



## **Determination of Nonylphenol and Its Ethoxylates by HPLC 1100 in Water Environment of Taiyuan City**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author KYS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CXZ and AKS managed the analyses of the study. Author AKS managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The aim of this work is to study the occurrence of nonylphenol and its ethoxylates in Taiyuan industrial area. The present study has firstly determined best conditions of Nonylphenol and its ethoxylates detection by using High Performance Liquid Chromatography (HPLC) 1100 series, varying solvent, mobile phase and flow: rate. These conditions let secondly the concentration determination of these pollutants in water media. Samples were collected from surface and groundwater in the industrial area of Taiyuan city (Shanxi province). Nonylphenol Ethoxylates (NPEOs) detection was better when solvent and Mobile phase were 2-propanol and Flow rate at 0.1 ml/min. Concentrations of Nonylphenol (NP) and NPEOs found in rivers and wastewaters collectors ranged from 80 to 933 µg/L and 38 to 743 µg/L respectively, while for groundwater,

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concentrations ranged from 24.6 to 151 µg/L and from 20 to 274 µg/L. These high concentrations found both in surface and groundwater, represent a risk of exposition to endocrine disruptors for humans and aquatic species. Actions should be taken to avoid or reduce the use of those compounds, or industries should apply some treatment before release their wastewater into environment. Attention should be paid especially to groundwater in case of human consumption. Introduction to groundwater way and degradation pathways from surface water to groundwater need to further study.

*Keywords: Wastewater; NP-NPEOs; HPLC; groundwater.*

## ABBREVIATIONS

*HPLC* : High Performance Liquid Chromatography;  
*NPEOs* : Nonylphenol Ethoxylates;  
*APEOs* : Alkylphenyl Polyethoxylates;  
*WWTPs* : Waste Water Treatment Plants;  
*CAN* : Acetonitrile;  
*LLE* : Liquid-liquid Extraction;  
*SPE* : Solid Phase Extraction;  
*SDE* : Steam Distillation Extraction;  
*DCM* : Dichloromethane;  
*EC* : Electrical Conductivity;

## 1. INTRODUCTION

In recent years, analytical chemistry and environmental chemistry have been used conjointly and no one of both domains can move or can be used without the contribution and support of the other discipline. Their conjoined development has contributed much to the knowledge, the understanding of environmental pollution, environmental processes, and to the development of measures and strategies to reduce contaminations. The determination of polar pollutants in samples (water, sludge, sediment and soil) use analytical processes follow these step: sample pretreatment, extraction and enrichment, clean up, derivatization, and separation and determination [1] Some analytical methods concerning certain classes of compound surfactants [2], herbicides and pesticides [3] pharmaceuticals, disinfection byproducts and complexing agents [4], have been the reported. Alkylphenyl polyethoxylates (APEOs), among the most commonly used nonionic surfactants, have been widely used in industrial processes as detergents in industrial and household applications [5].

After being used, NPEOs are discharged into wastewater to be in after treated in waste water treatment plants (WWTPs) and ultimately released into natural waters [6]. Currently, these compounds are on the US-EPA's priority testing list for evaluation of environmental and health effects [7].

The high toxicity of these chemicals led to many investigations about the fate in the environment. NPEOs are supposed to be degraded in NP and short chain ethoxylates which have been detected and reported in treated wastewater, sewage sludge, and soil. Studies reveal that these substances have varying estrogenic potencies [8]. Many works have studied the fate of these chemicals around the world in wastewaters, rivers and groundwaters (Japan [9], Netherland [10], Spain [11]) but few of them have been done in China especially in Taiyuan city where only Liao [12] has studied nonylphenol in sewage irrigated soil but no study concerning nonylphenol and its ethoxylates in water media. The aim of this work is to study the presence of nonylphenol and its ethoxylates in Taiyuan industrial water media by developing specific chromatographic method based on existing methods for separation and identification of Nonylphenol and its ethoxylates in water especially for Agilent 1100 series, and quantify them in surface and groundwater of this area.

