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# New sample treatment for the determination of alkylphenols and alkylphenol ethoxylates in agricultural soils

I. Jiménez-Díaz [a], O. Ballesteros [a], A. Zafra-Gómez [a], G. Crovetto [a], J.L. Vílchez [a], A. Navalón [a,*], C. Verge [b], J.A. de Ferrer [b]

[a] Research Group of Analytical Chemistry and Life Sciences, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain
[b] Cepsa Química. Avda. del Partenón 12, 28042 Madrid, Spain

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ABSTRACT

A new sample treatment for alkylphenols (AP) and alkylphenol ethoxylates (APEO) determination in agricultural soil samples has been developed. In a first stage these compounds were isolated from soil by pressurized liquid extraction (PLE) using methanol. In a second stage the extracts were cleaned up and pre-concentrated by solid-phase extraction (SPE) using ENV + cartridges. The effect of different variables on PLE and SPE was also studied. In the last place, separation and quantification of analytes were performed by liquid chromatography with fluorescence detection (LC–FD) and gas chromatography coupled to mass spectrometry (GC–MS). Quantification limits (QL) ranged from 20 to 200 ng $g^{-1}$ for LC–FD and from 3 to 126 ng $g^{-1}$ for GC–MS. This method was satisfactorily applied in a study field designed to evaluate the environmental behaviour of APEOs in agricultural soils.



## 1. Introduction

Alkylphenol ethoxylates are non-ionic surfactants widely employed for commercial and domestic use, with a worldwide production of about 500 kilotons (Petrovic and Barceló, 2001; Langford et al., 2007). In 1995, a voluntary ban on APEO use in household cleaning products was introduced in northern Europe, followed by restrictions on industrial cleaning use in 2000 (Renner, 1997; OSPAR, 2006). However, octylphenol ethoxylates (OPEO) and nonylphenol ethoxylates (NPEO) remain two of the most common surfactants in commercial use as evidenced by their continuous discharge into sewage treatment works (STW) (Cheng et al., 2006; Cailleaud et al., 2007) and into the environment (Gigson et al., 2005; Langford et al., 2005). Environmental concerns about these compounds derive from their potential endocrine effects (Jones-Lepp et al., 2000; Sumpter, 2002). APEO biodegradation during wastewater treatment can occur under both aerobic and anaerobic conditions resulting in more persistent and estrogenic metabolites: short chain APEO ($AP_1EO$, $AP_2EO$ and $AP_3EO$), carboxylated intermediates (APEC) and alkylphenols (AP) including nonylphenol (NP) and octylphenol (OP) (González et al., 2007; Langford et al., 2007). Although central fission is also possible, aerobic biodegradation typically occurs via sequential removal of ethoxylate units leading to lower mole ethoxylates. Only minor amounts of AP appear under aerobic conditions. In contrast, APEC and low mole APEO are often seen as dominant intermediates. Although the mechanism involved in the ultimate degradation of low mole ethoxylates and ether carboxylates is unclear, it is likely that ring fission occur at this stage (Naylor et al., 2006). The pathway for anaerobic biodegradation of APEO favours the formation of low mole ethoxylates and AP. Some investigations suggest AP can be further degraded to methane and carbon dioxide (Salanitro and Díaz, 1995; Chang et al., 2004).

Analysis of alkylphenolic compounds is complicated due to the different ethoxylate oligomers and alkyl-chain isomers present in their structure. In complex matrices is a complicated task not only because of the complexity of the samples but also because these compounds are generally found in very low concentrations. Therefore, there is a need to develop methods that permit to improve isolation and extraction of these compounds.

For AP and APEO extraction from solid samples (sewage sludge, sediment, soil) different analytical protocols have been proposed in literature. Soxhlet extraction and steam distillation have been replaced by efficient and versatile sonicated extraction systems (Blackburn et al., 1999; Petrovic et al., 2002a) and supercritical fluid extraction (SFE) (Kreisselmeier and Duerbeck, 1997; Bennett and Metcalfe, 2000). Recent articles report the use of efficient semi- or fully automated continuous-flow–high-temperature sonication (Lee Ferguson et al., 2000) and subcritical hot-water

* Corresponding author. Tel.: +34 958 243326; fax: +34 958 243328.
*E-mail address:* anavalon@ugr.es (A. Navalón).

