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Application of Gas and Liquid Chromatography in Determination of Nonylphenol in the Workplace Air

Dr. Małgorzata Szewczyńska, Elżbieta Dobrzyńska, Dr. Małgorzata Pośniak

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Abstract:

Background: The paper presents the application of two widespread chromatographic techniques for determination of nonylphenol air sample.

Material and methods: IOM sampler and adsorption tube with Amberlite XAD-2 resin connected to a 2 Lmin-1 aspirator were used to isolate the inhaled and respirable fraction of particles and the gaseous phase of 4-nonylphenol from the air. Nonylphenol was analyzed by high-performance liquid chromatography with fluorescence detection (UHPLC/FL) and gas chromatography with mass spectrometry detection (GC/MS).

Results: For 4-nonylphenol the calibration range in the HPLC/FL method was from 3.25 to 130 ng mL-1. At the air intake of 720 L the determinability of 4.5 ng m-3 was obtained. Co-existing substances did not interfere. The possibility of using GC/MS technique for determination of alkylphenols was also checked. The calibration curve was performed in the range of 0.04 - 3.3 µg mL-1. Determination at the level of 53 ng m-3 was obtained.

Conclusions: The method of air sampling shall ensure the quantitative separation of the substance present in the gaseous phase and in the particles. HPLC/FL has been chosen for the determination to give the lowest quantification for nonylphenol, which is important as it can occur in the working environment air at very low concentration levels.

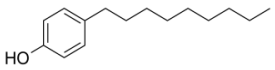
Keywords: Alkylophenols, Fine Particles, Respirable Fraction.

INTRODUCTION

Compounds which, apart from producing certain effects in the environment and the ability to bioaccumulate in fatty tissues, are capable of binding to estrogen receptors, are called endocrine active modulators, hormonal modulators or endocrine disrupting compounds (EDCs). Endocrine disruptors include among others, frequently monitored alkylphenol compounds, which include 4-tertooctylphenol, 4-nonylphenol and bisphenol A. Alkylphenols imitate the activity of naturally produced hormones, block hormone receptors in the cell, affect the synthesis, transport, metabolism and excretion of hormones because they are structurally similar to the natural sex hormone 17ß-estradiol [1]. The first evidence that alkylphenols may be estrogenic was published in 1938 by Dodds and Lawson [2]. Nonylphenol is a toxic xenobiotic compound classified as an endocrine disrupter that originates principally from the degradation of nonylphenolethoxylates widely used as industrial surfactants. Nonylphenolethoxylates reach sewage treatment works in substantial quantities where they biodegrade into several by-products including nonylphenol[3]. It is the most abundant derivatives of alkylphenolpolyethoxylate compounds demonstrated to stay biologically active for a longer period of time in the body than an endogenous estrogen [4]. Nonylphenol is a raw material or an intermediate in the manufacture of the non-ionic surfactants nonylphenolexthoxylates, which are used for industrial, commercial use and in households in detergents, paints, pesticides, personal care products, plastics and additives for lubricating oils. Table 1 presents basic physicochemical properties of nonylphenol and its classifications on the list of endocrine active substances (EDCs) and according to the CLP regulation.

Dr. Małgorzata Szewczyńska, Centralny Instytut Ochrony Pracy - PIB, Poland. E-mail: mapol@ciop.pl
Elżbieta Dobrzyńska, Centralny Instytut Ochrony Pracy - PIB, Poland. E-mail: eleki@ciop.pl
Dr. Małgorzata Pośniak, Centralny Instytut Ochrony Pracy - PIB, Poland. E-mail: mapos@ciop.pl

Table 1: Basic physicochemical properties of npnylphenol and its classification on the list of endocrine active substances (EDCs) and according to the CLP Regulation

