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Trace level determination of phenols as pentafluorobenzyl derivatives by gas chromatography–negative-ion chemical ionization mass spectrometry

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A method for the trace level determination of 11 phenols as pentafluorobenzyl (PFB) derivatives by gas chromatography–mass spectrometry (GC-MS) with negative-ion chemical ionization (NICI) is described. First, the conditions for the PFB derivatisation of phenols were optimized and were found to be reaction temperature 80 °C and reaction time 5 h. Second, the detection limits using selected ion monitoring (SIM) were compared between trimethylsilylated (TMS) derivatives in the electron ionization (EI) mode and PFB derivatives in the NICI mode. The responses for the PFB derivatives in the NICI mode were 3.3–61 times higher than those of the TMS derivatives in the EI mode. The instrumental detection limits using NICI-SIM ranged from 2.6 to 290 fg. This method was applied to the analysis of phenols in river water using solid-phase extraction. The recoveries of the phenols from a river water sample spiked with standards at 100 ng l^{-1} with 2-chlorophenol, 4-chloro-3-methylphenol and pentachlorophenol and at 1000 ng l^{-1} with phenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 2-nitrophenol, 2,4,6-trichlorophenol and 4-nitrophenol were 81.2–106.3% (RSD 5.1–8.0%), except for 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol, for which the recoveries were 5.8 and 4.2%, respectively, because water contained in the acetone eluate interfered with the derivatisation of these compounds with two electrophilic nitro groups.

Introduction

In recent years, many phenolic compounds have been discharged into water, soil and sediments from a variety of industrial activities. Phenol, alkylphenols, chlorophenols and nitrophenols are the most commonly discharged products and are toxic to many organisms and the environment. If the compounds are present in waters which are used to produce drinking waters, disinfection with chlorine produces chlorophenols, many of which give taste and/or odour problems. Furthermore, alkylphenols such as 4-octylphenol and nonylphenol are considered to be endocrine disrupters (chemicals which interfere with endocrine system function).[1,2] Since phenols are of concern, it is important to monitor them in the environment.

Chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC) have been used for determining phenols.[3–8] With regard to GC, a conventional or mass spectrometric detector is used for detection. Although free phenols can be determined by GC, the generally less favourable chromatographic behaviour makes this approach undesirable, especially for chlorophenols and nitrophenols. Some workers prefer to prepare derivatives such as methyl ethers, trimethylsilyl ethers, alkyl perfluoroacyl esters, pentafluorobenzyl (PFB) ethers and pentafluorobenzoate esters.[9,10] This approach provides improved chromatographic behaviour and good sensitivity. One of the most sensitive techniques is GC with electron capture detection (ECD) after preparing fluorine-containing derivatives. The US Environmental Protection Agency (EPA) reports the analysis of nine phenols as PFB derivatives in wastewater by GC-ECD.[11] However, electron capture negative-ion chemical ionization (NICI) also provides high sensitivity and selectivity for electrophilic compounds. Therefore, GC combined with NICI mass spectrometry (MS) has been applied for the trace level determinations of electrophilic compounds containing halogens, nitro groups or highly conjugated systems in environmental samples.[12–14] Compounds which are not inherently electrophilic, with active groups, can be determined by GC-NICI-MS after preparing derivatives such as PFB ethers and pentafluorobenzoate esters.[15,16]

On the other hand, LC-MS is the most powerful technique for the determination of phenols. Derivatisation is not required and good sensitivity and selectivity are provided. However, LC-MS is not available in all laboratories. Therefore, the alternative of using GC-MS with derivatisation can still be useful. In this study, 11 phenols (phenol, 2,4-dimethylphenol, five chlorophenols and four nitrophenols) were determined by GC-NICI-MS after PFB derivatisation. Very few studies on the determination of 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol by GC-ECD or GC-NICI-MS after PFB derivatisation have been reported. The method was applied to the determination of the phenols in river water.

