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Short communication

Analysis of nonylphenol polyethoxycarboxylates and their related metabolites by on-line derivatization and ion-trap gas chromatography–mass spectrometry

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Abstract

This study presents a modified method to analyze nonylphenol polyethoxycarboxylates (NPEC) and their related metabolites (carboxyalkylphenol ethoxycarboxylates (CNPEC)) in water samples. The method involves extraction of samples by a graphitized carbon black (GCB) cartridge, and direct derivatization in the GC injection-port using a large-volume (10–20 μl) direct sample introduction (DSI) device with tetraalkylammonium (TAA) salts. The analytes are identified and quantitated by ion-trap GC–MS. The large-volume DSI injection-port derivatization technique provides sensitivity, fast and reproducible results for NPEC and their metabolites, to quantitation at 0.1 μg/l in 200 ml of water samples. The retention effect of TAA salts in the injection-port is not detected. In addition, the significant $[M-29]^+$ ions and molecular ions of butylated NPEC and CNPEC residues are observed. Recovery of NP1EC in spiked water samples ranges from 90 to 108%. Moreover, relative standard deviations of replicate analyses ranges from 1 to 9%. However, unsatisfactory on-line derivatization of CNPEC residues is observed. This finding maybe owing to their lesser dissociation with the ion-pair reagent in chloroform.

Keywords: Derivatization, GC; Nonylphenol polyethoxylates; Carboxyalkylphenol ethoxycarboxylates; Tetraalkylammonium salts

1. Introduction

Nonylphenol polyethoxylates (NPEO) are widely used nonionic surfactants. In Taiwan, more than 48×10^6 kg of NPE-type surfactants were produced and used in diverse industrial, household and commercial applications in 1996 [1]. Taiwan's deficient municipal wastewater treatment leads to a significantly higher concentration of NPE-type residues in Taiwanese rivers than in those of other countries [2,3]. Also, due to the estrogenic activity of these biodegradation products [4,5] and their persistence in the aquatic environment [6–8], a convenient analytical technique must be developed to study the occurrence and composition of NPE-type residues which are directly discharged into the aquatic environment with untreated wastewater.

Nonylphenol polyethoxycarboxylates (NPEC) and carboxyalkylphenol ethoxycarboxylates (CNPEC), two major groups of biorefractory metabolites of NPEO, could be regarded as potential precursors to the formation of nonylphenol under various bio-

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transformation conditions. Herein, dicarboxylic acids of NPE residues (CNPEC) represented the biodegradation products which was oxidized on both side chains (the ethoxy and the alkyl chains) of NPEO [2,7,8]. The structures of branched nonylphenoxy ethoxy acetic acid (abbreviated as NP2EC) and branched nonanoic phenoxy ethoxy acetic acid (abbreviated as C9-CNP2EC) are presented in Fig. 1 as examples of this group of compounds. Liquid chromatography [9–12] and LC–MS techniques with thermospray [13], particle-beam [14] or electrospray ionization (ESI) interface [8,12] have been successfully applied to analyze such compounds in a variety of matrices. A large amount of NPEC residues with four to seven degrees of ethoxylation can be detected by LC–MS techniques without chemical derivatization. However, as GC–MS is a more readily available technique in many environmental laboratories, and provides a higher chromatographic resolution with a capillary column, considerable efforts have been made to overcome the volatility and polarity problems when applying GC to these compounds. One approach is to convert the analyzed compound to its corresponding esters.

Previous analytical approaches to determine the amount of NPEC and CNPEC residues in aqueous samples involved extracting the samples by a graphitized carbon black (GCB) cartridge. The extract would then be esterified by a propanol–acetyl chloride procedure off-line [2,3]. This procedure was generally time-consuming and required highly reactive reagents (i.e., acetyl chloride) to convert carboxylic acids into their corresponding ester derivatives. An alternative derivatization method has been studied with a direct GC injection-port derivatization using ion-pair reagents for aliphatic and aromatic acids [15–17]. The derivatization was initiated by reacting carboxylic acid groups with TAA salts (i.e., tetrabutylammonium hydrogen sulfate, $N(Bu)_4^+HSO_4^-$) to form carboxylate ion pairs [$RCOO^-N(Bu)_4^+$] in solution. Upon introduction to a high temperature (300°C) GC injection-port, the carboxylic acid groups were converted to their corresponding butyl esters [RCOOBu].

