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Optimization of comprehensive two dimensional gas chromatography-flame ionization detection–quadrupole mass spectrometry for the separation of octyl- and nonylphenol isomers

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ABSTRACT

In the present work, the separation of complex nonylphenol technical mixtures has been optimized using comprehensive two-dimensional gas chromatography coupled with a flame ionization detector and quadrupole mass spectrometer (GC × GC–qMS), using valve-based modulator. The optimization of GC × GC–qMS has been carried out using experimental designs and the optimal separation was obtained at the following conditions: 1st column flow: 1 mL/min; 2nd column flow: 17.75 mL/min, oven temperature ramp: 1 °C/min, modulation period: 1.5 s and discharge time: 0.12 s. These values have been used to determinate the previously synthesized 22OP, 33OP, 363NP and 22NP isomers in two different nonylphenol technical mixtures. Percentages obtained were as follows: 4.86% and 0.59% for 22OP, 4.91% and 2.82% for 33OP, 11.79% and 7.71% for 363NP and 2.28% and 1.98% for 22NP, in Fluka and Aldrich mixtures, respectively. The values obtained for NP isomers are in good agreement with the literature.

1. Introduction

Alkylphenol ethoxylates (APEOs) are surfactants that have been widely used as detergents, emulsifier and dispersing agents in industrial, commercial or household applications [1]. These compounds are degraded aerobically or anaerobically in wastewater treatment plants (WWTPs) and more toxic compounds, such as nonylphenols (NPs) and octylphenols (OPs), are obtained [2–4]. Although the main source of NPs and OPs is the degradation of APEOs, they have also been used in the production of plastics and phenolic oximes [5,6]. Commonly, NPs and OPs appear in a mixture of different isomers where the main isomers are *para*-substituted [7,8].

The interest in NPs and OPs has increased during the last decades due to their capacity to disrupt the endocrine system [9–14], and for this reason, they have been included in the water framework directive (WFD) as priority hazardous substances [15].

According to the literature, different isomers of alkylphenols show different estrogenicities [16–22] which can be attributed to the position and branching of the alkyl group [23] which varies the physic-chemical properties [7] (for instance, $\log k_{ow}$ can vary from 4.8 to 5.7 for NP isomers [24] and from 5.3 to 5.5 for OP isomers [25]). Therefore, an effort should be made on the analysis of individual isomers using techniques such as GC × GC since their separation is not possible by means of one-dimension GC or liquid chromatography [26–29] where, traditionally, the sum of the total concentration of the isomers is measured. Nevertheless, the synthesis and separation are not easy and the quantity of the isomers changes depending on the author and the technical mixture. Guenther et al. [30] have characterized up to 211 possible constitutional isomers of NP according to a hierarchical and logical system, although it is thought that not all the isomers are included in the commercial technical mixture. Wheeler et al. [8] were able to characterize 22 *para*-isomers using high resolution capillary gas chromatography–mass spectrometry (HRCGC–MS). Thiele et al. [31] separated 10 isomers using, as Wheeler and co-workers, a 100 m capillary column and a GC–MS. However, the works that provide the maximum number of separated isomers are based on comprehensive two dimensional gas chromatography (GC × GC). This technique is able to separate the analytes by compounds boiling point and polarity. This way, Ieda et al. [32] separated 102 peaks and 13 compounds were characterized using comprehensive two-dimensional gas chromatography-mass spectrometry (GC × GC–qMS). Moeder et al. [33] separated 40 peaks using GC × GC coupled to time-of-flight mass spectrometry (GC × GC–TOF-MS) and, more recently, Egan-

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house and co-workers [7] were able to separate from 153 to 204 peaks using a GC × GC–TOF-MS and around 59 to 66 were identified as *para*-NPs.

As illustrated above, GC × GC is a useful tool to separate a maximum number of compounds from a technical mixture. Two main types of modulators can be found in a GC × GC system: thermal desorption modulators, including cryogenic and phase ratio modulators, and valve based modulators [34]. Although nowadays thermal modulators are the most widely used [7,32,33], a valve-based modulator has been used in the present work. In this case, a three-way solenoid valve controls the fill of the collection channel and the discharge with H_2 at 18–20 mL/min flows. Afterwards, a splitter restrictor divides the flow to the FID and the qMS in order to decrease the flow entering in the qMS detector. The optimization of the GC × GC parameters is necessary in order to obtain the most favorable conditions for the separation and quantification of the octyl and nonylphenol isomers from commercially available technical mixtures.

