Bulgarian Chemical Communications, Volume 47, Number 4 (pp. 1163 – 1168) 2015

Simultaneous determination of nonylphenol and short-chain nonylphenolpolyethoxylates by DLLME-HPLC

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Received April 6, 2015

A method for the simultaneous determination of nonylphenol (NP) and short-chain nonylphenolpolyethoxylates (SCNPEOs) in water samples by dispersive liquid-liquid microextraction - high performance liquid chromatography (DLLME-HPLC) was developed. The six components of NP and SCNPEOs (n=1, 2, 3, 4, 5) were simultaneously extracted and enriched under the optimized microextraction conditions with enrichment factors of 86, 106, 93, 84, 76, and 62, respectively. The six components were simultaneously determined within 18 min under the optimized chromatographic conditions. The working curves of the six components are characterized with a linear concentration range of up to 3 orders of magnitude, correlation coefficients from 0.9991 to 0.9999 and detection limits ranging from 0.09 to 2.1 ng/mL. The spike recoveries and relative standard deviations (RSD) of this method were 88.9%~117% and 1.9%~4.3%, respectively.

Key words: dispersive liquid-liquid microextraction, high performance liquid chromatography, nonylphenol, short-chain nonylphenolpolyethoxylates, simultaneous determination.

INTRODUCTION

Recently, it was discovered that only a very small dosage of environmental endocrine disruptors could interact with the endocrine system of the receiver by seriously threatening humans' health [1]. Therefore, the monitoring of hormone-like substances in the environment attracted increasing attention.

Nonylphenol (NP) and short-chain nonylphenolpolyethoxylates (SCNPEOs, n=1, 2) are typical environmental hormones [2]. Most of them present in the environment are biodegradation products of nonylphenolethoxylates (NPEOs, n=1~20). NPEOs are stable, hard to hydrolyze, easy to dissolve in organic solvents, and gradually degrade++-+ to NP and SCNPEOs [3-8]. Studies [9-11] showed that NP and SCNPEOs had an estrogen-like property that could disrupt the endocrine and neuroendocrine systems of organisms, and therefore could affect reproductive development, reduce immunity and increase cancer risk. The harm of NP and SCNPEOs to organism, especially to human, obviously increased. Thereby, the detection and monitoring of NP and SCNPEOs in the environment had become a public focus.

Currently gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) are the main methods for NP and NPEOs detection [12,13]. GC-MS is suitable for the analysis of low-molecular compounds such as NP and NPEOs (n <4). But the high cost of GC-MS hinders its extensive application. Due to the presence of NP5EO in the water samples, HPLC was selected to detect the NP and SCNPEOs in this study.

NPEOs are usually in trace amounts in water samples and enrichment must be performed before instrumental analysis. Solid phase extraction has poor reproducibility; ordinary liquid-liquid extraction has a low enrichment factor. To solve this problems, dispersive liquid-liquid micro-extraction (DLLME) was adopted as pretreatment to enrich the samples. DLLME has good reproducibility and high enrichment factor. It could improve the detection sensitivity and lower the detection limit and was therefore suitable for the enrichment of NP and SCNPEOs in water samples.

In this study, the DLLME technology was used as a pretreatment method to enrich the NP and SCNPEOs before HPLC measurement. The new combined DLLME - HPLC method was developed to simultaneously determine NP and SCNPEOs in water samples.

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EXPERIMENTAL SECTION

Instruments and reagents

The pH meter was PHSJ-3F type from Shanghai Leici, China. Refrigerating centrifuge was SIGMA-3k15 type from Sigma, Germany. HPLC was Waters 2695 type from Waters, USA, combined with 2487 UV detector and Hypersil APS-2 amino chromatographic column (250*4.6 mm, 5 μm, Thermo Electron, USA).