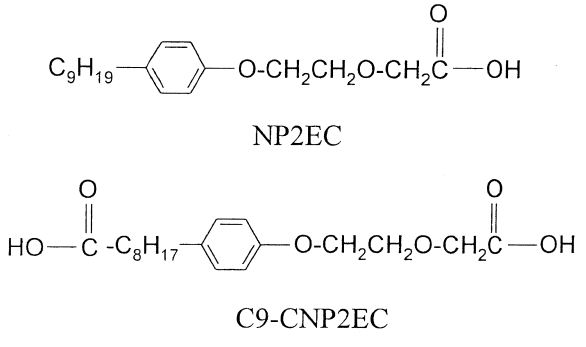

Fig. 1. The structures of NP2EC and C9-CNP2EC as examples of NPEO metabolites detected in the samples of river water.

This work presents the preliminary results of a modified method to rapidly and quantitatively determine NPEC and CNPEC residues in aqueous samples. The method involves extraction of NPEC and CNPEC residues from water samples by GCB-SPE and derivatization NPEC and CNPEC residues with TAA salts in the GC injection-port by a large-volume direct sample introduction (DSI) device.

2. Experimental

2.1. Samples

Water samples containing a high concentration of primary industrial effluents (specific conductance, 2180 μS/cm) were collected from a river that receives effluents from industrial wastewater treatment plants in Tai-Yuan Industrial Park, Tao-Yuan County (Taiwan). Samples of river water (specific conductance, 740 μS/cm), polluted by surfactants, were collected from Lao-Jie River at Chung-Li city (Taiwan). In this city, untreated municipal wastewater is discharged directly into the river. Duplicate 500-ml samples were collected and shipped to the laboratory in ice-packed containers. Upon arrival, the samples were immediately adjusted to pH 2–3 by adding concentrated HCl, and then stored at 4°C until analysis.

2.2. Chemicals and reagents

Unless specified otherwise, all high purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany), and were used

without further purification. Reagent-grade tetrabutylammonium hydrogen sulfate (TBA-HSO_4) was purchased from TCI (Tokyo Chemical Industry, Tokyo, Japan). The surrogate *tert.*-octylphenoxy acetic acid (OP1EC) and NP1EC mixture for NPEC recovery studies were synthesized as described by Fujita [18].

2.3. Sample extraction

Details of the procedure using graphitized carbon black cartridges (GCB or ENVI-carb, trade name from Supelco, USA) to extract NPEC and CNPEC residues from the water samples can be found elsewhere [2,3,12]. Acidified 200-ml spiked samples were passed through the GCB cartridge at a flow-rate of about 10–20 ml/min with the aid of a vacuum. The NPEC and CNPEC residues were eluted from the cartridge with 7 ml of methylene chloride–methanol (9:1, v/v) eluent modified with 25 m*M* formic acid. Next, the extract of NPEC and CNPEC residues was completely evaporated to dryness by a stream of nitrogen. The residues were then redissolved in 100 μl of chloroform with 10 m*M* TBA-HSO_4, and made ready for GC–MS analysis.

2.4. GC–MS analysis

Analyses were performed on a Varian 3400CX gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometry (Varian, USA). Large-volume samples were introduced with a direct sample introduction (DSI) device (or ChromatoProbe, from Varian, USA) as described by Ding and coworkers [2,19]. A 10–20 μl portion of sample solution was introduced into GC by a DSI device. The injection split ratio was 1:10. A DB-5MS capillary column (30 m×0.25 mm I.D., 0.25 μm film, from J&W, USA) connected to a 2-m deactivated fused-silica per-column, was used. The injector temperature, GC temperature program and the conditions of ion-trap MS system can be found elsewhere [19]. The precision of the injection-port derivatization and the GC–MS analysis, as determined from the relative standard deviation (RSD) of over 50 injections of NP1EC, ranged from 3 to 15%.

