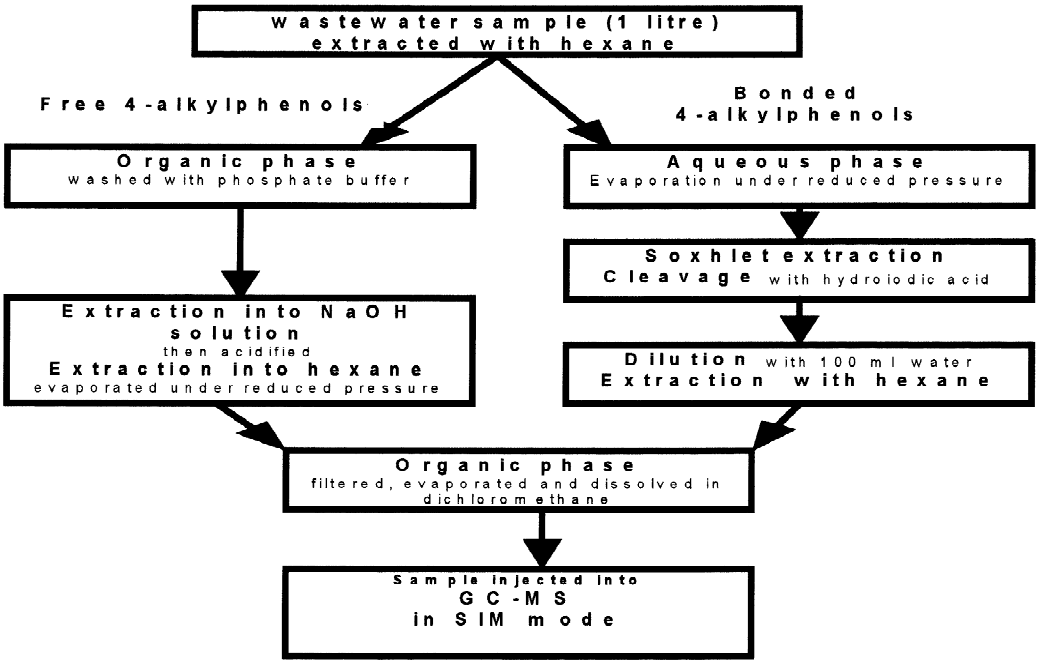

Fig. 3. Flow-chart of the complete method of determination of the free and the "bonded" 4-alkylphenols in wastewater.

Table 1
Retention times, monitored ions, and average concentrations of each free and bonded 4-alkylphenol in Geneva wastewater

Alkylphenol	%**	Group*	Retention time (min)	Monitored ion	Free 4-alkylphenols (ng/l)	Bonded 4-alkylphenols (μg/l)
4tOP	–	–	31.10	135	204 (167–301)	–
NP1	8.0	1	35.62	149	197 (62–583)	29.05 (0.36–86)
NP2	2.2	2	35.52	149	53 (18–154)	4.39 (0.12–12.7)
NP3	0.9	2	35.44	149	22 (8–64)	2.46 (0.04–7.2)
NP4	13.1	1	35.37	135	311 (121–833)	33.45 (0.63–98)
NP5	0.4	2	35.25	149	16 (3–51)	0.96 (0.03–1)
NP6	3.8	5	35.16	121	78 (23–234)	9.93 (0.16–29)
NP7	4.9	3	35.10	149	114 (37–327)	15.3 (0.24–45)
NP8	4.9	4	35.05	135	106 (37–302)	10.23 (0.16–30.1)
NP9	4.2	5	34.93	121	85 (25–254)	12.6 (0.22–37)
NP10	6.7	1	34.83	135	154 (57–423)	15.5 (0.23–46)
NP11	1.9	5	34.82	121	7 (2–17)	0.48 (0–1.4)
NP12	7.9	3	34.54	149	174 (59–495)	13 (0.18–38)
NP13	7.2	3	34.36	149	152 (54–440)	11.4 (0.15–36)
NP14	2.0	1	34.23	135	47 (19–125)	3.4 (0.06–10)
NP15	13.0	3	34.19	149	319 (112–896	26.6 (0.04–80)
NP16	6.8	1	34.43	135	172 (71–462)	14.8 (0.25–44)
NP17	11.0	1	33.59	135	281 (116–735)	18.5 (0.30–55)
NP18	1.0	1	34.07	135	38 (17–92)	3.1 (0.02–8.8)
Total	100				2532 (1010–6790)	225.1 (3.2–663)

* Approximate 4-nonylphenol isomer group numbering according to Wheeler et al. [6].
** Isomeric distribution of nonylphenols in the technical mixture.
4tOP=4-*tert*.-octylphenol; NP=4-nonylphenol.

which was determined by GC–FID [6] (see Table 1), the actual concentration of individual nonylphenol isomers in the first calibration solution (0.5 μg/ml) was in the range from 2 to 65 ng/ml. Internal standard (4-*n*-nonylphenol, 25 μg/ml) was added to the calibration solutions and to the samples. Suitable dilution was applied to the wastewater extracts, when necessary.