Experimental

Chemicals

Phenol, 2,4-dimethylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, 4-chloro-3-methylphenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2-methyl-4,6-dintrophenol were obtained from Wako (Osaka, Japan). Dichloromethane and acetone, both of pesticide grade, were purchased from Wako. Stock standard solutions of the individual phenols were prepared by diluting each compound to a concentration of 1.0 mg ml^{-1} in dichloromethane. Anhydrous potassium carbonate (K_2CO_3) was obtained from Wako, and 2

g of K_2CO_3 was dissolved in 20 ml of water obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Pentafluorobenzyl bromide (PFBBr) was obtained from GL Sciences (Tokyo, Japan), and 0.25 g of PFBBr was dissolved in 5 ml of acetone for each derivatisation. 3M Empore disks (SDB-XD, 47 mm) were used for solid-phase extraction (SPE).

Sample preparation

Solid-phase extraction. River water was collected from the Kanzaki River (Osaka, Japan), and was filtered through a 0.45 μm filter. The sample was adjusted to pH 2.0 with 1 M hydrochloric acid. The Empore disk was washed with acetone (10 ml) and was then conditioned with methanol (10 ml) and water (10 ml). The sample (100 ml) was loaded on the disk. After the extraction was complete, the disk was dried by applying a vacuum. The extract was eluted with acetone (7 ml) and the eluate was evaporated to 2 ml with a gentle stream of nitrogen.

Derivatisation. A 1 ml volume of the sample extract was transferred into a 2 ml glass vial, 10% aqueous potassium carbonate (100 μl) and an acetone solution of 5% PFBBr reagent (100 μl) were added and the vial was maintained at 80 °C for 5 h. After cooling, the volume was reduced to about 100 μl with a gentle stream of nitrogen. Dichloromethane (1 ml) was added and the organic phase was washed with Milli-Q-purified water (0.5 ml).

Instrumentation

All GC-MS analyses were carried out on an Agilent 6890/5973 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-5.625 capillary column (J&W Scientific, Folsom, CA, USA) 30 m × 0.25 mm id, 0.25 μm film thickness). Helium was used as the carrier gas with a column flow rate of 1.2 ml min^{-1} in the constant flow mode, and methane served as the CI reagent gas with a flow rate of 3 ml min^{-1}. The GC oven temperature was kept at 90 °C for 1.5 min, followed by a ramp to 160 °C at 20 °C min^{-1}, further ramp to 280 °C at 8 °C min^{-1} and a final hold for 1 min. The injection port and transfer line temperatures were kept at 250 and 280 °C, respectively, and the ion source was kept at 190 °C. Pulsed splitless injection was used with a pulse pressure of 206.8 kPa (1.1 min) and a purge time delay of 1.0 min. The mass spectrometer was operated in the NICI mode and with a scan range of *m*/*z* 10–500 at 1.55 scans s^{-1}. In the selected ion monitoring (SIM) mode, $[M-PFB]^-$ ions and the second largest ions were monitored for all compounds with a dwell time of 30–150 ms per ion. Table 1 shows SIM monitoring ions of the PFB derivatives of phenols. The injection volume was 2.0 μl.

Table 1 SIM monitoring ions of the PFB derivatives of phenols

Compound	Target ions (*m*/*z*)	Qualifier ions (*m*/*z*)
Phenol	93	94
2,4-Dimethylphenol	121	122
2-Chlorophenol	127	129
4-Chloro-3-methylphenol	141	143
2,4-Dichlorophenol	163	161
2-Nitrophenol	138	139
2,4,6-Trichlorophenol	197	195
4-Nitrophenol	138	139
2-Methyl-4,6-dinitrophenol	197	198
2,4-Dinitrophenol	183	184
Pentachlorophenol	265	263