## 2. MATERIALS AND METHODS

### 2.1 Presentation of Study Area

Located at latitude 37°27' to 38°25' and longitude 111°30' to 113°09', Taiyuan city is one of the great industrial cities of China and the capital of Shanxi province in North. It is located in the middle reaches of the Yellow River and is surrounded by hills in its west, north and east. It covers an area of 6998 km<sup>2</sup>. At the 2010 census, it had a total population of 4,201,591 habitants whom 3,212,500 are urban on 1,460 km<sup>2</sup>. The name of the city literally means "Great Plains", referring to the location where Fen River leaves the mountains. The Fen River, a key tributary of the Yellow River, runs through the territory of Taiyuan City with a long distance of 100 kilometers. It commands the north-south route through Shanxi, as well as important natural lines of communication through the mountains to Hebei province in the east and (via Fenyang) to

northern Shanxi province in the west. Taiyuan lies in the valley basin of both mountains of Taihang Mountain Ranges in east and Luliang Mountain in west. It has continental climate in North Temperate Zone, no bitterly cold days in winter and no broiling hot days in summer, with a quite temperature difference between day and night, and a long

frost-free period and plenty of sunshine hours in a year. Water samples were collected from wastewater collectors, 2 rivers and groundwater. Fig. 1A and Fig. 1B present the sampling locations for surface and ground water respectively. Samples were collected using pre-rinsed glass bottles (4L) and shipped to the laboratory.