The obtained results were treated by linear regression, the slope showing the average recovery rate for each individual isomeric alkylphenol.

Method detection and quantification limits were calculated from GC–MS-SIM noise and checked using spiked Millipore water sample at the corresponding concentration because a nonylphenol free wastewater sample was not available. The results are given in the following section.

3. Results and discussion

Prior to their GC–MS and LC–MS analyses, the ''bonded'' 4-alkylphenols in non-degraded and various partially degraded alkylphenol polyethoxylates, were liberated by cleavage with hydroiodic acid. Several reagents for cleavage of phenol–ether bonds [hydrobromic acid 49% and 62%, sodium sulfide in dimethylformamide (DMF) and hydroiodic acid 56%] have been tested at different temperatures and reaction times. A similar procedure described in the literature using hydrobromic and acetic acids resulted in an incomplete cleavage [2]. Of these reagents, only the hydroiodic acid (56%) produced complete splitting of the ethoxylate chain giving 1,2-diiodoethane and 4-alkylphenols. The best conditions found for cleavage of wastewater samples were 24 h at 100 °C with an average recovery rate of 85% in the range of concentrations from 20 ng to 2 mg/l. The cleavage of *tert*.-octylphenol based alkylphenol polyethoxylates gave very low recovery rates because of a practically complete elimination of the *tert*.-octyl group under our experimental conditions. This cleavage of the C–C bond is due to the acid catalyzed retro-electrophilic substitution. Harder cleavage conditions (24 h at 120 °C) also produced a

slight removal of the nonyl chain as the small cluster of peaks of various nonyl iodides was observed in the GC–MS chromatogram. Therefore, the relative proportions of isomeric 4-nonylphenols were also carefully controlled. Only small changes of relative concentrations were noticed for two 4-nonylphenols of minor importance (NP 11 and NP 18).

In our preliminary experiments, LC–MS proved to be suitable only for total quantification of 4-nonylphenols because of incomplete separation of all isomers. We have tested several columns as well as solvent systems (acetonitrile–water; methanol–water).

The best separation was obtained on a C_{18} Discovery (column 150×2.1 mm I.D., 3 μm), but the ABZ+ 250×2.1 mm I.D., 5 μm column was best suited for total quantification of 4-nonylphenol isomers, giving only two partially separated peaks which were easy to integrate. Under these conditions, LC–MS was mainly used to evaluate the optimal clean-up approach and preliminary estimation of recovery rates. We have not tested a graphitized carbon column, which reportedly can give a better resolution [5]. Chromatograms published in this paper showed that the separation of these compounds still remains incomplete. Therefore, we opted for GC which allows the separation of practically all alkylphenol isomers. Their partial coelution was observed only in a few cases. Use of the MS-SIM mode allowed correct quantification even of these coeluting substances. Under our experimental conditions, the peaks of isomeric 4-nonylphenols were found between 33 and 36 min (see Figs. 4 and 5). The MS spectra practically corresponded to those published by Wheeler et al. [6]. There were only slight differences as concerns the intensity of characteristic fragments. For analysis, the most intense fragment was chosen. In case of coelution, less intense but more characteristic ions had to be monitored which lead to a slight increase of minimum detection limit (MDL) and quantitation (MQL) values.

The results show that strong fluctuations of concentrations of the free and the ''bonded'' 4-alkylphenols were observed in Geneva wastewater (see Table 1). Some variations of their relative isomeric proportion were also detected. The total concentration of free 4-alkylphenols ranged from 1.0 to 6.8 μg/l (average 2.5 μg/l). The whole concentration of ''bonded'' 4-alkylphenols can reach 0.66 mg/l. Consequently, the alkylphenol fraction is composed mainly of 18 differently branched 4-nonylphenol derivatives and 4-*tert*.-octylphenol. 2-Nonylphenols, 2,4-dinonylphenols, various branched 4-octyl- and 4-decylphenols which are common impurities in