Results and discussion

PFB derivatisation conditions

The procedure reported by Lee *et al.*[10] for the derivatisation of chlorophenols with PFBBr was applied to the 11 phenols chosen for this study. The conditions were optimized by derivatising the phenols (2 μg ml^{-1} each) with PFBBr. GC combined with electron ionization (EI) and SIM was used to evaluate the results. First, the reaction was carried out for different times (1, 2, 3, 4 and 5 h) at 60 °C. Eight phenols reacted completely after 1 h (the same conditions as in Lee *et al.*). The remaining three compounds, pentachlorophenol, 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol, partially reacted, and after 1 h the proportions remaining were 1.1, 80.0 and 80.7%, respectively. The high proportion of unreacted 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol is due to the interference of the two electrophilic nitro groups with the introduction of the the PFB group to the phenolic hydroxy group. The reaction of entachlorophenol was complete after 2 h. 2,4-Dinitrophenol and 2-methyl-4,6-dinitrophenol were not completely reacted after 5 h, the proportions remaining after this time being 32.2 and 52.0%, respectively. Second, the reaction was carried out at 80 °C, *i.e.*, 20 °C higher. The reaction times were every hour up to 12 h. The proportions of the two compounds remaining decreased considerably until 4 h, and then gradually decreased until 9 h. Although the proportions remaining were constant after 9 h, the reaction was still not complete. The proportions of 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol remaining after 9 h were 3.4 and 16.1%, respectively. Since the reaction after 9 h was not complete, a reaction time of 5 h was chosen in order to shorten the reaction time. The proportions of 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol remaining after 5 h were 9.3 and 23.8%, respectively. Fig. 1 shows the proportions of 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol remaining under the above conditions. After the derivatisation reaction, Lee *et al.* performed a complicated clean-up with silica gel column chromatography to remove the excess of derivatisation reagent when using ECD. However, by using the high selectivity of NICI, the simple clean-up

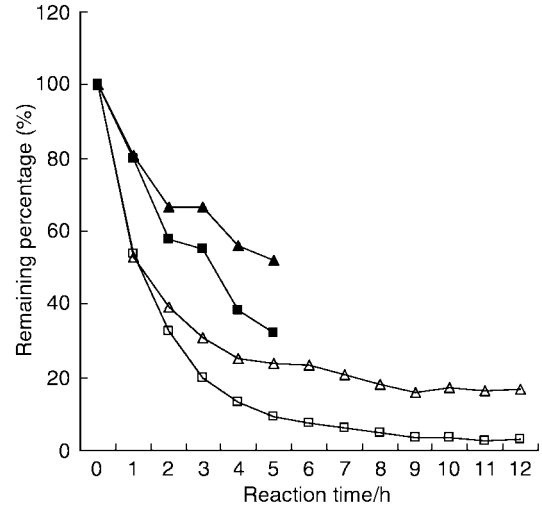

Fig. 1 Proportions of 2,4-dinitrophenol and 2-methyl-4,6-dinitrophenol remaining under the different derivatisation conditions. ▲, 2-methyl-4,6-dinitrophenol (60 °C); ■, 2,4-dinitrophenol (60 °C); △, 2-methyl-4,6-dinitrophenol (80 °C); □, 2,4-dinitrophenol (80 °C).

procedure as shown in the Experimental section provided good results.

Mass spectra of PFB derivatives (NICI) and TMS derivatives (EI)

In the NICI mode, the PFB derivatives of all the target compounds produced an $[M-PFB]^-$ ion as the sole peak by a dissociative electron capture process. The $[M-PFB]^-$ ion is, of course, characteristic of the original phenols and should be useful for quantification by SIM. In the EI mode, the TMS derivatives of all the target compounds produced a high abundance $[M-15]^+$ ion. This ion is useful for quantification by SIM. Fig. 2 shows NICI mass spectrum of the PFB derivative of 4-nitrophenol and the EI mass spectrum of the TMS derivative of 4-nitrophenol.

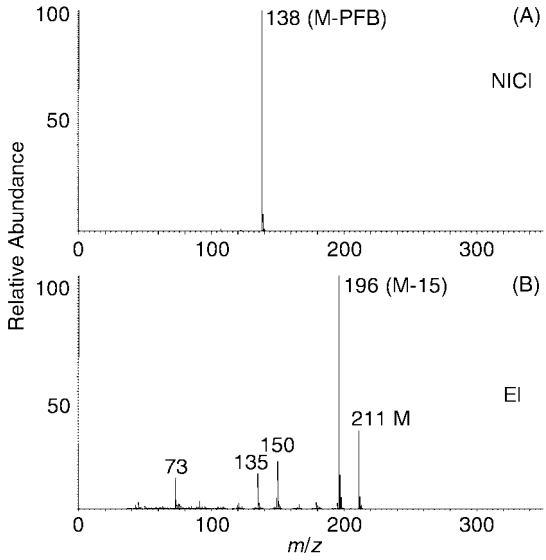

Fig. 2 NICI mass spectrum of the PFB derivative of 4-nitrophenol (A) and EI mass spectrum of the TMS derivative of 4-nitrophenol (B).