Name of the compound	4-Nonylphenol
shortcut	(4-NP)
Formula	
CAS#	104-40-5
	25154-52-3 (CLP)
Molar mass	220.36 g/mol
Densities	0.94 g/cm^3
Appearance	White crystals
Melting point	43 – 45 ^{o}C
Boiling point	180 – 181 ^{o}C
Solubility in water	0.06 g/100mL (25 ^{o}C)
Solubility	Alcohols, ketones, esters
CLP Classification	
Hazard Class and Category Code(s)	Repr. 2 Acute Tox. 4 Skin Corr. 1B AquaticAcute 1 Aquaticchronic 1
Hazard Statement Code(s)	H302 Harmful if swallowed. H314 Causes severe skin burns and eye damage. H361fd Suspected of damaging fertility. Suspected of damaging the unborn child H400 Very toxic to aquatic life. H410 Very toxic to aquatic life with long-lasting effects.
Labeling	
Pictograms,	
Signal Word Code(s)	GHS09 GHS08 GHS05 GHS07 Dgr
Classification as endocrine active substance (EDCs)	Included in the EDCs list, without classification

Since the first synthesis of nonylphenol in 1940, its use and production have increased very rapidly [5] and the scientific opinions on the environmental effects of nonylphenolethoxylates and nonylphenol itself were very controversial. Already in 1983-84 concerns were raised about the effects of nonylphenol, when

nonylphenolethoxylates and degradation products were found to be more toxic to aquatic organisms than their precursors [6]. Soto at el. [7] noted that nonylphenol, applied to produce the test tubes used in their experiments, was capable of initiating proliferation in breast cancer cells as if estrogens were present. It was found that nonylphenol mimics the natural hormone 17β-estradiol, competing for the place of estrogen receptor binding [8, 9]. It is therefore assumed that this compound will initiate various reactions in organisms. Recently it has also been established that nonylphenol is able to interfere with the proper functioning of androgens, which are essential for the proper development of males and their reproductive systems [10]. Due to these worrying observations, European countries have begun to take actions against nonylphenol compounds, first through the introduction of voluntary agreements with the industry to limit the use and production of nonylphenol derivatives.

Major pathways of exposure to EDCs may include dietary and non dietary ingestion, dermal absorption from product use, and inhalation [11]. Thus, it should be also noted that alkylphenols are important air pollutants both in indoor and outdoor areas. Moreover, emissions in the workplace can have harmful effects on employees. Even cleaning products as well as personal care products can act as a potential source of air pollution with nonylphenol. Existing studies show that indoor concentrations are generally about 100 ng ml-3, while outdoor concentrations are about 10 times lower [11, 12]. EDCs exist in air as volatile or semi-volatile compounds in the gas phase or attached to particulate matter [13]. Since the place of deposition of particulate matter in the body has an impact on health effects, two fractions of particles - inhalable and respirable fraction should be considered. Inhalable aerosol fraction is the fraction of total airborne particles that enters the body through the nose and/or mouth during breathing. This fraction corresponding to particles with aerodynamic diameter ≤100 μm is relevant to health effects anywhere in the respiratory tract. Respirable aerosol fraction is the sub fraction of the inhaled particles <10 μm that penetrates into the alveolar region of the lung (i.e., includes the respiratory bronchioles, the alveolar ducts and sacs) and is pertinent to the development of such chronic diseases as pneumoconiosis and emphysema. Hence the route of nonylphenol in the respiratory system seems to be extremely important.

Nonylphenol occurs in the air at very low concentration levels. In order to obtain the lowest possible limit of quantification, two analytical methods of gas chromatography with detection of mass spectrometry and liquid chromatography with fluorescence detection were compared to determine these compounds in the workplace air. High-performance liquid chromatography with fluorescence detection (HPLC-FL) [14] and technique of gas chromatography with mass spectrometry [15, 16] were used to optimise and validate the method of determination 4-nonylphenol in air samples.

MATERIALS AND METHOD

Material and Reagents

The following reagents used in the presented study*:* acetonitrile (ACN), dichloromethane (DCHM), acetone, ethanol were purchased from J.T. Baker (USA).High purity water was obtained from Milli-Q equipment (Millipore Corporation, USA). Analytical standard of 4- nonylophenol(NP) was from Supelco (USA); bisphenol A from Aldrich (USA) and 4-tert-oktylphenol from Sigma (USA).

Fibreglass filters of 25 mm diameter from Waters (England), polyurethane foam (PUF) and adsorption tube filled with Amberlite XAD-2 resin from Sigma Aldrich(USA) were used for air sampling.