3. Results and discussion

3.1. GC–MS of NPEC and CNPEC residues

Details on how to evaluate the tetraalkylammonium salts and the conditions of the injection-port can be found elsewhere [19]. Herein, TBA-HSO_4 reagent was chosen because butylated NP1EC produced the highest average peak areas and quantitative results. The studies employed a sample volume of 20 μl and an injection temperature of 300°C.

Fig. 2 depicts the full-scan EI mass spectra of the butylated NPEC residues by TBA-HSO_4. The characteristic ions are produced by benzylic cleavages of $[M-71]^+$ and $[M-85]^+$, corresponding to the ions of m/z 249 and 263 for NP1EC isomers, ions of m/z 293 and 307 for NP2EC isomers, and ions of m/z 337 and 351 for NP3EC isomers. The molecular ions were weak, but observed for all butylated NPEC residues. The relative abundance of $[M-29]^+$ ions was also determined and was attributed to the loss of an ethyl group from the butyl ester side. This double-hydrogen rearrangement mechanism, resulting in the loss of an alkyl group, is common for propyl or a higher ester [20]. Fig. 3 shows the EI mass spectra of the butylated CNP1EC and CNP2EC, labeled as C9 (eight-carbon unit plus one carboxylic group) and C7 (six-carbon unit plus one carboxylic group) of CNPEC, from the sample of industrial effluent. The most significant ions in EI-MS are also produced by benzylic cleavages of $[M-71]^+$ and $[M-85]^+$, corresponding to the ions of m/z 249 and 263 for CNP1EC isomers and ions of m/z 293 and 307 for CNP2EC isomers. The molecular ions were observed for all butylated CNPEC residues. They can be used to distinguish between NPEC and CNPEC residues of the same benzylic cleavages. The molecular mass determination can be easily confirmed by the ions of $[M-29]^+$ and $[M]^+$ in the complex environmental samples. Herein, the C7-CNP1EC and C7-CNP2EC were determined by the molecular ions at m/z 392 and 436, while C9-CNP1EC and C9-CNP2EC were determined by the molecular ions at m/z 420 and 464, respectively. Fig. 4 displays the selected mass chromatograms of the butylated NPEC and CNPEC residues from industrial effluent. The patterns of NP1EC, NP2EC, NP3EC