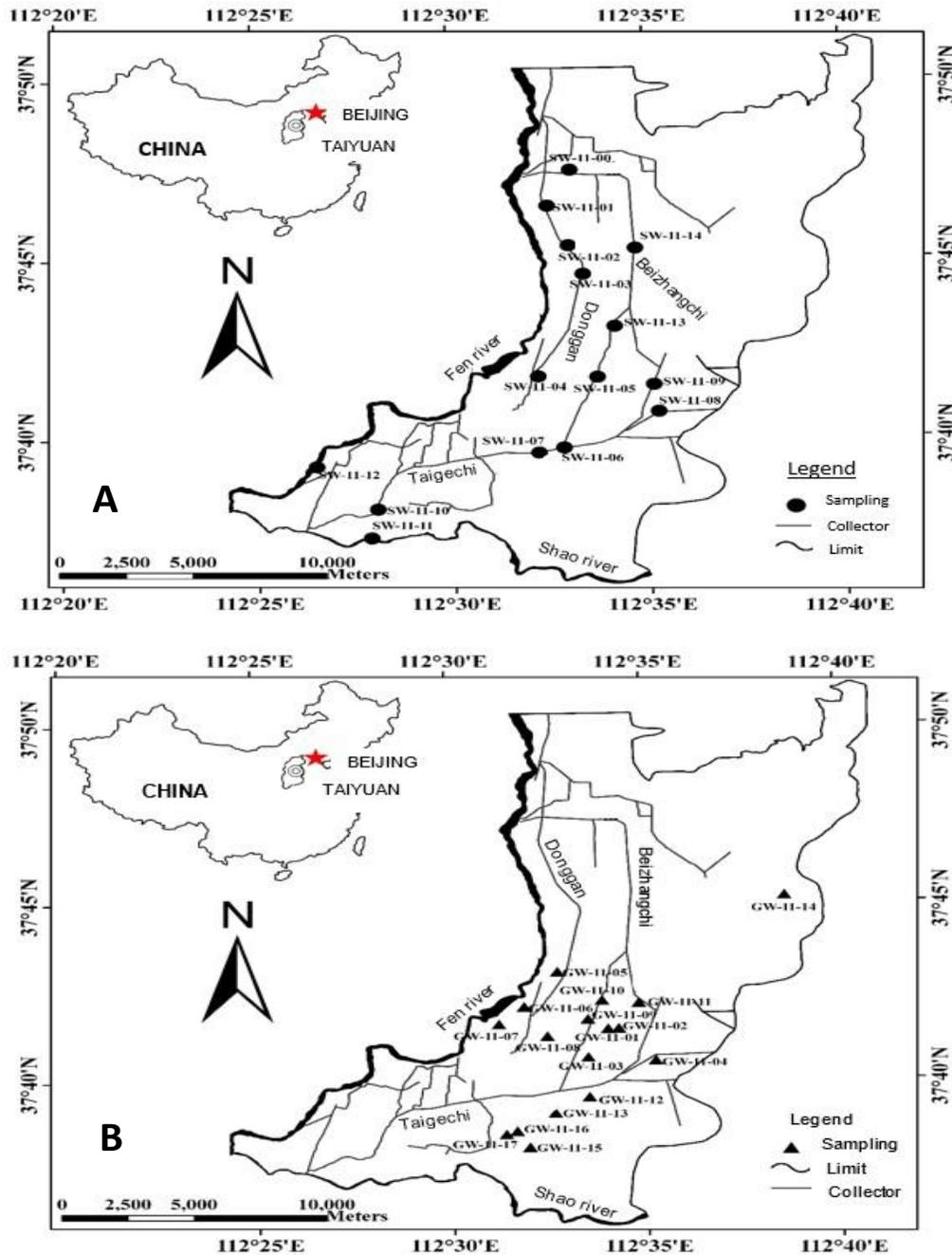


Fig. 1. Area of study

## 2.2 Reagents and Chemicals

Technical Nonylphenol (Pestanal, analytical standard,  $\geq 98\%$  purity) was obtained from Fluka Chemicals (Sigma-Aldrich, St. Louis, Missouri). Envi-grade Dichloromethane (DCM) (99.5% purity) was provided by ROWE Scientific Pty. Ltd. (Victoria, Australia). 2-propanol, Acetonitrile and Methanol (HPLC (High-performance liquid chromatography)/Spectro grade with a purity of 99.9%) were purchased from Tedia Company Inc. (Fairfield, Ohio, USA). The stock solutions (1 g/L) was prepared by dissolving 100 mg of nonylphenol in 100 ml of solvent. All solvents were used without further purification. NPEO (2 g/L) stock solutions was made by dissolving 200 mg in 100 ml of solvent.

## 2.3 Equipment

Equipment and laboratory glassware used for experiments were made of glass. The clean glassware was washed with organic solvent before using it to avoid eventual contamination. Evaporation of extracts with a larger volume ( $>10$  mL) was performed in rotary evaporator (Buch, Switzerland) and for the extract with a smaller volume ( $<5$  mL), evaporation under a stream of nitrogen was used. The glass column used for extracts purification by liquid chromatography had an internal diameter of 10 mm and the top of the column contained a reservoir for elution solvents. Microsyringes Hamilton, Sge or Rheodyne of various volumes were used for the injection and the dilution. Equipment used for NPEOs analyses was HPLC Agilent series 1100 using UV detector and Normal phase 150x4.6 mm i.d. column packed with 5  $\mu$ m particles of APS-2 Hypersil.

## 2.4 Method for Determination of NP and NPEOs

Firstly, the choice of the solvent which can better dissolve standards and can give the best result for HPLC detection was done by dissolving targeted chemicals in water, Methanol, Isopropanol, and Acetonitrile (ACN). Different mobile phases and variation of flow rate were also investigated. Then NP detection position was highlighted. To confirm results repetition has been done 2 to 3 times.

## 2.5 Extraction Method

### 2.5.1 Extraction solvent and recovery

Different extraction methods and elution solvents were used to evaluate the extraction recovery.

Liquid-liquid extraction (LLE), solid phase extraction (SPE) and steam distillation extraction (SDE) were extraction methods used. Elution solvents were methanol, Dichloromethane (DCM), hexane and acetonitrile. A specific volume (10 mL) of stock solutions, according to the final wished concentration, was added to the experimental sample (1L of pure water).