Due to the impossibility to separate octyl- and nonylphenol isomers using unidimensional chromatography, the synthesis, identification and quantification of two OP isomers, 4-(2′-methyl-2′-heptyl)phenol (22OP) and 4-(3′-methyl-3′-heptyl)phenol (33-OP) and two NP isomers, 4-(2′-methyl-2′-octyl)phenol (22NP) and 4-(3′,6′-dimethyl-3′-heptyl)phenol (363-NP) in two different nonylphenol mixtures from Fluka and Aldrich has been carried out in this work after the optimization of GC × GC parameters (1st column flow, 2nd column flow, oven temperature ramp, modulation period and discharge time) by means of an experimental design approach using GC × GC-FID–qMS and a valve based modulator.

2. Experimental

2.1. Reagents and materials

Nonylphenol technical mixtures (NPs) were purchased from Fluka (Pestanal®, Steinheim, Germany) and Aldrich Chemistry (Steinheim, Germany) and 2,3,5,6-d_4-4-nonylphenol (NP-d_4, min 97 atom% D) from Isotec (Miamisburg, OH, USA). 4000 mg/L stock solutions of analytes were individually prepared in methanol (99.9%, Alfa Aesar, Karlsruhe, Germany) and stored in amber vials at −20 °C.

Ethyl acetate (EtOAc) and *n*-hexane used in the synthesis and the purification procedures of individual isomers were supplied by Panreac (Barcelona, Spain) and anhydrous ligroin and anhydrous diethyl ether by Aldrich (Steinheim, Germany).

Magnesium, crystal iodine, 1-bromobutane, 1-bromopentane, 1-bromohexane, 3-methyl-1-bromobutane, 2-propanone, 2-butanone and BF_3–Et_2O complex were supplied by Aldrich.

Calcium chloride, ammonium chloride and anhydrous sodium sulfate were supplied by Panreac.

TLC silica gel sheets (0.040–0.063 nm) were supplied by Merck (Darmstadt, Germany).

The synthesis of 2-methylheptan-2-ol, 3-methylheptan-3-ol, 2-methyloctan-2-ol and 3,6-dimethylheptan-3-ol, 4-(2′-methyl-2′-heptyl)phenol (22OP), 4-(3′-methyl-3′-heptyl)phenol (33-OP), 4-(2′-methyl-2′-octyl)phenol (22NP) and 4-(3′,6′-dimethyl-3′-heptyl)phenol (363-NP) and their RMN data are described in the Supplementary Data.

2.2. GC × GC-FID–qMS detection

The analytes were analyzed in a 7890A gas chromatograph (Agilent Technologies, Avondale, PA, USA) equipped with an Agilent flame ionization detector (FID), an Agilent 5975C electron impact ionization quadrupole mass spectrometer (qMS) and a 7683B Agilent autosampler. 2 μL of the solution was injected in the splitless mode at 300 °C into a primary HP5 MS (30 m × 0.25 mm, 0.25 μm) capillary column, coupled with a DB-17MS (5 m × 0.25 mm, 0.25 μm) capillary column. The following oven temperature program was used for the separation of the analytes: 60 °C (2 min), temperature increase at 20 °C/min to 205 °C, a second increase of 1 °C/min up to 220 °C, and a final increase of 30 °C/min to 300 °C, where it was finally held for 2 min. H_2 (Hydrogen generator AD-1020, Cinel Strumenti Scientifici, Padova, Italy) was used as carrier gas at a constant flow of 1 mL/min in the first column and 17.75 mL/min in the second. The flow was divided by a splitter to the FID with a tube of 70 cm length × 0.32 mm i.d., and to the MS with a tube of 45 cm length × 0.10 mm i.d. FID worked at 270 °C, 20 mL/min H_2 flow and 350 mL/min air flow (99.9992%, Carburos Metálicos, Barcelona, Spain) and data acquisition was set at 200 Hz. The qMS transfer line temperature was maintained at 310 °C, and the ion source and quadrupole at 230 °C and 150 °C, respectively. Data acquisition was set at 36.14 Hz. Measurements were performed both in the scan (105–220 m/z) and in the SIM (Selected Ion Monitoring) modes. The m/z values monitorized for each analyte were the following: 22-OP (135/107), 33-OP (107/149), 363-NP (149/107), 22-NP (135/107) and NP-d_4 (111/110). The first ion was used as quantifier and the second one as qualifier.