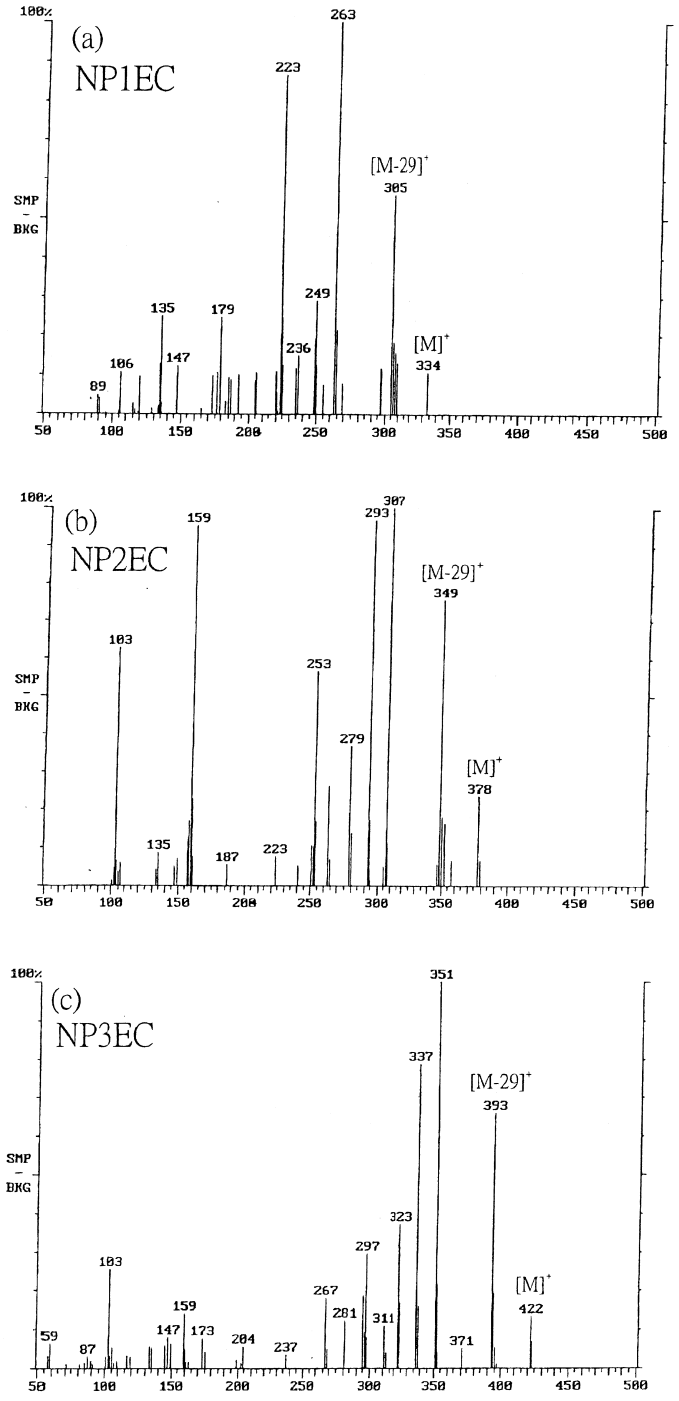

Fig. 2. Full-scan EI mass spectra of the butylated NPEC residues detected in the sample of river water polluted by industrial effluent.