Nylon syringe filters of diameter 25 mm and pore size of 0.45 μm (Alltech, USA) for filtering solution before HPLC analysis were used. Additional equipment used in the presented study consisted of glassware, volumetric flasks, 10 mL conicalflasks with stoppers, test tubes, pipettes, and syringes.

Apparatus

Gas chromatograph 7890A with 5975C mass spectrometry detector from Agilent Technologies was used. RTX-5 silMS column length 30 m, internal diameter 0.25 mm and film thickness 0.25 μm (Restek USA) was applied. EliteLaChrom Ultra liquid chromatograph with VWR Hitachi Fluorescent Detector (HPLC) was used in the experiment. Eclipse XDB C_{18} column (150 mm long, 4.6 mm inside diameter with 1.8 μm grain size) from Agilent Technologies(USA)was applied.

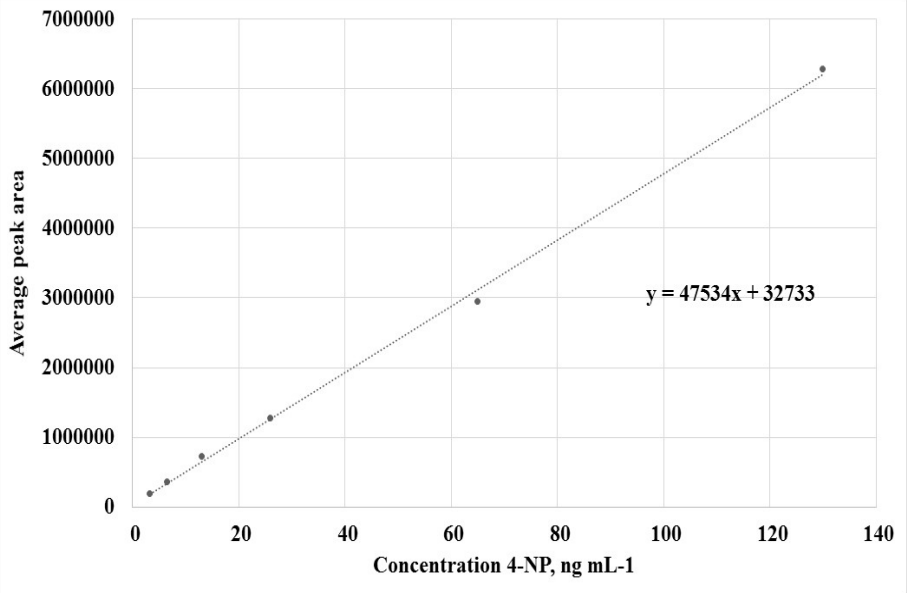
Gilair 5 aspirator operating at 2 L/min(Sensidyne, USA) was used for collecting air samples. Sartorius TE214Sanalytical balance (Sartorius Corporation, USA) to weigh standard substances. EKS series cabinet desiccator (WSL, Poland) was used to store filters.

RESULTS AND DISCUSSION

In order to determine the conditions of air sampling, the type of sampler, the volume of extracted air, as well as the flow of air volume were determined.