Valve based modulator parameters were settled as follows: 1.5 s modulation period and 0.12 s discharge time according to the optimized results.

The chemical structures and mass spectra of the synthesized compounds are illustrated in Fig. 1.

3. Results and discussion

3.1. Optimization of the GC × GC-FID–qMS separation

In order to obtain the best separation of the OP and NP isomers from the NP technical mixtures, a full factorial design (FFD) was developed using Statgraphics Centurion XV. ~1700 mg/L stock solution from Fluka NPs was injected and five variables were studied: 1st column flow (0.7–1.2 mL/min), 2nd column flow (18–25 mL/min), oven ramp (0.5–5 °C/min), modulation period (1.4–1.5 s) and discharge time (0.05–0.1 s). The modulation period is the time elapsed from the moment that the modulator channel starts filling with mobile phase until the modulator channel is emptied. The discharge time is the time consumed by the mobile phase from the moment that the modulator channel is filled until the modulator channel is emptied.

Three different responses were checked during the optimization: one dimension symmetry (symmetry) in three different points of the chromatogram (beginning, middle, end), the blob number and the blob volume. Symmetry was qualitatively defined: 1 for good symmetry, 25 to acceptable symmetry and 50 to bad symmetry. The volume of 15 peaks around the chromatogram selected due to their high volume was checked, which guaranteed their presence in all the experiments performed. The identification of these blobs was performed by means of their mass spectra. The blob number was quantified.

During the whole optimization process the nature of the columns was kept constant. The 2nd dimension column (DB-17MS) was chosen since it gives satisfactory results according to Ieda et al. [32].

The responses obtained for the FFD were analyzed by means of an analysis of variance and parameters with a p-value <0.05 were chosen as significant. Thus, observing Pareto-charts obtained for symmetry (see Supplementary Data), it could be concluded that the 2nd column flow, the discharge time and the interactions