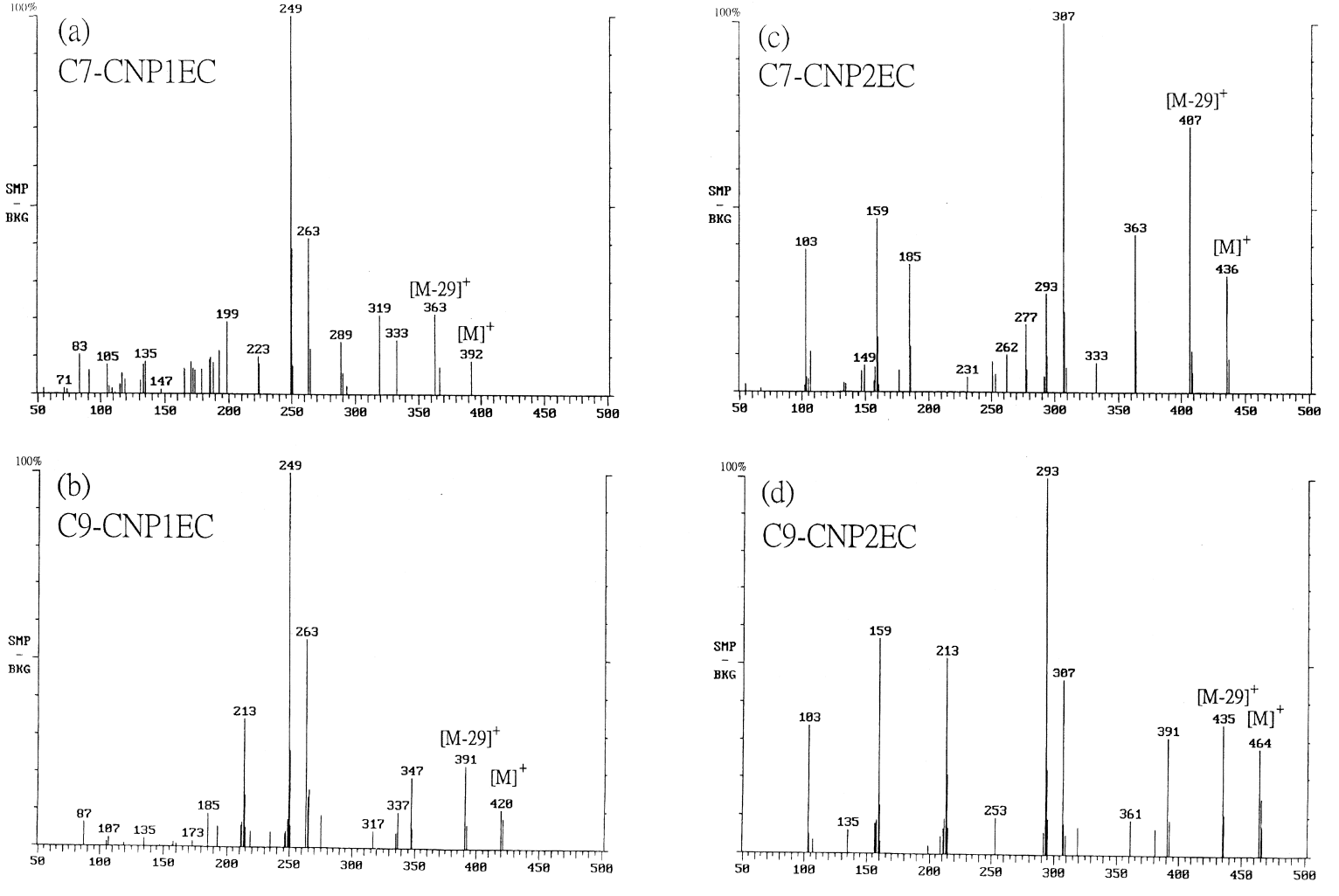

Fig. 3. Full-scan EI mass spectra of the butylated CNPEC residues detected in the sample of river water polluted by industrial effluent.