N-hexane (C_6H_{14}), isopropanol (C_3H_8O), methylene chloride (CH_2Cl_2), methanol (CH_3OH) and acetonitrile (C_2H_3N) were chromatographically pure chemicals from Tianjin Concord, China. Acetone (C_3H_6O), carbon tetrachloride (CCl_4) and chloroform ($CHCl_3$) were analytically pure chemicals from Tianjin Damao, China. Standard NP and SCNPEOs (average n≈2) reagents were from TCI (Shanghai), China.

Experimental methods

The water sample was centrifuged for 5 min at 6000 r/min. The supernatant was filtered through a 0.45 μm organic membrane. 0.5 g sodium chloride (NaCl) was added into 8 mL permeate. After complete dissolution, a mixture of 80 μL carbon tetrachloride and 0.6 mL methanol were quickly added to the permeate. The mixed permeate was treated by ultrasound for 3 min and was then centrifuged for 5 min at 4500 r/min. After centrifugation, the lower organic phase was moved into a glass cannula using a microsyringe. The glass cannula was put into the lidded sample bottle which was then put at the designated position of the sample tray for measurement.

RESULTS AND DISCUSSION

Determination of DLLME parameters

DLLME is equivalent to miniaturized liquid-liquid extraction. It is characterized by simple operation, low cost, high enrichment factor and small dosage of organic solvent. As a kind of environmentally friendly liquid phase microextraction technique, DLLME is increasingly used for sample preparation in chromatography measurements. In DLLME process, extractant type and dosage, dispersant type and dosage, salt dosage, pH and ultrasound-assisted extraction time needed to be optimized. The volume of the water sample used in this study was 8 mL.

Extractant and its dosage

In DLLME process, the type of extractant is one of the most important parameters that affects the enrichment factor. Methylene chloride, chloroform and carbon tetrachloride were used as extractants in this study. The results in Fig. 1 show that all three extractants had good extraction properties. Carbon tetrachloride presented a little higher effect on the six target objects and had no influence on component separation and HPLC measurement. So carbon tetrachloride was selected as the extractant.

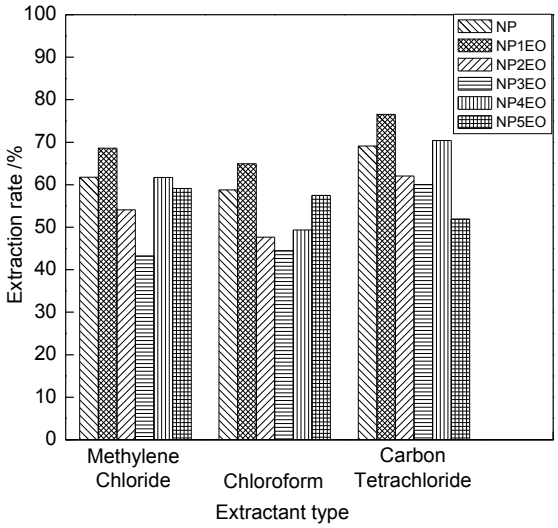

Fig. 1. The influence of extractant type on the extraction rate.

Furthermore, the extractant dosage also affects the enrichment factor of the target objects. Smaller extractant dosage means higher sensitivity. But too small extractant dosage can lead to incomplete extraction and troubles with organic phase separation. Different volumes (80, 100, 150, 200 μL) of carbon tetrachloride were added to the water samples (8 mL) to investigate its effect on extraction efficiency and enrichment factor (Fig. 2).