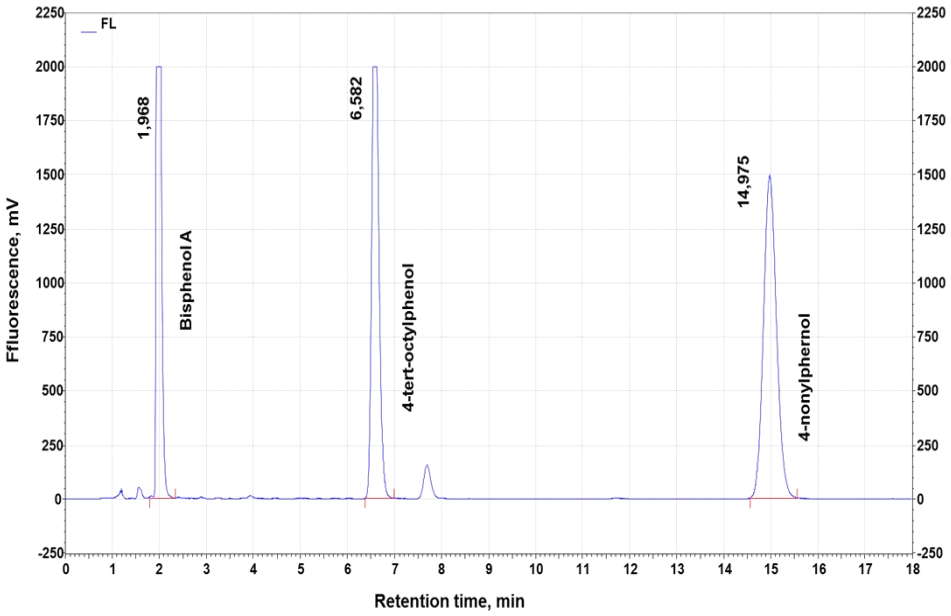
The air sampling kit consisted of a sampler developed by Vincent and Mark at the Institute of Occupational Medicine (IOM) which contained a glass fibre filter and polyurethane PUF foam to retain nonylphenol in the inhalable and respirable fractions and adsorption tube with Amberlite XAD-2 to separate the gas fraction. In order to take up an appropriate volume of air, the sampler was combined with an aspirator (pumps) with an appropriate volume flow rate. The sampling rate for the inhalable and respirable fractions is 2 L min-1 for the sampler "IOM Personal Inhalable PM Sampler". To determine nonylphenol in the air using the developed method, 720 L of air had to be collected in 360 minutes.

In order to elaborate the conditions of nonylphenol extraction before HPLC/FL analysis from a glass fibre filter, PUF and XAD-2 resin, methanol (MeOH), acetonitrile (ACN) and acetonitrile/water mixture (ACN/H_20, 70:30 v/v) were used. Thus, 100 µL of nonylphenol standard at a concentration of 3.33 µg mL-1 was injected separately into glass fibre filters, PUF foam and glass tubes containing XAD-2. After drying, the filters and PUF foam were placed in the IOM sampler, connected to the XAD-2 tube and the aspirator. Air was passed through the sampler for 6 hours. After this time, nonylphenol was desorbed from each of the sampling media with 3 mL of methanol. The samples were shaken in ultrasound for 30 minutes. The control sample was stored and shaken under the same conditions as the samples. The same procedure was applied for recovery tests using the remaining solvents. Figure 1 shows the results of the recovery test with use of different solvents.