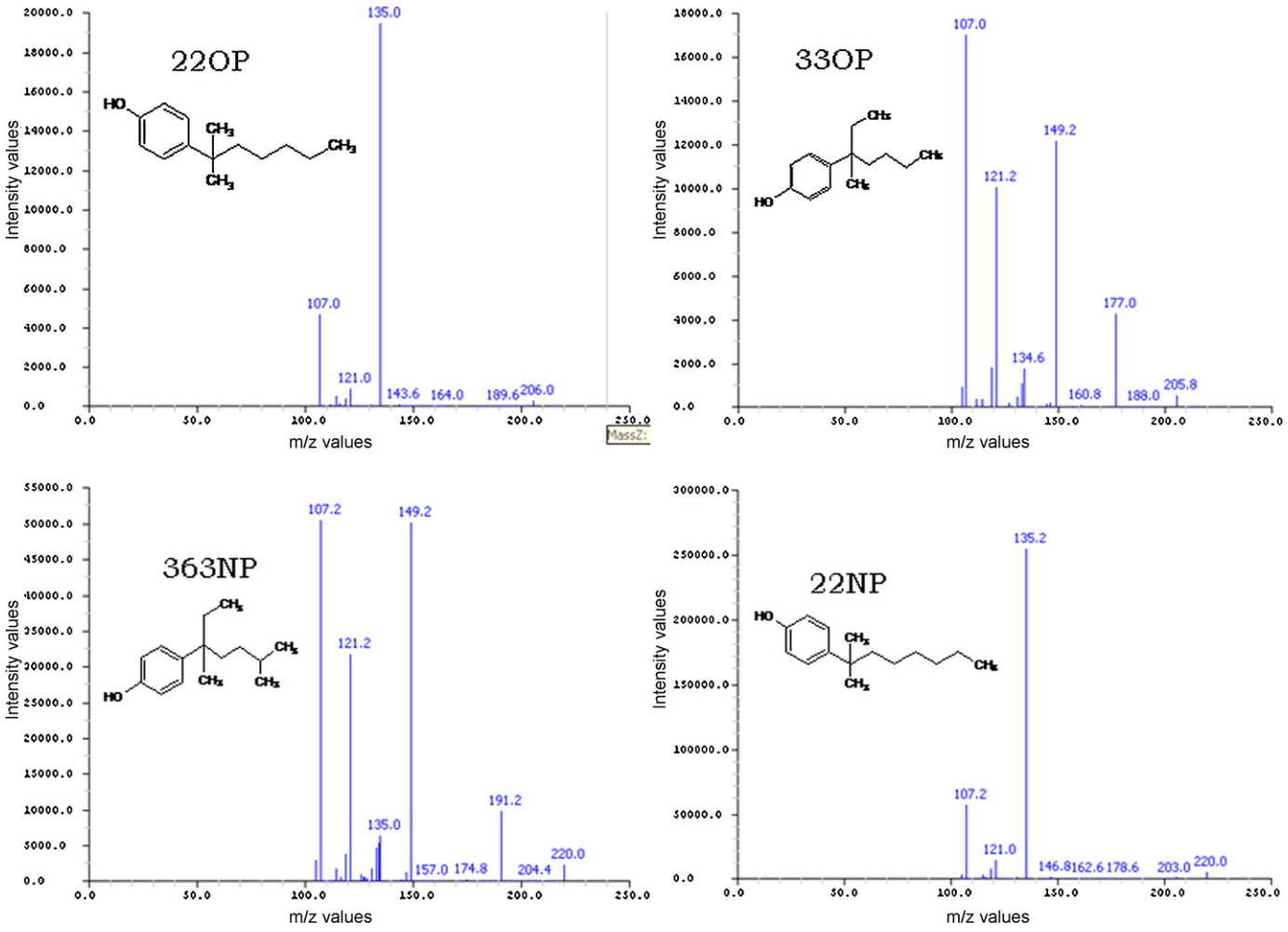

Fig. 1. Structure and mass spectra of the 22OP, 33OP, 22NP and 363 NP isomers.

between the 1st column flow-discharge time and the 2nd column flow-discharge time had a significant influence in the symmetry response.

The Pareto charts of 4 blobs have been illustrated as an example (see Supplementary Data). In the case of the blob volume, the 1st column flow, the 2nd column flow, the discharge time and the interactions between the 1st column flow and the discharge time, affected significantly to most of the blobs chosen.

Finally, the blob number was significantly affected by the 1st column flow, the 2nd column flow and the discharge time (see Supplementary Data).

Thus, the 1st column flow, the 2nd column flow and the discharge time were analyzed in a central composite design (CCD), while the slope of the oven temperature ramp and the modulation time were fitted at a low (1 °C/min) and at the highest (1.5 s) values, respectively. In order to fit these values, we have taken in consideration that low values of the oven temperature ramp are better for the analyte separation. Some experiments were performed at low values and no significant differences were found between 0.5 and 1 °C/min. Thus, 1 °C/min was chosen in order to reduce the analysis time. In the case of the modulation period, a high value was chosen since it is mostly used with this modulator.

A CCD was built with the rest of the variables: 1st column flow (0.53–1.37 mL/min), 2nd column flow (15.6–27.4 mL/min) and discharge time (0.033–0.12 s).

As for the FFD, the symmetry, the blob volume and the blob number were used as responses.

In order to optimize the symmetry a target value of 1 was set for optimization and according to the results obtained (see Fig. 2a), the discharge time, the squared value of the discharge time and the squared value of the 1st column flow were significant for a 95% confidence interval. According to the response surface obtained for symmetry, optimum values should be set at 1 mL/min for the 1st column flow and 0.11 s for the discharge time. The flow of the 2nd column was set at an intermediate value (17.75 mL/min) since it was not significant.

In the case of the blob volume the results obtained for two of the selected peaks are shown in Fig. 2b. The rest of the blobs studied showed a similar behavior. According to the results obtained in the CCD for the blob volume variables should be fitted at the highest value for the 1st column flow (1.37 mL/min) and at the highest value for discharge time (0.12 s). The 2nd column flow was fitted at an intermediate value (17.75 mL/min) since it was not significant.

The results obtained for the number of blobs are shown in Fig. 2c and it could be concluded that the highest values for the 1st column flow and the discharge time provided the highest number of blobs, while the flow of the 2nd column had not significant effect.