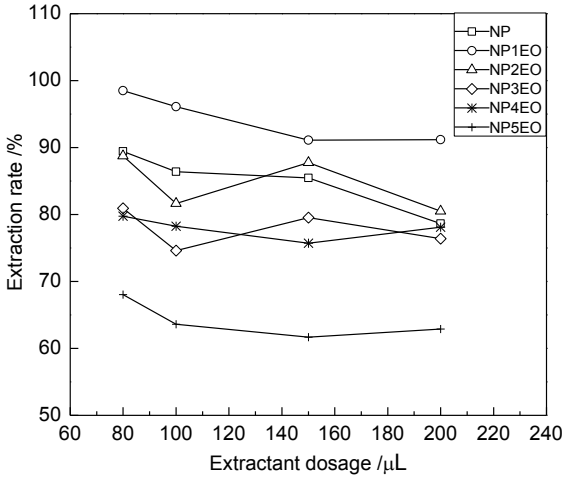

Fig. 2. The influence of extractant dosage on extraction rate.

The extraction efficiencies of NP, NP2EO and NP5EO obviously decreased on increasing extractant dosage. With carbon tetrachloride volume of 80 μL, the best extraction efficiencies of

all components were achieved. So the extractant dosage was eventually determined as 80 μL.

Dispersant and its dosage

In DLLME process, the dispersant should not only have a good solubility in the extractant but also be miscible with water, and thus play a bridging role in the system. An appropriate dosage of dispersant contributes to the formation of a homogeneous water/dispersant/extractant system. An appropriate dispersant with appropriate dosage can significantly increase the extraction efficiency of the components. Isopropanol, acetonitrile, acetone, and methanol were used as dispersants to investigate the extraction efficiency of the components. The results showed that acetone seriously interferes with the analysis. Therefore the extraction efficiency with acetone was not further investigated.

The results in Fig. 3 show that the three dispersants have similar extraction effects. Methanol presented the best effect of more than 60% on the six target objects. So methanol was selected as the dispersant. Different volumes (0.2, 0.4, 0.6, 0.8, 1 mL) of methanol were added to the water samples (8 mL) to investigate its effect on extraction efficiencies (in Fig. 4). The extraction efficiencies increased on increasing dispersant dosage in the range of 0.2~0.6 mL. But in the range of 0.6~1 mL, the extraction efficiencies showed slight variations and even downward trends for some components. So the dispersant dosage was eventually determined as 0.6 mL.

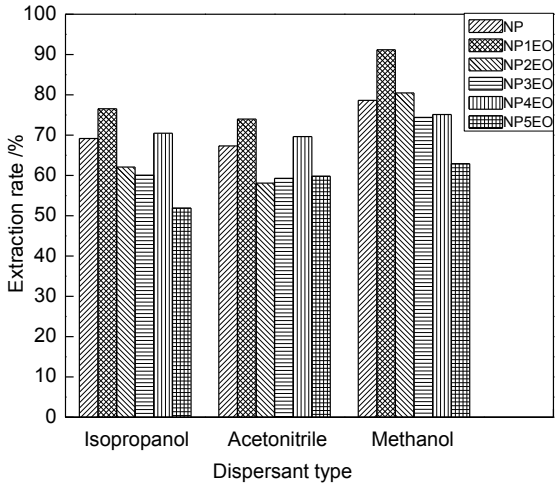

Fig. 3. The influence of dispersant type on extraction rate.

Salt dosage

The addition of an appropriate amount of sodium chloride (NaCl) during extraction can reduce the solubility of components and extractant in the liquid phase, increase the ionic strength of the sample, decrease emulsification, and thus increase the extraction efficiency. The effect of different amounts of salt on the extraction efficiency of the components was investigated. Different amounts (0.1, 0.2, 0.3, 0.4, 0.5 g) of salt were added to the extraction system. The results in Fig. 5 show that the salt dosage didn't notably affect the extraction efficiency. But with increasing salt dosage, emulsification significantly decreased. So, the salt dosage was determined to 0.5 g in the study.