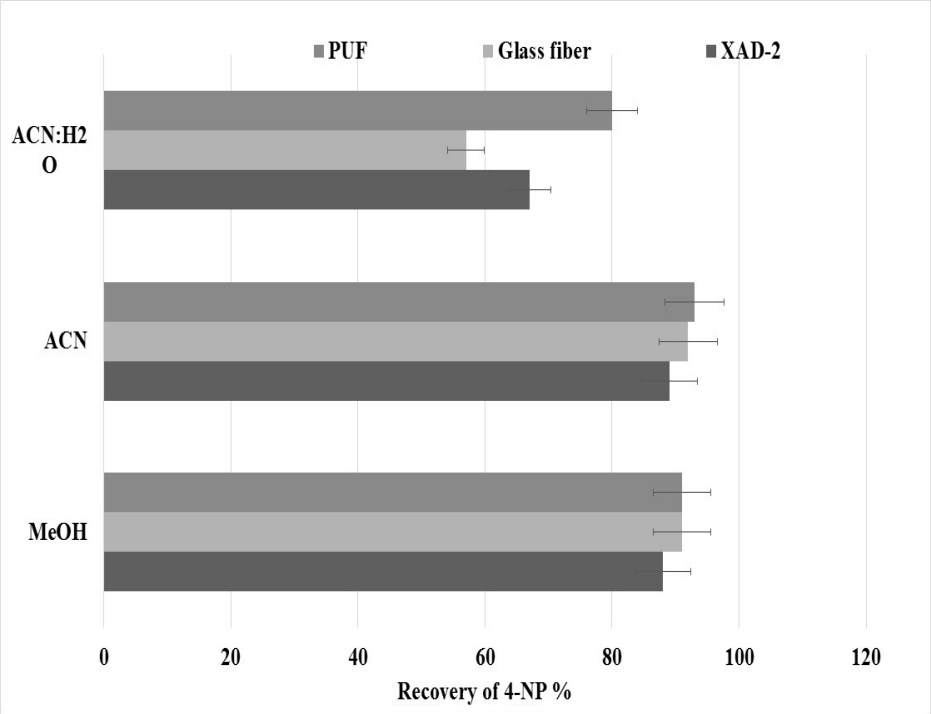


Based on the above results (Fig 1.), the best recovery values were obtained using acetonitrile and methanol. However, the use of 100% methanol as solvent resulted in high peak asymmetry, which was caused by the solvent effect. Taking into account the recovery values and the signal on the chromatogram for 4-nonylphenol, acetonitrile was used for further studies.

For analysis of nonylphenol by high performance liquid chromatography with a fluorescent detector,Eclipse XDB-C18 analytical column of 150 mm long with ULTRA C18 pre-column was used. The column operated at 40^0C. Acetonitrile and water in various volume ratios were used as the mobile phase and a change of 4-NP retention time was observed as the acetonitrile content increased with the flow rate of 1 mL min-1. As a result of the study it was found that the ratio of 70% ACN to 30% water (V/V) gives an optimal retention time of 15 min. The fluorescence detector worked at excitation wavelength: 226 nm and emission at 315 nm. The injection volume was 90 µL. Under these conditions the separation of 4-nonylphenol from co-occurring substances, i.e. bisphenol A and 4-tert octylphenol is obtained(Fig. 2).



Under the developed conditions of chromatographic analysis, a linear calibration graph of 4-nonylphenol in the range of 3.25-130 ng/mL was made and the correlation coefficient was 0.998 (Fig. 3).



The described method of determination of nonylphenol in workplace air has been validated according to PN-EN 482:2012 [17] in terms of linearity, sensitivity, selectivity and precision. The detection limit (LOD) and quantification limit (LOQ) were determined on the basis of the results of the blank test. The total uncertainty, the expanded uncertainty of the developed method were also calculated. The degree of nonylphenol recovery from the filter, from PUF and XAD-2 were also investigated. For three levels of concentration 13; 65 and 130 ng ml-1 the overall test precision as a mean coefficient of variation for the range of the method was determined. The results of validation are presented in Table 2.

Table 2: Validation parameters 4-NP by HPLC-FL method

Parameter	Value for 4-NP
Range of calibration [ng/ml]	3.25 - 130
Determinability	
for fiber glasses and PUF [ng m-3]	13.5(720 L)
XAD-2[ng m-3]	4.5 (720 L)
Average efficiency of the desorption/recovery coefficient. %	98
Overall test precision - mean coefficient of variation for the range	6.54
Relativetotaluncertainty [%]	14.06
Extended uncertainty[%]	28.12
Limit of detection *[ngmL-1]*	0.15
Limit of quantification *[ngmL-1]*	0.45

In order to establish the working conditions of GC/MS for the determination of 4-NP and co-occurring compounds, the analytical conditions proposed by Berkner and co-workers [15] and Xie with col.[16] have been optimized. The RTX-5 silMS column, 30 m x 0.25 m x 0.25 m x 0.25 µm and programmable furnace temperature was applied. The column operated at programmed furnace temperature from 60°C (1 min), temperature increase from 10°C/min to 320°C (10 min). Injection chamber temperature was 300 °C, transfer line temperature 280°C, carrier gas was helium with a flow rate of 1 mL/min, injection volume was 5 µL, sample splitter was determined at 10:1. Ion monitoring (m/z) was 107, 135 (NP); 107, 220 (4-t-OP); 213, 228 (BPA), respectively. Acetone was used for extraction, as it was used, among others, in the works of Saito et al. [18] and Li Luo et al. [19]. At this stage, no other solvents have been tested and the results of these publications have been taken as a basis. For the recovery of 4 nonylphenol from a fibreglass filter, PUF polyurethane foam and glass tubes containing XAD-2 with acetone, the same procedure as described in the HPLC-FL method was followed. The results of the 720 L air permeation recovery test are presented in Table 3.