Concerning SPE, elution of targeted chemicals was done by using 3 mL Chromabond ODS-C<sub>18</sub>, 200 mg adsorbent, 45  $\mu$ m particule size and 3 mL Waters Oasis HLB 3cc cartridges, 60 mg sorbent per cartridge, 30  $\mu$ m particule size. Before extracting the samples, the cartridges were cleaned up by washing them subsequently with 6 ml of DCM then 6 ml of methanol and finally with 12 ml of pure water. Precautions were taken to avoid cartridge adsorbent getting dry before and during the percolation of the water sample. The glass tubes used to collect solvent wastes from washing the cartridges were taken out from the box and discarded. The samples were then poured into the cartridges and percolated through them slowly using a slight vacuum. The calibration flask that contained the sample was rinsed with 10 ml of pure water and this washing was also percolated through cartridges. Finally, cartridges reservoir itself was rinsed with 5 ml of water. After percolation, cartridges were allowed to get dry under vacuum for 1 min. To remove the last water residues from the sorbent, exactly 1 ml of methanol was passed slowly through cartridges. Subsequently, cartridges traps were air-dried under vacuum again. Clean glass tubes (25 ml) were put in the percolation box under cartridges and analytes were desorbed from the sorbent by 18 ml of ACN, DCM or 2-propanol. Concerning LLE, 30 ml of solvent was poured on funnel containing targeted chemicals and shaken during 5 min. Then leave the two liquid-phases to separate. After that, the extraction solvent was retrieved in clean glass tubes (25 ml). Adding of extraction solvent was done 3 times. Closed-loop apparatus (modified Nielson-Kryger unit, Ace Glass, USA) was used for continuous steam-distillation and solvent-extraction of the distillate. Water samples were refluxed for 6 hours. 10 ml of hexane was used as extraction solvent. The extracts were then dried over anhydrous sodium sulfate. Collected extracts from those different extraction methods, were evaporated to dryness using rotary evaporation and transferred, using multiple washing with dichloromethane, into a 1.8 ml glass vial equipped with a Teflon-lined screw cap. The sample was brought to dryness under the stream of nitrogen and re-dissolved in an exact

volume (typically 1 mL) and take directly to HPLC analysis.

### 2.5.2 Water samples extraction

Upon arrival, the samples were immediately filtered. Extraction was made by modifying a procedure by Ahel [13]. 500 mL of sample was taken from each bottle and extracted by solid phase extraction (SPE) to concentrate the chemicals in solution with 6 mL Waters Oasis HLB 6cc cartridges, 200 mg sorbent per cartridge, 30  $\mu$ m particule size. Before extracting samples, cartridges were cleaned up by washing them subsequently with 12 mL of dichloromethane /methanol (1:1) then 24 mL of pure water. Precautions were taken to avoid cartridge adsorbent getting dry before and during the percolation of the water sample. Glass tubes used to collect wasted solvent from cartridges washing were taken out from the box and discarded. Samples were then poured into the cartridges and percolated through at a flow rate similar to 10–15 mL/min using a slight vacuum. The calibration flask that contained the sample was rinsed with 10 mL of pure water and this washing water was also percolated through the cartridge. After percolation, cartridges were allowed to get dry under vacuum for 1 min. Cleaned glass tubes (25 mL) were put in the percolation box under the cartridges and elution was made by 36 mL of acetonitrile through  $\text{NaSO}_4$  to remove water residue. The collected extracts were evaporated to dryness using rotary evaporation (R-210, Swiss Buchi) and

transferred, using multiple washing with acetonitrile, into a 1.8 mL glass vial equipped with a Teflon-lined screw cap. The sample was brought to dryness under the stream of nitrogen and re-dissolved in an exact volume (typically 1 mL) 2-propanol. Such final extract was directly analyzed by normal-phase HPLC as described below.

## 3. RESULTS AND DISCUSSION

### 3.1 NP and NPEOs Detection

NPEO were analyzed by using a normal phase column. Column temperature and wavelength were respectively according to Ahel [14] and [15] with flow rate set at 1 mL/min. Results in Fig. 2 show that only 2-propanol used as solvent can well separate NPEOs (solvents and mobile phase were here the same).