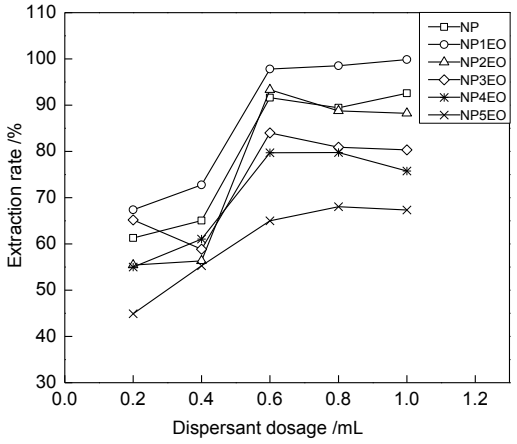

Fig. 4. The influence of dispersant dosage on extraction rate.

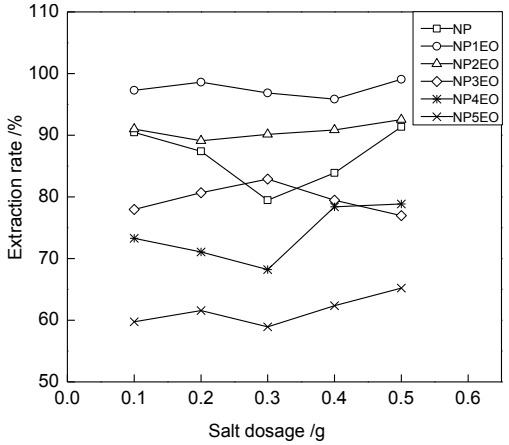

Fig. 5. The influence of salt dosage on extraction rate.

Determination of sample pH

The pH value of the sample system can affect the existence form of the test substance. An appropriate pH value can benefit the process of extraction and improve the extraction efficiency. The extraction efficiency of each component was measured at pH values of 5.0, 6.0, 7.0, 8.0, and 9.0. The results in Fig. 6 show that the extraction efficiency of the components is relatively higher in the pH range from 6 to 8. In this study, the pH values of the samples were 6.5~7.8 and no pH adjustment was needed.

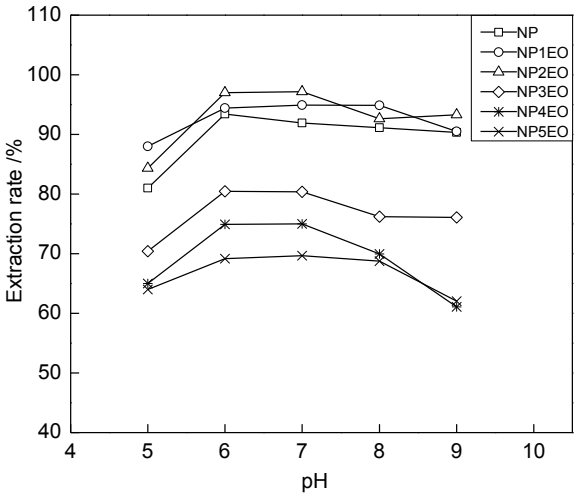

Fig. 6. The influence of pH on the extraction rate.

Ultrasound-assisted extraction time

Ultrasound can promote the contact and mixing of extractant and sample solution, and thus improve the extraction efficiency. But overlong ultrasonication time may increase the volatilization of reagents and cause emulsification, and thus affect the extraction efficiency. The effect of ultrasound-assisted extraction time on the extraction efficiency of the components was investigated (Fig. 7). The results indicated that on increasing the ultrasonication time, the extraction efficiency of each component showed a rising trend. Except for NP4EO, the other five components reached the highest extraction rate with 3 min of ultrasonication. The extraction rate began to decrease when the ultrasonication time was 4 min. The optimal ultrasonic time was 3 min in this study.