Table 1 summarizes the optimum values for the variables studied in the case of each of the responses studied. The flow in the 2nd column was not significant and it was finally fitted at 17.75 mL/min intermediate value. In the case of the discharge time, the best responses were obtained for the discharge time in the 0.11–0.12 s range for the three responses considered and it was set at 0.12 s. Finally, in the case of the 1st column flow, the best values for

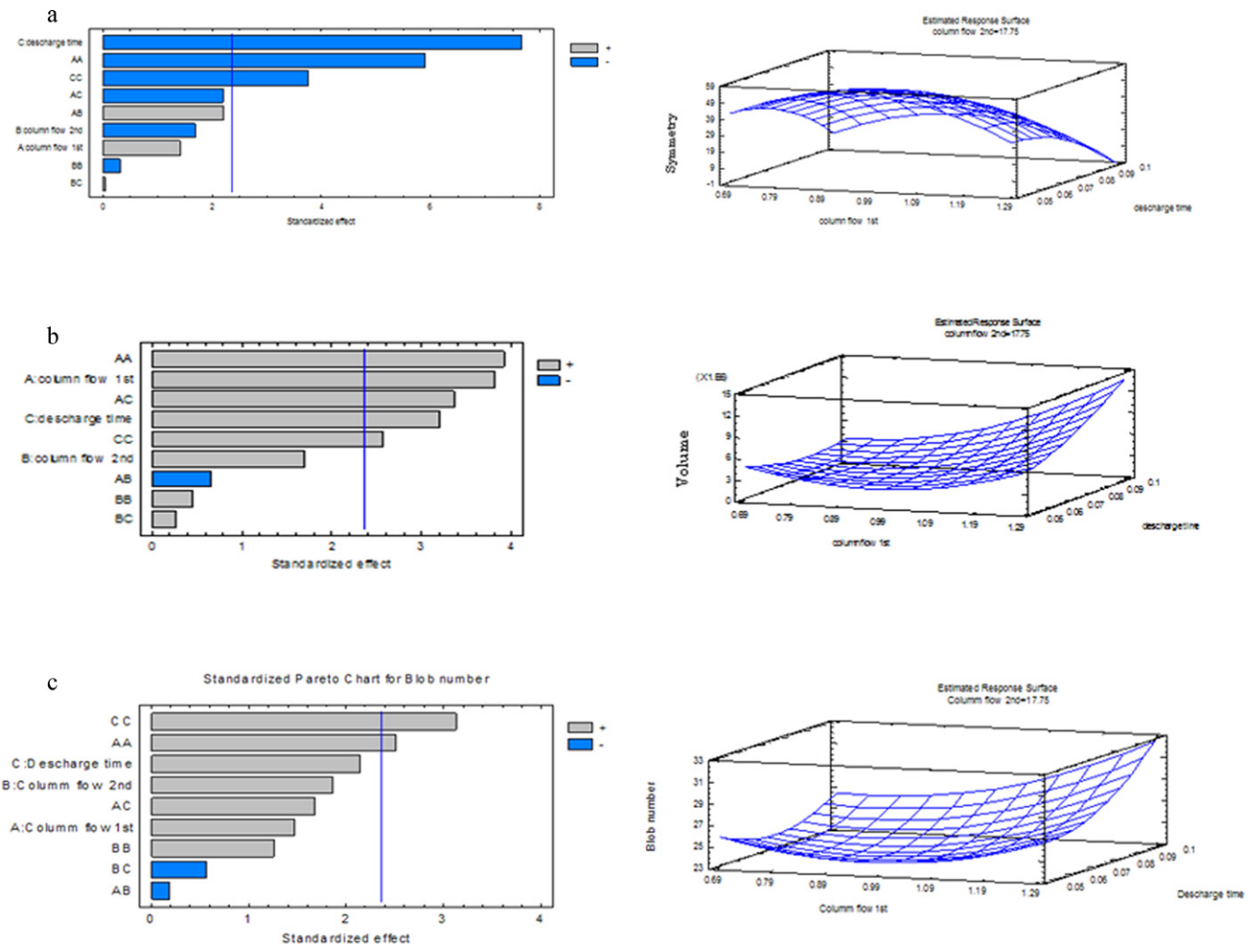

Fig. 2. Pareto chart and the response surface obtained during the CCD for (a) symmetry, (b) blob volume and (c) blob number. The flow of the 2nd column was fitted at 17.75 mL/min.

blob number and volume were obtained for 1.37 mL/min, while 1 mL/min was best for symmetry. Since the symmetry obtained at 1.37 mL/min was not good, 1 mL/min was finally chosen.