Determination of phenols in SIM mode

A comparison of the detection limit levels (at a signal-to-noise ratio of 3) between PFB derivatives by NICI-MS and TMS derivatives by EI-MS is shown in Table 2. The sensitivities of PFB derivatives were 3.3–61 times higher than those of TMS derivatives. When a blank test was run in the NICI mode, phenol, 2-nitrophenol and 4-nitrophenol were detected at levels of 870, 74 and 17 ng l^{-1}, respectively. Fig. 3 shows SIM chromatograms of PFB derivatives of the standard mixture of 11 phenols (1 ng ml^{-1} each) obtained by GC-NICI-MS and TMS derivatives of the standard mixture of 11 phenols (10 ng ml^{-1} each) obtained by GC-EI-MS.

The linearity and reproducibility of the NICI method were tested and the results are given in Table 3. The calibration curves for all the phenols as PFB derivatives were linear at 10, 20, 50, 100, 200, 500, 1000, 5000, 10 000, 50 000 and 100 000 pg ml^{-1} with correlation coefficients between 0.9981 and 1.0000. The reproducibility, expressed as relative standard deviation (RSD) (n = 6), for peak areas of all the phenols as PFB derivatives were between 3.2 and 7.2% at 50 pg ml^{-1}, 3.3 and 18.5% at 200 pg ml^{-1}, and 4.3 and 9.4% at 1 ng ml^{-1} (2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol were not detected at 50 pg ml^{-1}).

The NICI-MS method provided a wide range of linearity (over five orders of magnitude) and good reproducibility. The

Table 2 Comparison of instrumental detection limits (fg) (at S/N = 3) between PFB derivatives and TMS derivatives

Compound	PFB derivatives (NICI-SIM)	TMS derivatives (EI-SIM)
Phenol	2.6	19
2,4-Dimethylphenol	2.6	67
2-Chlorophenol	4.4	67
4-Chloro-3-methylphenol	6.9	71
2,4-Dichlorophenol	12	170
2-Nitrophenol	9.4	150
2,4,6-Trichlorophenol	40	130
4-Nitrophenol	10.5	170
2-Methyl-4,6-dinitrophenol	290	5400
2,4-Dinitrophenol	120	7400
Pentachlorophenol	12	220