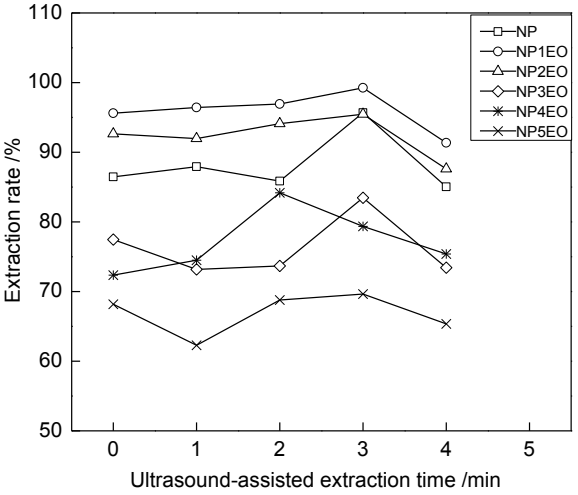

Fig. 7. The influence of ultrasound-assisted extraction time on extraction rate.

Under the above optimal DLLME pretreatment parameters (water sample volume of 8 mL, carbon tetrachloride extractant of 80 μL, methanol dispersant of 0.6 mL, ultrasound-assisted extraction time of 3 min, salt dosage of 0.5 g), the effective enrichment factors of NP, NP1EO, NP2EO, NP3EO, NP4EO and NP5EO were 86, 106, 93, 84, 76 and 62, respectively.

HPLC measurement

HPLC operational parameters

In this study, mobile phase mixture ratio and change rate, UV detection wavelength, flow velocity, injection volume and column temperature were optimized as follows: mobile phase of isopropyl (A)-n-hexane (B)-methylene chloride (C), gradient elution procedure (Table 1), UV detection wavelength of 277 nm, flow velocity of 1.0 mL/min, injection volume of 10 μL and column temperature of 35 °C.

Table 1. Gradient elution program

Time (min)	A%	B%	C%
0	7	92	1
5	7	92	1
8	75	24	1
11	75	24	1
13	7	92	1
18	7	92	1

The chromatogram of NP and SCNPEOs standard solution under the optimal operation parameters is shown in Fig. 8. The results show that each component is clearly separated. The peak shapes meet the experimental requirement.

Working curve and detection limit

Different amounts of the standard solution were added to blank water samples. After DLLME enrichment and HPLC measurement, the working curves of NP, NP1EO, NP2EO, NP3EO, NP4EO and NP5EO were obtained. The detection limits of the six components were simultaneously determined. The detailed results are shown in Table 2. It follows from the results that the linear concentration range of each component was up to 3 orders of magnitude. The correlation coefficients were from 0.9991 to 0.9999 and the detection limits ranged from 0.09 to 2.1 ng/mL. This means that the method is stable, accurate, and can be used for the analysis of real samples.

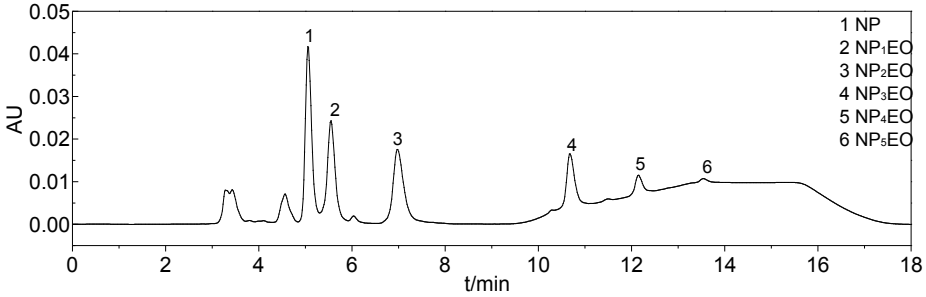

Fig. 8. Chromatogram of NP and SCNPEOs standard solution

Table 2. Regression equation, correlation coefficient, linear range and detection limit of 6 components.