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extraction (Field and Reed, 1999). PLE has proven a suitable alternative method for AP and APEO extraction (Shang et al., 1999; Valsecchi et al., 2001; Petrovic et al., 2002b) and also has the advantage of the small amounts of solvent required, the reduction in extraction time and the considerable improvement in operator safety compared to other extraction procedures.

Quantification of these compounds has been usually performed using GC–MS and LC coupled to different detection systems. The use of GC for direct analysis is limited to APEO with lower numbers of ethoxy groups, while long-chain ethoxylates require derivatization in order to increase volatility (Jungclaus et al., 1978; Bjoerseth and Angeletti, 1986; Rivera et al., 1987; Bhatt et al., 1992). LC–FD (Ahel et al., 2000; Marcomini et al., 2000) and LC–UV (Zhou et al., 1990) have been also widely used. However, these techniques sometimes lack the sensitivity and specificity required at low concentrations. LC coupled with tandem mass spectrometry (LC–MS/MS) is increasingly being used and several methods have been recently reported (Houde et al., 2002; Loyo-Rosales et al., 2003; Petrovic and Barceló, 2003; Schitz-Afonso et al., 2003; Jahnke et al., 2004).

In the present study, a sensitive analytical method for simultaneous determination of 4-*n*-nonylphenol (4-*n*-NP), 4-*n*-octylphenol (4-*n*-OP), 4-*t*-octylphenol (4-*t*-OP) and 4-*t*-octylphenol ethoxylates (4-*t*-OPEO) was developed. We focussed in the optimization of extraction and clean up parameters. Quantification was performed using LC–FD and GC–MS. The proposed method was applied to determine AP and APEO's presence in agricultural soil samples taken from various sites of the irrigated plain of Granada (Spain). Since none of the target compounds were found in these samples, we designed a study field that allow us to study their behaviour in agricultural soil.

## 2. Materials and methods

### 2.1. Chemicals

All chemicals were analytical grade unless otherwise specified. Water (18.2 MΩ $cm^{-1}$) was purified using a Milli-Q plus system from Millipore (Bedford, MA, USA). 4-*n*-NP was obtained from Alfa Aesar (Karlsruhe, Germany); 4-*t*-OP from Fluka (Buchs, Switzerland) and 4-*n*-OP from Dr. Ehrenstorfer (Augsburg, Germany). OPEO were only available in the form of technical mixtures containing a range of oligomers. These mixtures were Igepal CA-520 and Igepal CA-210, both obtained from Sigma–Aldrich (Madrid, Spain). The composition of these mixtures was supplied by Cepsa Química S.A. (Igepal CA-210: 4-*t*-$OP_1$EO 76.82%, 4-*t*-$OP_2$EO 20.37%, 4-*t*-$OP_3$EO 2.14% and 4-*t*-$OP_4$EO 0.67%; Igepal CA-520: 4-*t*-$OP_1$EO 0.68%, 4-*t*-$OP_2$EO 8.54%, 4-*t*-$OP_3$EO 18.85%, 4-*t*-$OP_4$EO 22.58%, 4-*t*-$OP_5$EO 18.79%, 4-*t*-$OP_6$EO 13.35%, 4-*t*-$OP_7$EO 8.57%, 4-*t*-$OP_8$EO 4.76%, 4-*t*-$OP_9$EO 2.26%, 4-*t*-$OP_{10}$EO 0.98%, 4-*t*-$OP_{11}$EO 0.31% and 4-*t*-$OP_{12}$EO 0.34%). Acenapthene, obtained from Sigma Aldrich, was used as internal standard.