When different mobile phases were tested with NPEOs dissolved in 2-propanol, the best result was obtained when mobile phase were 2-propanol in Fig. 3.

Effect of flow rate was seen by varying it from 0.1 to 1 mL/min. On Fig. 4, 0.1 mL/min gives the best result because peaks are better separated, the highest and areas are the biggest when used 0.1 as mobile. It can be seen that peaks high and area become respectively higher and bigger with reducing flow rate. Here the elution time was 90 min which can be considered as slow detection compare to rapid detection of Ahel works [14,15] where flow rate was 1.5 mL/min in almost 20 min.

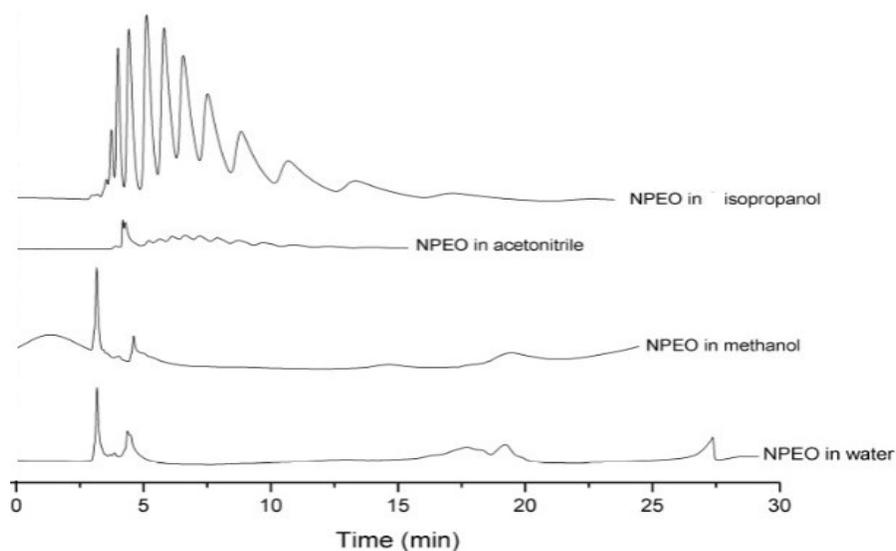
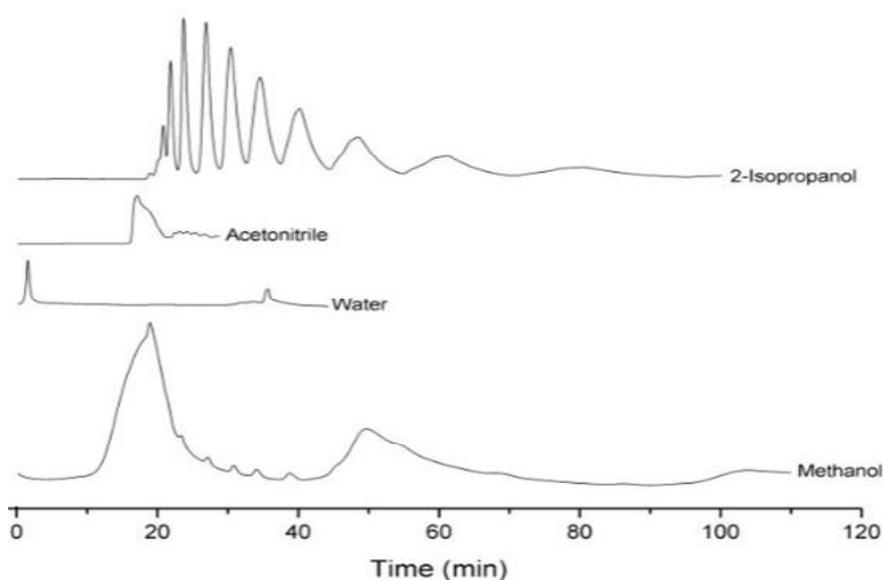
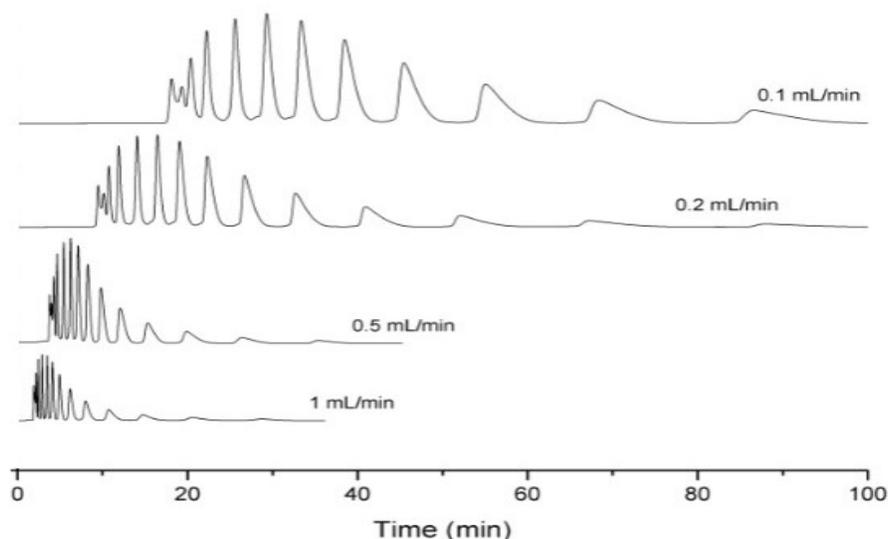