Actually, these parameters did not saturate the modulator (the 1st column flow is good to fill the modulator in the charge period. Besides, the analytes are removed completely from the modulator during the discharge time), avoiding the front distortion and the bleeding of the peak. Furthermore, 17.75 mL/min of the 2nd column gave a good second dimensional separation and avoided the wraparound, confirming that the analytes crossed the 2nd column before the next discharge. Thus, we obtained the best parameters for the separation and quantification of octyl- and nonylphenols with the best symmetry and the highest number of blobs.

Fig. 3 shows the chromatogram obtained under optimized conditions in qMS. Obviously, a higher number of compounds (up to 79) are detected in the FID, while only 38 were confirmed in the qMS as NP or OP isomers. The signal obtained in the qMS was used for the identification of the isomers synthesized, while the FID signal was used for quantification.

Although an optimum condition was established after the optimization, the resolution of all OP or NP isomer was impossible. In some cases the mass spectra obtained changes around the blob and in others the peak observed in the FID does not appear in qMS, thus the identification is not possible.

Table 1
Optimum values of optimized variables according to the variables studied.

	1st column flow (mL/min)	2nd column flow (mL/min)	Discharge time (s)
Symmetry	1.00	–	0.11
Blob volume	1.37	–	0.12
Blob number	1.37	–	0.12

3.2. Quantification of 22OP, 33OP, 363NP and 22NP in technical mixtures

In order to identify the 22OP, 33OP, 363NP and 22NP isomers in two technical mixtures, individual injections were performed and retention times and mass spectra of single isomers were obtained. Fig. 1 shows the mass spectra of the target isomers.

The synthesized isomers were identified in two NP technical mixtures based on the retention time and mass spectra in the case of qMS (see Fig. 3) and according to the retention times for the FID.

In the area corresponding to the 22OP isomer blobs, two different mass spectra could be detected in the case of the technical mixtures. At one end of the blob, the mass spectrum of the 22OP isomer was observed while at the other edge of the blob an unknown NP isomer can be detected. That means, that the 22OP isomer coelutes with an unknown NP isomer. In order to quantify, SIM mode could be an alternative, but the mass spectra are similar, except for the 220 and 206 fractions, which have a low abundance.