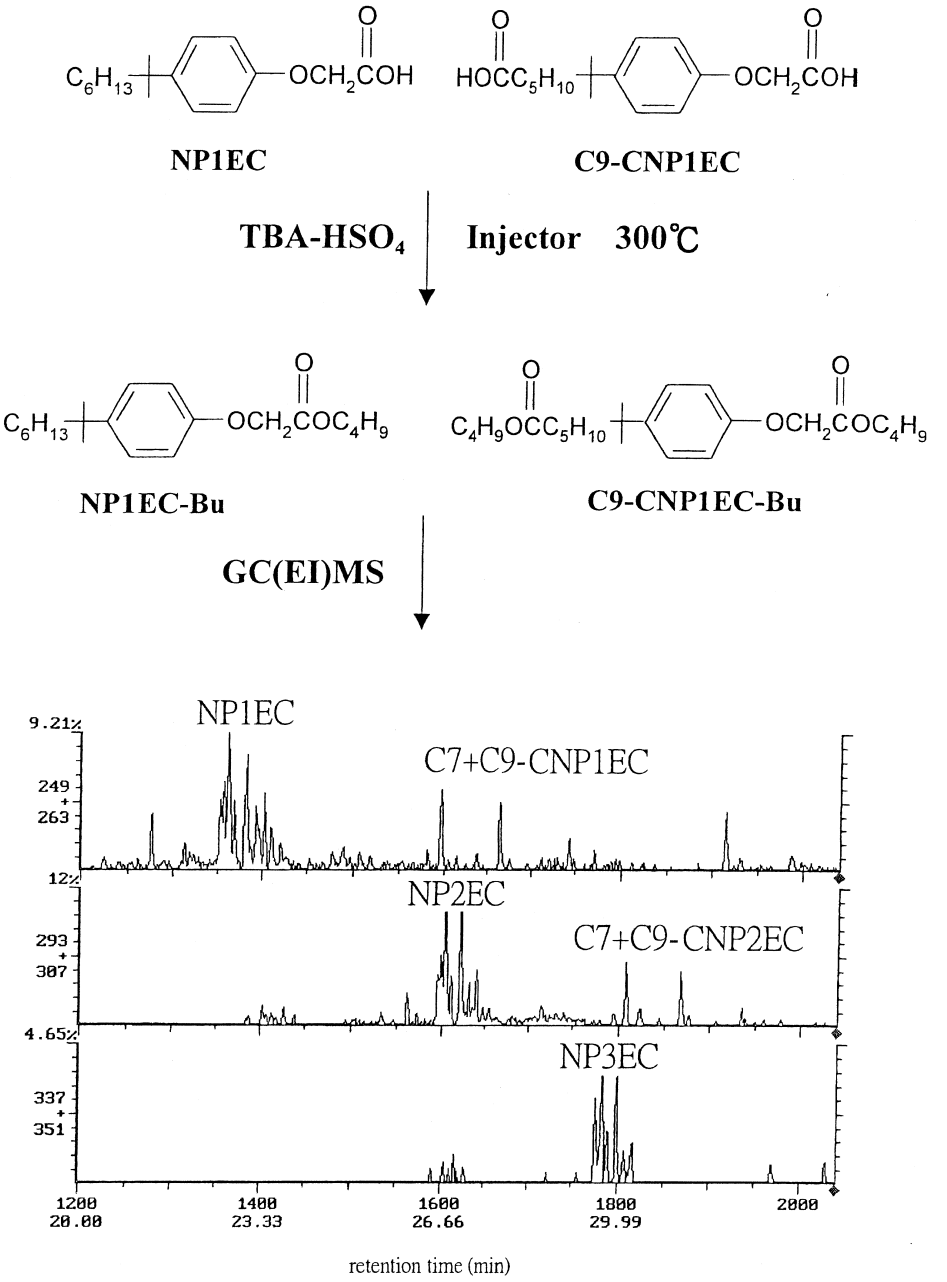

Fig. 4. Flow diagram of the on-line derivatization and the selected characteristic mass chromatograms of the butylated NPEC and CNPEC residues isolated from the sample of river water polluted by industrial effluent.

and their corresponding C7- and C9-CNPEC residues are efficiently derivatized and clearly depicted. The NPEC and CNPEC residues in water samples were semi-quantified by comparing the butylated OP1EC peak area to the sum of total peak areas of two EI selected characteristic ion chromatograms (i.e., ions of m/z 249+263 for NP1EC and C7+C9-CNP1EC) as described elsewhere [2,3].

3.2. Recovery study and application to environmental samples

The quantitation limit of butylated OP1EC by EI-MS was 0.1 ng/μl, defined at a signal-to-noise ratio (S/N)≥10. The method for 1.0–100 ng/μl of the total butylated NP1EC was accurate to within 6% RSD, based on the total peak areas of the quantitation ions at m/z 249+263. The recovery from GCB-SPE was evaluated by a spiked known amount of synthetic OP1EC (as surrogate) and NP1EC mixture in deionized water. Three replicate 200-ml deionized water samples were each spiked to obtain the final concentrations of 5 μg/l of OP1EC and 50 μg/l of NP1EC mixture. The degree of recovery of NP1EC was calculated by the calibration curve (or average standard response factor) that was calculated from four levels of NP1EC mixture from 1.0 to 50 ng/μl, each divided by the fixed concentration of the OP1EC surrogate. The average recovery was 97% with 1% RSD. The recoveries of NP1EC from environmental samples were evaluated by standard addition experiments. Three replicate 200-ml samples of river water polluted by industrial effluents and surfactants, were used in the investigation. Table 1 summarizes the average percentage recoveries of NP1EC in the environmental samples with different specific conductances, as well as their estimated average background concentrations. Recovery of NP1EC ranged from 90 to 108%, with RSD ranging from 7 to 9%. The concentrations given were estimated on the assumption that the response factors for the OP1EC and the analytes were equal because the authentic standards for NP2EC, NP3EC and corresponding CNPEC residues were unavailable. The RSDs of three replicate environmental sample analyses ranged from 1 to 8%. Analyses results indicated that neither the high ionic contents nor the variable compositions of environmental samples affected the extraction efficiency of GCB. The direct injection-port derivatization with the ion-pair reagent procedure, simplified the preparation of the calibration standard and quantitation of the sample.