Fig. 2. NPEOs analysis using different solvents



**Fig. 3. NPEOs (dissolved in 2-propanol) analysis using different mobile phases**



**Fig. 4. Flow rate influence on NPEOs separation**

To highlight NP eluting on NPEOs chromatograph, an amount of 10  $\mu$ L of NP stock solution has been added to 1 mL of NPEOs stock solution. Fig. 5 shows clearly a rising of first peak, which is probably NP peak (NP added to NPEOs (A)). That means in a mixture of nonylphenol ethoxylates (B), there is a very small amount of nonylphenol. In the other hand, nonylphenol elute before its ethoxylates.

Various extraction solvents were used in association with extraction methods in order to

compare their extraction recovery. Averages have been given in Table 1. For liquid-liquid extraction, 2-propanol, hexane and DCM were used. DCM gives the best result with 80.3% of recovery. Extraction by LLE was not possible with 2-Isopropanol because it was totally mixed with water. Hexane gives 2% as recovery. SDE extraction is not convenient for NPEOs extraction because no peaks separation was detected on chromatograph. For SPE extraction, firstly two cartridges (HLB and ODS-C18) were used with DCM. Results show that HLB is more suitable

with 88% of recovery while ODS-C18 gives 75.4% recovery. Secondly, with HLB cartridge, extraction solvent was changed by ACN and 2-propanol. ACN has the best recovery with 95.6% while 2-propanol gives 89.7% as recovery percentage.

**Table 1. Extraction solvent and recovery**

Extraction method	Recovery %
LLE ISO	-
LLE HEXANE	2
LLE DCM	80,3
SPE HLB DCM	88
SPE ODS DCM	75,4
SPE HLB ISO	89,7
SPE HLB ACN	95,6
SDE	-

## 3.2 Water Samples

### 3.2.1 Surface water

As it can be seen below, only short chain (1 to 3) of NPEOs and NP have found in rivers and waste water collectors around the industrial area of Taiyuan city. This could be explained by the fact that NPEOs long chains are degraded into short chains under certain conditions in natural environment which confirms results of many studies [16,17,18,19,20,21,22]. Concentrations of NP and NPEOs in surface water ranged from 99.3 to 1113.4 µg/L and 38 to 743 µg/L respectively as shown in Table 2. Highest NP and NPEOs concentrations have been detected respectively at the point SW14 and sw01 (in the same area). This could mean a nearest source of pollution situated in this area. The increase in NP concentration on Taigechi (sw06-sw10) collector, results in water reception from others secondary collectors. In the other hand, the decrease in NP concentrations from Donggan (sw04-sw01) and Beizhangchi sw14-sw06 may be attributed to a degradation process of NP during the water flow respectively in south-north and north-south direction. On Fen river NP (180.4 µg/L) concentration is higher than Shao river (99.3 µg/L), which could be due to the fact that Fen river runs through the territory of Taiyuan City, therefore receives more waste water. Some authors have calculated the non-effect concentrations for NP which are 0.33–0.6 µg/L [23,24]. All water samples exceed so far this critical level and concentrations found in developed countries [25,22], and may be hazardous to aquatic populations in those 2 rivers of Taiyuan city. The concentrations