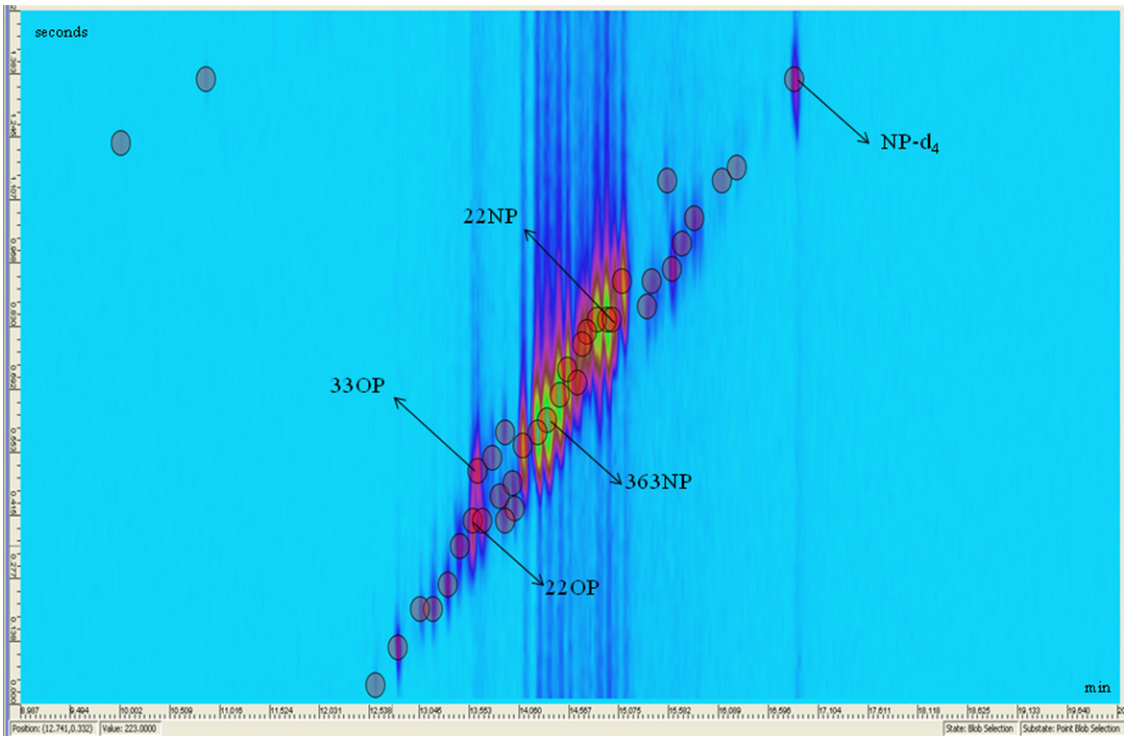

Fig. 3. Identification of the 22OP, 33OP, 363NP, 22NP isomers and NP-d_4 in the qMS chromatogram.

Table 2
Percentage of OP and NP isomers in two different NPs technical mixtures and comparison with data observed in literature.

Isomers	Fluka		Aldrich	
	This work	[7]	This work	[7]
22OP	4.86 ± 0.02	–	0.59 ± 0.06	–
33OP	4.91 ± 0.12	–	2.82 ± 0.25	–
363NP	11.70 ± 0.61	10.6 ± 1.0	7.71 ± 0.82	8.4 ± 0.7
22NP	2.28 ± 0.15	1.5 ± 0.1	1.98 ± 0.09	1.0 ± 0.1

(–) no data.

Calibration curves were built for each analyte in the 0.50–150 mg/L range, obtaining determination coefficients higher than 0.998 after correction with NP-d_4 for FID and higher than 0.990 for qMS. The precision ($n = 3$) was below 10% in all the cases for FID and below 13% for qMS.

Table 2 shows the percentages of the synthesized isomers in the two different technical mixtures.

These values are similar to those obtained by Eganhouse et al. [7] by a GC × GC–TOF-MS (see Table 2). No data has been obtained in the literature for OP isomers.

4. Conclusions

This is the first time where separation of OP and NP isomers using GC × GC-FID–qMS coupled with a valve-based modulator has been optimized by means of experimental designs. Up to 79 OP and NP isomers have been separated using the FID detector and 39 have been undoubtedly identified using the mass spectra obtained from the qMS detector. The 22OP, 33OP, 363NP and 22NP isomers were synthesized in the laboratory and were quantified in two different technical mixtures. The results obtained for NP isomers were in good agreement with the literature and it is the first time that 22OP and 33OP are quantified in NP technical mixtures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.03.016.

References

[1] A. Soares, B. Guieysse, B. Jefferson, E. Cartmell, J.N. Lester, Environ. Int. 34 (2008) 1033.
[2] M. Ahel, W. Giger, M. Koch, Water Res. 28 (1994) 1131.
[3] V.K. Sharma, G.A.K. Anquandah, R.A. Yngard, H. Kim, J. Fekete, K. Bouzek, A.K. Ray, D. Golovko, J. Environ. Sci. Health A: Tox. Hazard. Subst. Environ. Eng. 44 (2009) 423.
[4] C. Sonnenschein, A.M. Soto, J. Steroid Biochem. Mol. Biol. 65 (1998) 143.
[5] A.M. Soto, H. Justicia, J.W. Wray, C. Sonnenschein, Environ. Health Perspect. 92 (1991) 167.
[6] R. Vazquez-Duhalt, F. Marquez-Rocha, E. Ponce, A.F. Licea, M.T. Viana, Appl. Ecol. Environ. Res. 4 (2006) 1.
[7] R.P. Eganhouse, J. Pontolillo, R.B. Gaines, G.S. Frysinger, F.D.R.L.P. Gabriel, H.-P.E. Kohler, W. Giger, L.B. Barber, Environ. Sci. Technol. 43 (2009) 9306.
[8] T.F. Wheeler, J.R. Heim, M.R. LaTorre, A.B. Janes, J. Chromatogr. Sci. 35 (1997) 19.
[9] L.A. Ashfield, T.G. Pottinger, J.P. Sumpter, Environ. Toxicol. Chem. 17 (1998) 679.
[10] R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, M.G. Parker, Endocrinology 135 (1994) 175.
[11] A.D. Vethaak, J. Lahr, S.M. Schrap, A.C. Belfroid, G.B.J. Rijs, A. Gerritsen, J. de Boer, A.S. Bulder, G.C.M. Grinwis, R.V. Kuiper, J. Legler, T.A.J. Murk, W. Peijnenburg, H.J.M. Verhaar, P. de Voogt, Chemosphere 59 (2005) 511.
[12] Y. Jin, R. Chen, W. Liu, Z. Fu, Fish Shellfish Immunol. 28 (2010) 854.
[13] X. Domene, S. Chelinho, J. Sousa, J. Soils Sediments 10 (2010) 556.
[14] N. Bechi, F. Ietta, R. Romagnoli, S. Jantra, M. Cencini, G. Galassi, T. Serchi, I. Corsi, S. Focardi, L. Paulesu, Environ. Health Perspect. 118 (2009).
[15] Directive 2000/60/EC of The European Parliament and of the Council 23 October 2000 establishing a framework for Community action in the field of water