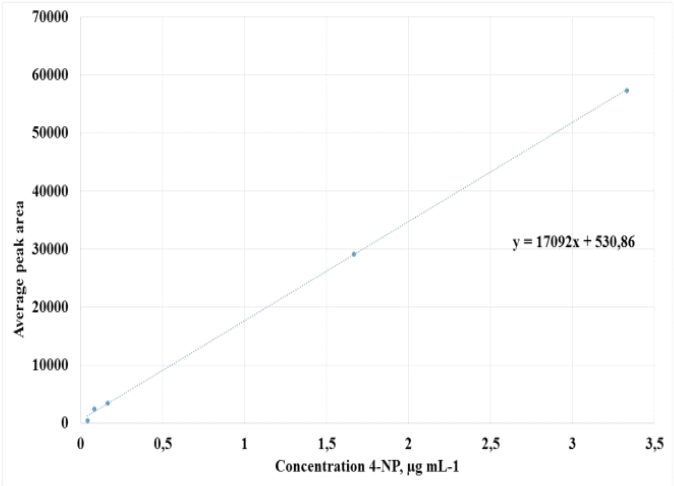
Table 3: Recovery test results for nonylphenol

Sampling medium	**PeakArea for 4-nonylphenol**			**RSD [%]**	**Recovery [%]**
	Average for sample (n=6)	Average for blank (n=3)	Average for control solution (n=3)		
Glassfiber	2812	n.d	3099	4.52	**91**
PUF	2763	n.d	3258	3.57	**85**
XAD-2	815	n.d	1137	2.25	**72**

n.d - not detected

Using acetone for 4-NP the recovery coefficients of 91; 85; 72%. for glass fibre filter, polyurethane foam PUF and Amberlite XAD-2 resin, respectively, were obtained.

In the GC/MS/SIM method the calibration curve was obtained in the concentration range from 0.04 to 3.33 µg mL-1 and the correlation coefficient was 0.999 (Fig. 4).



As in the case of using the HPLC/FL method, the validation parameters of the developed method were determined. The precision of the method for three concentration levels of 0.166, 1.66 and 3.33 µg mL-1 was determined. The results of validation are presented in Table 4.

Table 4: Validation parameters 4-NP by GC/MS-SIM method

Parameter	Value for 4-NP
Range of calibration [ng/ml] Determinability for fiber glasses and PUF [ng m-3] XAD-2[ngm-3]	40 – 3300 160 (720 L) 53 (720 L)
Average efficiency of the desorption/recovery coefficient. % Overall test precision - mean coefficient of variation for the range Relativetotaluncertainty [%]	96 2.74 6.46
Extended uncertainty[%]	12.92
Limit of detection *[ng mL-1]*	8
Limit of quantification *[ng mL-1]*	24

By comparison of both GC and HPLC methods for determination of 4-nonylphenol in the workplace air, better results and lower limits of quantification were obtained for the method using high-performance liquid chromatography. For the HPLC method the determinability of 13.5 for fiber glasses and PUF and 4.5 ng ml-3for XAD -2 at 720 L of air intake was obtained, whereas for the GC-MS method this determination was found to be unsatisfactory and almost 12 times higher. In the HPLC-FL method the limit of detection and the limit of quantification of the method was significantly lower (0.15 ng mL-1 and 0.45 ng mL-1) than in the GC-MS method (8 ng mL-1, 24 ng mL-1).

CONCLUSIONS

The proposed method of air sampling for nonylphenol determination ensures quantitative separation of the substance present in the gaseous phase and on the solid particles of the inhalable and respirable fraction through the use of a combined IOM sampler equipped with glass fibre filter and polyurethane foam sampler connected to adsorption tube filled with Amberlite XAD-2. This is important for health safety and human exposure assessment. Two chromatographic techniques were used for the analytical determination and a high performance liquid chromatography method with fluorescence detection giving the lowest determinability for nonylphenol was selected. This is important in the determination method because nonylphenol in the air of the working environment may occur at very low concentration levels.

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