measured in these developed countries decreased significantly over the past decade because of restrictions on the use of NPEOs in laundry detergents and the voluntary phase-out of NPEO surfactants in industrial cleaning formulations [26]. In contradiction, high concentrations of NP and NPEOs, ranging from 1.7 to 7.3 µg/L and 2.5 to 98 µg/L, respectively, have been detected in Yangtze River at Chongqing area in China [27]. Also concentrations of NP ranging from n.d. to 41.3µg/L were detected in Lake Shihwa in Korea [28] for freshwater. In comparison with those studies and EPA standards for freshwater [29], Fen and Shao rivers concentrations are higher so that they can be consider as polluted rivers considering these chemicals and industries in this city should apply some treatments or degradation processes before introducing their wastewater into environment in order to release non or less toxic products from degradation processes as Kouakou [30] found in their works.

Table 3 shows almost neutral pH of groundwater samples which are ranged between 6.43 and 7.88. Temperatures are ranged from 12.1 to 19.9, which may confer the psychrophilic characteristic to the samples. Samples present high level of conductivity in their majority. Groundwater in these areas has then a high salinity. Ec results are ranging from 1905 to 20900 µS/cm. This shows that Ec values of groundwater in this region are very high compared to pure water with a very slight electrical conductivity (0.055 µS/cm, 25°C). Electrical Conductivity (Ec) is the ability of an electric current to pass through water proportionally to the amount of dissolved salts in the water, specifically the amount of charged (ionic) particles. The concentration of salts in surface and groundwater can increase in several ways [31]: Increased dissolution can increase salinity levels; evaporative enrichment is the process of increasing salinity levels in surface or groundwater by removing water via evaporation [32]; Human activities also affect salinity levels in ground and surface water. Indeed, the EC high values of groundwater samples indicate saline water or anthropogenic contamination [33]. As it has been seen at the sampling period, famers in many places use surface water from collectors for farms irrigation and fertilizers were also applied. Those human activities added to the natural dissolution occurring during the infiltration of water may have as result a high salinity in this region as explained above. Groundwater concentrations of NP and total NPEOs are respectively ranged from 25.5 to 166.4 µg/L and