Standard stock solutions were prepared in methanol. Acenapthene was prepared in ethyl acetate. They were stored in the dark at 4 °C. The solutions were stable for at least six months. All glassware was cleaned with chromic acid in order to avoid contamination. Working standards were prepared right before use diluting standard stock solutions with methanol.

Methanol (HPLC gradient-grade), tetrahydrofurane, acetone, *n*-hexane and formaldehyde (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Sodium dihydrogen phosphate, o-phosphoric acid and dichloromethane were supplied by Panreac (Barcelona, Spain). Diethyl ether was purchased from J.T. Baker (Deventer, Holland) and ethyl acetate from Riedel-de-Haën (Madrid, Spain). N,O-bis(trimethylsilyl)trifluoro acetamide (BSTFA) was supplied by Sigma–Aldrich.

Isolute $C_{18}$ (500 mg/3 mL) and ENV + (200 mg/3 mL) SPE sorbent cartridges were purchased from Isolute Sorbent Technologies (Mid Glamorgan, UK).

### 2.2. Instrumentation and software

LC–FD analysis was carried out on an Agilent Technologies (Palo Alto, CA, USA) 1100 series high performance liquid chromatograph equipped with a quaternary pump, an on-line degasser, an autosampler, an automatic injector, a thermostated column compartment and a fluorescence detector (flow-cell volume 8 μL) connected on-line. ChemStation for LC 3D software (Agilent) was used for instrument control, data acquisition, and analysis.

GC–MS analysis was performed on a 6890 Agilent gas chromatograph with a 7683 series injector and a quadruple mass filter 5976 network mass selective detector (MSD). HPCHEM chromatography software was used for data acquisition and integration.

All pH measurements were made with a Crison (Crison Instruments S.A, Barcelona, Spain) combined glass-saturated calomel electrode using a Crison 2000 digital pH-meter previously calibrated. A Büchi R-200 Rotavapor (Flawil, Switzerland) equipped with a B-490 heating bath and a vacuum pump unit and a Hettich Universal 32 centrifuge (Tuttlingen, Germany) were also used. A Supelco (Madrid, Spain) vacuum manifold for 12 columns connected to a Supelco vacuum tank and to a pump was used for SPE.

Statgraphics Centurion XV, vs. 15.1.02 software package (1982–2006) Statpoint Inc., was used for statistical and regression analysis (linear mode).

### 2.3. Sampling and sample pre-treatment

Soil samples were collected from 15 plots at different locations in the fertile plain of Granada: Belicena (two samples); Churriana de la Vega (three samples); Cúllar Vega (two samples); Granada (one sample); Las Gabias (three samples); La Malahá (one sample); Otura (one sample) and Vegas de Genil (one sample). All the samples were collected from the arable layer.

The study field was carried out in a 4 $m^2$ plot of land situated in the irrigated plain of Granada (commonly known as "Vega de Granada"). The first day of the study the plot was irrigated with 140 L of water containing 100 and 40 g of Igepal CA-520 and Igepal CA-210, respectively. Subsequent irrigations were made with the same quantity of water alone, twice a day during the first week, and then once a day until the end of the study. Soil samples were collected at different depths (from ground surface to 60 cm) and times after irrigation with the analytes.

The soil had not received pesticides or herbicides in the last 5 years nor ever received sewage sludge. Soil samples were collected at different depths (from ground surface to 60 cm) using a drill. They were then placed in plastic containers, 3% (v/v) formaldehyde immediately added for conservation. In the laboratory the soil was dried at room temperature for 48 h, then ground in a mortar with a pestle and passed through a 30-mesh sieve in order to enhance analytes' extractability. Soil samples were stored in the dark at 4 °C until analysis.

### 2.4. Preparation of spiked soil samples

Soil samples were accurately weighed and placed in glass flasks. Spiked soil samples were prepared by adding 2 mL of a solution containing the analytes (4-*n*-OP, 4-*t*-OP, 4-*n*-NP, Igepal CA-520 and Igepal CA-210) to 5.0 g of each sample. The bulk of solvent was slowly evaporated at room temperature during 12–15 h and