Di Corcia et al. recently showed that di-acid NPE residues persisted in the test liquor for more than 5 months after their generation [9]. The concentration pattern of CNPEC residues should be higher than that of NPEC residues, which finding is consistent with our field studies [2,3]. The CNPEC residues are the most abundant NPEO biodegradation products in the environmental water samples. However, the relatively lower concentration pattern of CNPEC residues was determined in this study, using the on-line derivatization, as shown in Table 1 and Fig. 4. No improved results were detected with increased concentrations of the TBA-HSO_4 reagent. The *n*-butanol–acetyl chloride derivatization procedure was applied to the same water samples to compare the results of on-line and off-line derivatization. Interestingly, off-line derivatization yielded CNPEC residues with concentrations which were three- to five-times higher than those yielded by on-line derivatization (Table 2). This result may be explained by the CNPEC residues' being more hydrophilic and less dissociated in chloroform, than mono-acids. Therefore, fewer carboxylate ion pairs [$RCOO^-N(Bu)_4^+$] of CNPEC residues were produced in the solution, and they were unsatisfactorily

Table 1
Estimated average concentrations of NPEC and CNPEC residues, and recovery results of NP1EC spiked in different water samples[a]

Sample	NP1EC	NP2EC	NP3EC	C7+C9-CNP1EC	C7+C9-CNP2EC
Deionized water					
Spiked recovery	97% (1%)				
Surfactant polluted water					
Average conc. (μg/l)	10.2 (4%)	30.5 (2%)	1.0 (8%)	4.3 (5%)	19.8 (4%)
Spiked recovery (%)	90% (9%)				
'*Industrial effluent*'					
Average conc. (μg/l)	27.0 (2%)	78.3 (1%)	1.4 (3%)	1.8 (2%)	5.3 (6%)
Spiked recovery (%)	108% (7%)				

[a] The relative standard deviation (RSD) is given in parentheses, n=3.

Table 2
Comparison between on-line and off-line derivation for the concentrations (μg/l) of analytes in the sample of river water polluted by industrial effluent[a]

Analytes method	On-line derivatization	Off-line derivatization
NP1EC	29 (2%)	27 (9%)
NP2EC	32 (7%)	30 (15%)
NP3EC	6 (1%)	8 (11%)
C7+C9-CNP1EC	21 (17%)	66 (2%)
C7+C9-CNP2EC	19 (12%)	92 (5%)

[a] The relative standard deviation (RSD) is given in parentheses, $n=3$.

derivatized with less than 20–30% in GC injection-port. Our work strongly suggests that the on-line derivatization technique could facilitate a convenient analytical technique for trace determination of NPEC residues in complex environmental samples, but not for trace determination of CNPEC residues.

In conclusion, this work demonstrates that GCB solid-phase extraction and injection-port derivatization using a large-volume direct sample introduction (DSI) device with tetraalkylammonium salts, is a rapid and quantitative method for determining trace levels of NPEC residues in aqueous samples. The method significantly reduces the solvent waste and simplifies the sample preparation requirements. The method can be applied to other carboxylated environmentally important chemicals. In our laboratory, chlorinated herbicides can be easily esterified and routinely determined in water samples without involving hazardous reagents when using the same method.

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