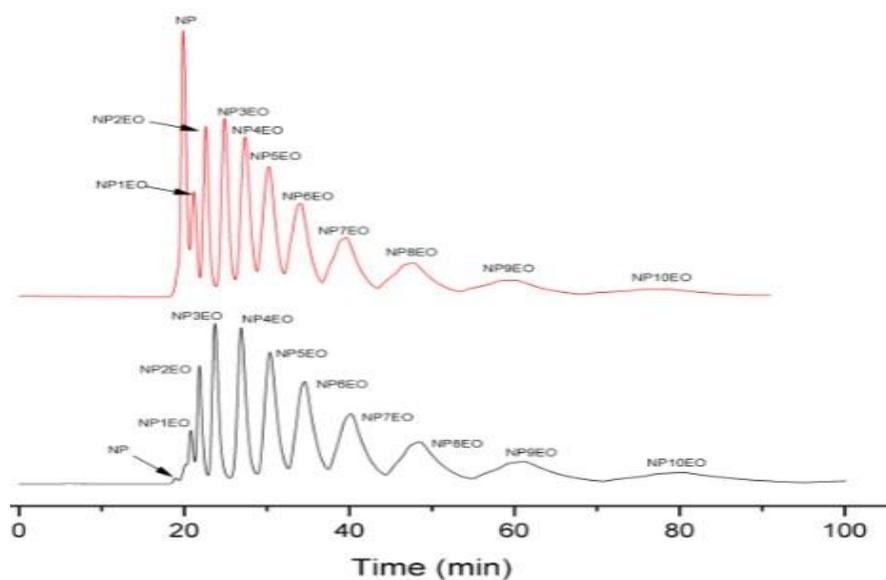


Fig. 5. Identification NP in NPEOs commercial mixture

Table 2. Concentrations ( $\mu\text{g/L}$ ) of NP and NPEOs in surface water samples

Samples	[NP]	[NP1EO]	[NP2EO]	[NP3EO]
SW00	221.8	-	-	-
SW01	138	743	-	96.9
SW02	132	-	-	38
SW03	161	-	-	42
SW04	289.7	-	-	69.7
SW05	282.4	-	-	272.8
SW06	270.7	-	-	189.7
SW07	118	684	-	143.9
SW09	182.3	-	227.5	78.3
SW10	380	-	-	205.8
SW11	99.3	-	-	-
SW12	180.4	-	-	90.5
SW14	1113.4	-	589.2	248

Table 3. Concentrations ( $\mu\text{g/L}$ ) of NP and NPEOs in groundwater samples

Samples	pH	EC	Temp	NP	NP1EO	NP2EO	NP3EO
GW01	7.88	3010	30.3	25.5	-	30.3	-
GW02	7.05	12900	24.2	35	-	24.2	-
GW03	6.79	20900	77.5	53.4	-	77.5	-
GW05	7.2	1902	-	33	-	-	-
GW06	7.73	1905	96.7	37.2	20	76.7	-
GW08	7.1	3300	94.6	44.9	94.6	-	-
GW10	7.28	4450	-	109.6	-	-	-
GW11	6.95	10100	275.8	47.7	275.8	-	-
GW12	6.92	9680	-	48.9	-	-	-
GW13	6.7	17300	111.4	159	-	111.4	-
GW14	7.47	2300	146.3	27.6	123.3	23	-
GW15	6.64	7500	-	54.4	-	-	-
GW16	6.68	5000	-	56.3	-	-	-
GW17	6.43	14500	66.8	166.4	-	-	66.8

from 24.2 to 275.8 µg/L. The presence of nonylphenol and its ethoxylates in aquifers is related invariably to anthropogenic activities. It can be seen that like surface water only short chain of NPEOs have been found as reported by Ahel [34] about the occurrence of NP, NP1EO and NP2EO in groundwater from three different field sites in the alluvial aquifer of Sava River, Croatia. It is understandable to find NP in Taiyuan groundwater because the removal of contaminants in groundwater is usually very slow since the chemical and biological characteristics in the aquifers are not favorable to secure degradation [35]. In addition, groundwater temperatures are in the psychrophilic range and both carbon sources and oxygen are then limited. As a consequence, the microbiological resources of such ecosystems are restricted and the contaminants undergo extremely slow degradation rates allowing contaminants to disperse up to several km from the contamination source and to exist for decades [36]. Considering Taiyuan city groundwater concentration in NP, attention should also pay to tap water NP concentration in case of human consumption.

#### 4. CONCLUSION

It can be retained that NP elute before NPEO in HPLC analysis and best conditions for HPLC detection are: 2-propanol as standards solvent and mobile phase, and 0.1 ml/min as flow rate. Solid phase extraction gives better results than extraction methods especially liquid-liquid extraction. Stream distilled extraction DE method is not suitable for NPEO extraction. These conditions have been used to determine NP and NPEOs concentrations in water environment of Taiyuan city industrial area. High concentrations have been found both in surface and groundwater which represents a high risk of exposition to endocrine disruptors for humans and aquatic species. Actions should be taken to avoid or reduce the use of those compounds, or industries should apply some treatment before release their wastewater into environment. Attention should be paid especially to groundwater in case of human consumption.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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