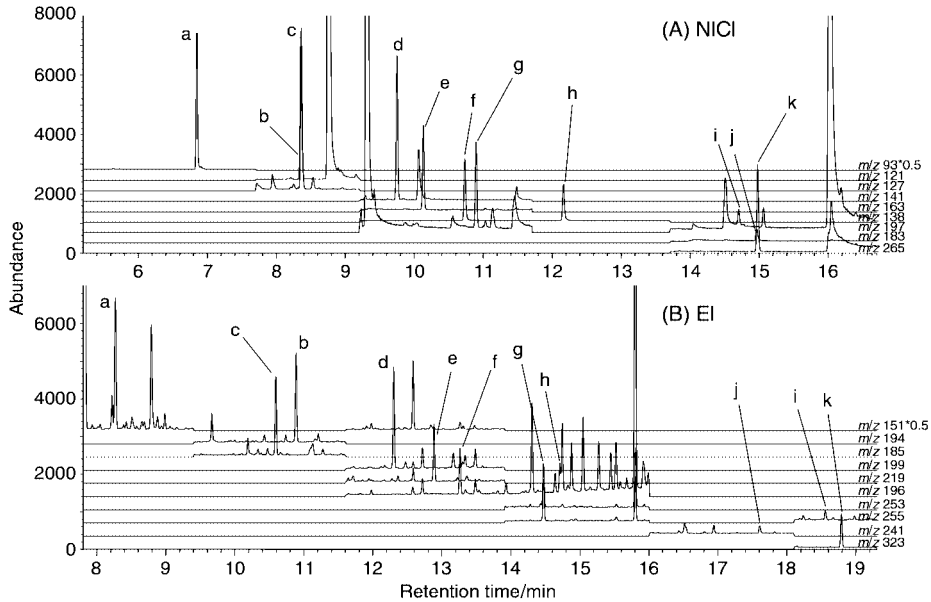

Fig. 3 SIM chromatograms of (A) the PFB derivatives of the standard mixture of 11 phenols (1 ng ml^{-1} each) and (B) the TMS derivatives of the standard mixture of 11 phenols (10 ng ml^{-1} each). Peaks: a, phenol; b, 2,4-dimethylphenol; c, 2-chlorophenol; d, 4-chloro-3-methylphenol; e, 2,4-dichlorophenol; f, 2-nitrophenol; g, 2,4,6-trichlorophenol; h, 4-nitrophenol; i, 2-methyl-4,6-dinitrophenol; j, 2,4-dinitrophenol; k, pentachlorophenol.

method also provided high sensitivity (3.3–61 times higher than the EI-MS method).

Application to river water

Interference of river water matrix. A river water sample was treated by the SPE method described in the Experimental section, and then standards (4-chloro-3-methylphenol and pentachlorophenol, 0.5 ng; 2,4-dimethylphenol, 2-chlorophenol, and 2,4-dichlorophenol, 5 ng; phenol, 2-nitrophenol, 2,4,6-trichlorophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol, 50 ng; 10–1000 ng l^{-1} as the concentrations in the river water) were added to 1.0 ml of the extract. The extract spiked with the standards and the non-spiked extract were derivatised as described in the Experimental section, and then analysed by GC-NICI-MS. Although all the phenols except 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol were detected in the non-spiked sample, these compounds could be determined without interference from the river water matrix. Fig. 4 shows NICI-SIM chromatograms of the PFB derivatives of the phenols in the river water extracts spiked with the standards and in the non-spiked river water extracts.

Recovery. The standards were added to 100 ml of a river water sample (see Table 4 for the spiked amounts; 100–1000 ng l^{-1} as the concentrations in the river water), and then the spiked sample was treated by the SPE method. Subsequently, the extract was analysed by GC-NICI-SIM after derivatisation with PFBBr. The recovery and reproducibility were tested and the results are given in Table 4. Good recoveries were obtained for 2,4-dimethylphenol, 2-chlorophenol, 4-chloro-3-methylphenol, 2,4-dichlorophenol and pentachlorophenol, with values between 85.4 and 106.3%. The recoveries of phenol, 2-nitrophenol, 2,4,6-trichlorophenol and 4-nitrophenol were higher than 100% (between 122.6 and 174.3%) because these compounds were originally present in the river water at several hundred ppt levels. For these compounds, Table 4 also shows the recovery without the influence of the originally existing amounts; the recovery was calculated by comparing the target peak areas obtained from the spiked river water with those obtained from the extract that was spiked with the standards after the SPE of the non-spiked river water. The recoveries of 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol were 5.8 and 4.2%, respectively, because water contained in the acetone eluate interfered with the derivatisation of these compounds with two electrophilic nitro groups. There was about 200 μl of water in the acetone eluate (1 ml). However, the water did not interfere with the derivatisation of the other phenols. Practical

Table 3 Correlation coefficients of calibration curves and reproducibilities (n = 6).

Compound	Correlation coefficient	RSD (%) At 50 pg ml^{-1}	At 200 pg ml^{-1}	At 1 ng ml^{-1}
Phenol	0.9986	4.6	3.3	5.4
2,4-Dimethylphenol	1.0000	4.0	4.4	4.3
2-Chlorophenol	0.9999	6.9	8.2	4.5
4-Chloro-3-methylphenol	0.9996	3.2	3.6	4.3
2,4-Dichlorophenol	0.9999	3.7	6.6	4.5
2-Nitrophenol	0.9985	6.1	5.0	5.8
2,4,6-Trichlorophenol	0.9999	7.2	7.3	4.8
4-Nitrophenol	0.9981	5.9	4.4	5.9
2-Methyl-4,6-dinitrophenol	0.9993	n.d.[a]	18.5	9.4
2,4-Dinitrophenol	0.9997	n.d.[a]	11.2	7.7
Pentachlorophenol	0.9996	6.5	6.8	7.6

[a] Not detected. Concentration range: 10 pg ml^{-1}–100 ng ml^{-1}. Injection volume: 2 μl.