policy. Amended by Directive 2009/31/EC of the European Parliament and of the Council of 23 April 2009.
[16] U. Kammann, M. Vobach, W. Wosniok, A. Schäffer, A. Telscher, Environ. Sci. Pollut. Res. Int. 16 (2009) 227.
[17] T. Katase, K. Okuda, Y.-S. Kim, H. Eun, H. Takada, T. Uchiyama, H. Saito, M. Makino, Y. Fujimoto, Chemosphere 70 (2008) 1961.
[18] J. Lalah, G. Severin, K.W. Schramm, D. Lenoir, A. Behechti, K. Guenther, A. Kettrup, Arch. Environ. Contam. Toxicol. 52 (2007) 104.
[19] T.G. Preuss, J. Gehrhardt, K. Schirmer, A. Coors, M. Rubach, A. Russ, P.D. Jones, J.P. Giesy, H.T. Ratte, Environ. Sci. Technol. 40 (2006) 5147.
[20] T.G. Preuss, H. Gurer-Orhan, J. Meerman, H.T. Ratte, Toxicol. In Vitro 24 (2010) 129.
[21] T. Sato, H. Saito, T. Uchiyama, Y. Fujimoto, T. Katase, O. Kai, Arch. Toxicol. 83 (2009) 1097.
[22] H. Shioji, S. Tsunoi, Y. Kobayashi, T. Shigemori, M. Ike, M. Fujita, Y. Miyaji, M. Tanaka, J. Health Sci. 52 (2006) 132.
[23] E.J. Routledge, J.P. Sumpter, J. Biol. Chem. 272 (1997) 3280.
[24] J.L. Gundersen, J. Chromatogr. A 914 (2001) 161.
[25] C. Harman, K.E. Tollefsen, O. Bøyum, K. Thomas, M. Grung, Chemosphere 72 (2008) 1510.
[26] L. Brossa, E. Pocurull, F. Borrull, R.M. Marcé, Chromatographia 56 (2002) 573.
[27] K. Quednow, W. Püttmann, Environ. Pollut. 152 (2008) 476.
[28] T. Vega Morales, M.E. Torres Padrón, Z. Sosa Ferrera, J.J. Santana Rodríguez, TrAC-Trends Anal. Chem. 28 (2009) 1186.
[29] V. Andreu, E. Ferrer, J.L. Rubio, G. Font, Y. Picó, Sci. Total Environ. 378 (2007) 124.
[30] K. Guenther, E. Kleist, B. Thiele, Anal. Bioanal. Chem. 384 (2006) 542.
[31] B. Thiele, V. Heinke, E. Kleist, K. Guenther, Environ. Sci. Technol. 38 (2004) 3405.
[32] T. Ieda, Y. Horii, G. Petrick, N. Yamashita, N. Ochiai, K. Kannan, Environ. Sci. Technol. 39 (2005) 7202.
[33] M. Moeder, C. Martin, D. Schlosser, J. Harynuk, T. Górecki, J. Chromatogr. A 1107 (2006) 233.
[34] M. Adahchour, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, TrAC-Trends Anal. Chem. 25 (2006) 438.