Component	Linear equation	Correlation coefficient	Linear range (μg/mL)	Detection limit (ng/mL)
NP	Y=387588x+28346	0.9991	0.005~10	1.5
NP1EO	Y=302278x+36103	0.9992	0.007~6.5	2.1
NP2EO	Y=255272x+13869	0.9996	0.004~7.7	1.2
NP3EO	Y=240353x-705.36	0.9992	0.005~2.3	1.5
NP4EO	Y=217875x+1030.5	0.9998	0.001~1.3	0.3
NP5EO	Y=200798x+945.47	0.9999	0.0003~0.3	0.09

Table 3. Spike recoveries of water sample.

Component	Original (μg/mL)	Added (μg/mL)	Recovered (μg/mL)	Recovery (%)	RSD (%)
NP	1.05	0.500, 1.00, 1.50	1.58, 1.99, 2.61	106, 94.0, 104	1.9, 2.2, 3.2
NP_1EO	1.49	1.31, 1.96, 3.27	2.74, 3.50, 4.78	95.4, 103, 101	3.5, 4.1, 4.3
NP_2EO	2.65	1.53, 2.30, 3.84	4.17, 4.90, 6.51	99.3, 97.8, 101	4.2, 3.3, 2.8
NP_3EO	1.89	0.93, 1.39, 2.32	2.85, 3.30, 4.25	103, 101, 102	3.1, 3.4, 2.8
NP_4EO	0.48	0.26, 0.38, 0.64	0.73, 0.89, 1.14	96.2, 108, 103	2.7, 1.9, 2.5
NP_5EO	0.09	0.06, 0.09, 0.15	0.16, 0.17, 0.25	117, 88.9, 107	4.2, 4.3, 3.8

Spike recoveries

High, medium and low concentrations of the standard solution were added to the water samples to study spike recoveries, the results are showed in Table 3. The spike recoveries were from 88.9% to 117% and the relative standard deviations (RSD) were less than 5%, showing that the method was accurate, reliable, and appropriate for common analytical testing.

CONCLUSIONS

A method for the simultaneous determination of NP and SCNPEOs in water samples by DLLME-HPLC was developed. The DLLME pretreatment parameters were determined as water sample volume of 8 mL, carbon tetrachloride extractant of 80 μL, methanol dispersant of 0.6 mL, ultrasound-assisted extraction time of 3 min and salt dosage of 0.5 g. The HPLC operation parameters offering wide linear concentration range, low detection limits, good correlation coefficients, high spike recovery and low RSD were also determined. This method can be applied to the separation and determination of NP and SCNPEOs from microbial and bacterial degradation water. The combined method is sensitive, accurate, and friendly to the environment.

Acknowledgements: *This study was jointly supported by National Natural Science Foundation of China (No. 21107011, No. 51178098) and the Fundamental Research Funds for the Central Universities of China (No. N100303006).*

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ЕДНОВРЕМЕННО ОПРЕДЕЛЯНЕ ЧРЕЗ DLLME-HPLC НА НОНИЛ-ФЕНОЛ И НОНИЛ-ФЕНОЛ-ПОЛИЕТОКСИЛАТИ С КЪСА ВЕРИГА

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Постъпила на 5 април, 2015 г.

(Резюме)

Създаден е метод за едновременно определяне на нонил-фенол (NP) и нонил-фенол-полиетоксилати с къса верига (SCNPEOs) във водни проби чрез дисперсивна течно-течна микроекстракцияивисоко-ефективна течна хроматография (DLLME-HPLC).Шестте компонентиот NP и SCNPEOs (n=1, 2, 3, 4, 5) са екстрахирани едновременно и обогатени чрез оптимизирана микро-екстракция. Факторите на обогатяване са съответно 86, 106, 93, 84, 76и 62. Шестте компонента са определени едновременно в продължение на 18 минути при оптимизирани хроматографски условия. При тези условия калибрационните линии са прави в рамките на три порядъка от концентрации с корелационни коефициенти от 0.9991 до 0.9999 и предели на откриване от 0.09 до 2.1 ng/mL. Добивите при инжектиранеи относителното стандартно отклонение достигат съответно 88.9%~117% и 1.9%~4.3%.