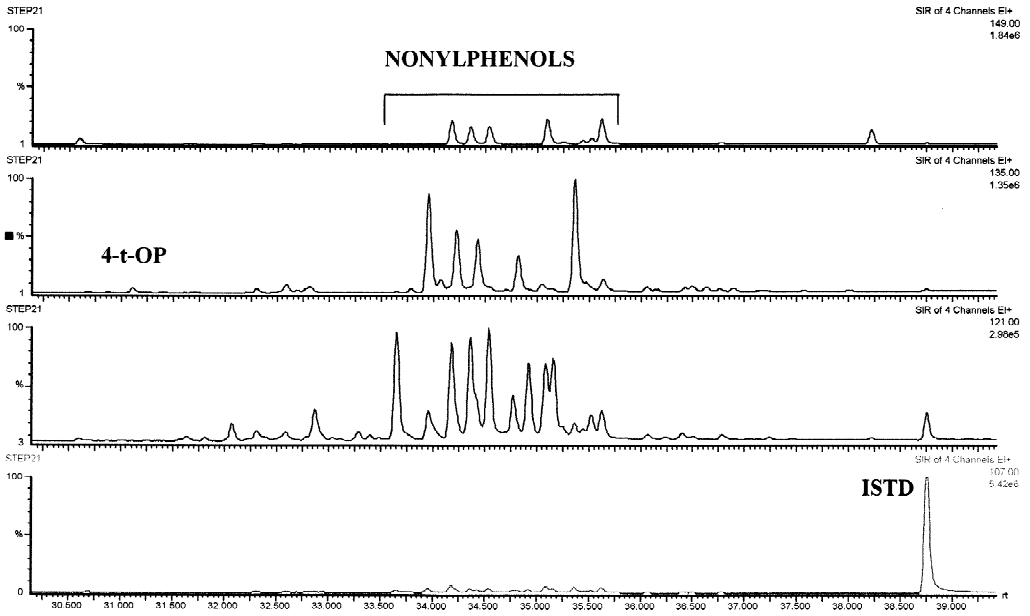

Fig. 4. GC–MS chromatogram in SIM mode of isomeric 4-nonylphenols in a typical wastewater sample.

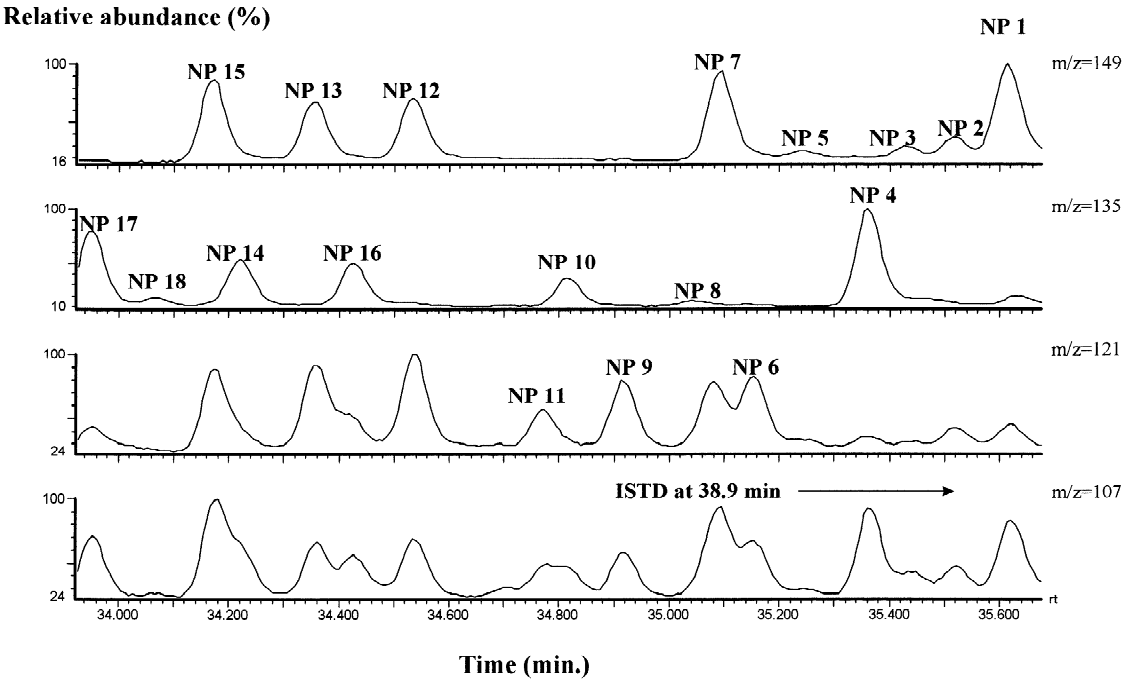

Fig. 5. Enlarged chromatogram of the isomeric 4-nonylphenol fraction from Fig. 4.

technical products (see Fig. 1) could not be detected in wastewater (see also Figs. 4 and 5).

The precision of the method and its accuracy were satisfactory with average recovery rates for all individual isomeric free and ''bonded'' 4-alkylphenols ranging from 74 to 79% and 80 to 85%, respectively.

All calibration curves were linear with correlation coefficients better than 0.988. The detection limits were slightly different depending on analyzed 4-alkylphenol isomer (0.4 to 6 ng/l), typically 2 ng/l and the quantification limits for all analyzed individual nonylphenol isomers were in the range of 2 to 22 ng/l (typically 10 ng/l). The standard deviation was also determined for each analyzed compound (below 6%).

4. Conclusion

After a primary treatment, the wastewater from the sewage plant in Aïre, Geneva, contains in average 2.5 μg/l (1.0 to 6.8 μg/l) of free 4-alkylphenols and up to 0.66 mg/l of these compounds are ''bonded'' in form of readily hydrosoluble 4-alkylphenol polyethoxylates and their metabolites. These latter compounds are degraded principally to free 4-alkylphenols in the environment [1–3]. Hence, the publications dealing only with 4-alkylphenols have probably underestimated the environmental impact of 4-alkylphenol polyethoxylates and their various hydrosoluble degradation products dissolved in wastewater. For this reason, our method of determination of the free and ''bonded'' 4-alkylphenols will be very useful for monitoring the efficiency of their biological elimination in a number of sewage plants.

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