Table 4 Recovery (%) of phenols from river water and reproducibility (n = 5). River water sample: 100 ml

Compound	Spiked amount/ng	Recovery (%)[a]	RSD (%)
Phenol	100	174.3 (81.2)	7.1
2,4-Dimethylphenol	100	98.9	5.1
2-Chlorophenol	10	106.3	5.3
4-Chloro-3-methylphenol	10	103.7	5.6
2,4-Dichlorophenol	100	98.1	5.5
2-Nitrophenol	100	148.1 (88.4)	7.1
2,4,6-Trichlorophenol	100	131.4 (95.7)	6.0
4-Nitrophenol	100	122.6 (92.4)	8.0
2-Methyl-4,6-dinitrophenol	100	5.8	18.4
2,4-Dinitrophenol	100	4.2	23.5
Pentachlorophenol	10	85.4	7.7

[a] Values in parentheses are recoveries without the influence of the originally existing amount in the river water.

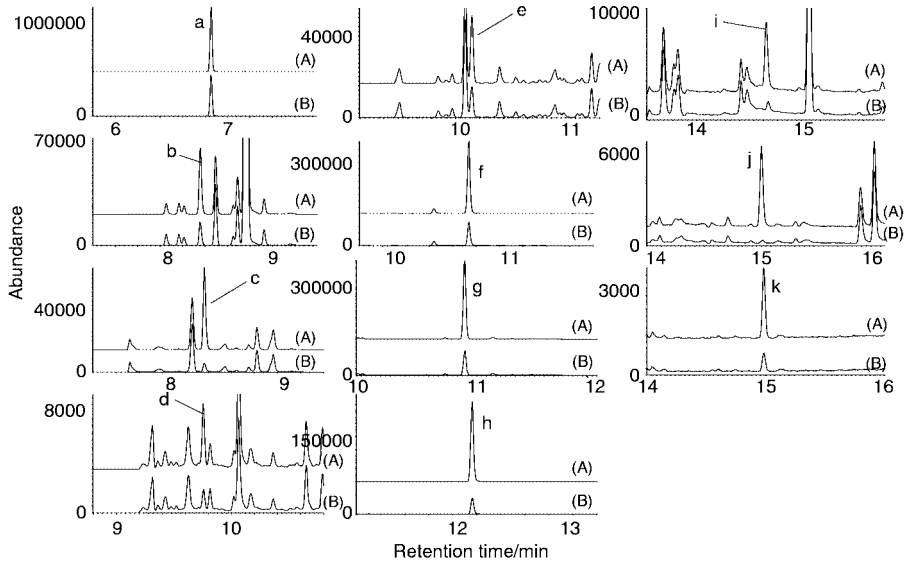

Fig. 4 NICI-SIM chromatograms of the PFB derivatives of the phenols extracted from (A) the river water spiked with the standards and (B) the non-spiked river water. Peaks: a, phenol; b, 2,4-dimethylphenol; c, 2-chlorophenol; d, 4-chloro-3-methylphenol; e, 2,4-dichlorophenol; f, 2-nitrophenol; g, 2,4,6-trichlorophenol; h, 4-nitrophenol; i, 2-methyl-4,6-dinitrophenol; j, 2,4-dinitrophenol; k, pentachlorophenol.

reproducibility was obtained for all the target compounds with RSD values ($n = 5$) between 5.1 and 8.0% for the peak areas, except for 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol.

Conclusions

A GC-NICI-MS method for the trace level determination of nine phenols in river water was developed. NICI-MS allows the detection of levels in the range 0.2–2 ng l^{-1} of the phenols as PFB derivatives in river water using SPE (50-fold concentration). For 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol, the detection limits were 290 and 120 fg for a standard mixture. However, the recoveries of these two compounds from river water were only 5.8 and 4.2%, respectively.

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