*Pol. J. Environ. Stud. Vol. 21, No. 1 (2012), 129-138*

*Original Research*

# Screening of Trace Organic Compounds in Municipal Wastewater by Gas Chromatography-Mass Spectrometry

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*Received: 4 January 2011*
*Accepted: 21 July 2011*

## Abstract

This research proposes the simple and reliable procedure of screening a wide range of polarity trace organic pollutants in influent and effluent municipal wastewater. Such a method includes concentrations of analytes on C18 bonded phase cartridges and its subsequent eluting with organic solvents of different polarity: *n*-hexane, ethyl ether, and methanol. Each eluate is directed to gas chromatography/mass spectrometry (GC/MS) separation and detection. Silylation of compounds eluted with ethyl ether and methanol was done prior to chromatographic analysis. For reliable identification of the unknown organic compounds a combination of three independent parameters: mass spectra, retention indices and partition coefficients in *n*-hexane/ acetonitrile system was employed. More than 120 compounds were identified and semi-quantitatively determined in municipal wastewater from the Wastewater Treatment Plant in Białystok, mainly from the groups of aliphatic acids, alcohols and polyalcohols, and carbohydrates. Compounds potentially harmful to the environment and found in the analyzed municipal wastewater are: 2,6-ditertbutylhydroxytoluene, phosphoric acid, phthalic acid esters, phenol, and drug remnants (ibuprofen, naproxen, and caffeine).

**Keywords:** wastewater analysis, identification, distribution coefficients, gas chromatography/mass spectrometry

## Introduction

The quality of municipal wastewater needs attention as it is one of the main sources of surface water pollution. Municipal (domestic) wastewater is the outflow that comes from households, offices, laundries, hospitals, and small industrial plants. Also, rain waters and snow-melt alongside with impurities washed away from streets and adjacent areas access municipal wastewater. Municipal wastes are channeled to purification plants, where they undergo processes designed to remove excess organic and inorganic matter and where their chemical analyses are carried out. Standard procedures used to mark organic compounds employed in sewage-treatment plants, e.g. biochemical and chemical oxygen demand, allow us to establish the total content of all or a certain group of organic substances.

A considerable number of studies devoted to the subject of marking chosen organic trace pollutants in influent and effluent municipal wastewater can be found in scientific literature. Some of the most often marked analytes are: different kinds of pharmaceuticals, endocrine-disrupting compounds, sex hormones, and volatile organic compounds [1-9]. Personal care products, corticosteroids, polychlorinated dibenzo-p-dioxins and furans, chlorinated flame retardants, chlorinated harmans, and polycyclic aromatic hydrocar-

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bons (PAHs), alkyl substituted PAHs, carbazoles and alkyl-substituted carbazoles, were also marked in wastewater [1, 3, 6, 7, 10, 11].

Target compound monitoring is often insufficient to assess and maintain wastewater quality. Many (as yet) unknown compounds can be present, some of them harmful to the environment and also to humans. Only a few papers have been devoted to the complete identification of the wide range of non-target organic compounds in wastewater samples [12-16].

Identification of the unknown analytes is a key element in the process of screening analysis of wastewater samples, which are complex mixtures of organic compounds. Screening analysis of wastewater samples was carried out only by matching registered mass spectra with the spectra in a reference library [13-15]. The application of a single identification parameter is insufficient to acknowledge the results as reliable. The reliability of GC/MS identification is substantially increased by the use of both GC and MS identification approaches [17-18]. However, in some cases mass spectra and retention data alone may be insufficient for positive identification. In such circumstances additional spectroscopic methods such as nuclear magnetic resonance spectroscopy or recordings of ultraviolet absorption spectra or infrared absorption spectra are usually applied [19].

The main purpose of this work was the qualitative study of trace organic compounds in municipal wastewater. To increase reliability of organic pollutant identification, the use of partition coefficients ($K_p = C_1/C_2$) between two immiscible liquids has been proposed. The $K_p$ value of compound is a physico-chemical constant as, for instance, its boiling temperature. Determination of partition coefficients is simple and no additional equipment is needed to do it [16, 20, 21].

## Experimental

### Materials

Acetonitrile, *n*-hexane (Merck, HPLC grade), anhydrous pyridine (Fluka), diethyl ether and methanol (POCH, Poland) were used without additional purification. The derivatization reagent was bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (Sigma). Series of $C_{10}$-$C_{40}$ *n*-alkanes and *n*-butyl benzene (internal standard) were purchased from Sigma. Solid phase extraction cartridges C18 were obtained from Supelco Inc.

### Sample Collection

Wastewater samples were obtained from the Wastewater Treatment Plant in Białystok. The plant receives municipal wastes from the territory of Białystok, a city with the population of more than 300,000 people. Approximately 15 mln $m^3$ of wastewater are disposed to the plant each year. The purification process consists of two stages: mechanical purification, and biological purification and lasts 24 to 30 h. After being purified, the wastewater is disposed to the local Białka River that flows into the Supraśl River. Average daily samples of influent and effluent wastewater were taken five times between February and November. Wastewater samples were drawn into glass bottles. Upon arrival the samples were immediately acidified (pH ~2) with the use of hydrochloric acid, filtered to obtain clear solution, and then stored at 4ºC until analyzed (no longer than 2 days).

### Solid Phase Extraction

C18 SPE cartridges containing 1 g of sorbent were conditioned by eluting 2·10 mL of acetone, followed by 2·10 mL of methanol and 2·10 mL of distilled water. The 350 mL of raw wastewater or 1000 mL of treated wastewater sample was allowed to pass through the cartridge at a flow rate of 10 mL·$min^{-1}$ by applying a slight vacuum. The cartridges were washed by distilled water (50 mL) and air-dried for 5 min. The analytes were eluted with 2·10 mL of hexane, followed by 2·10 mL of ethyl ether and 2·10 mL of methanol. *n*-Hexane eluates were concentrated to 1 mL using a rotor evaporator and submitted for GC/MS analysis. Solvents were removed from ethyl ether and methanol eluates on a rotor evaporator and 40 µL of pyridine and 100 µL of BSTFA were added to the dry residues. The mixture was heated for half an hour at 70ºC to form trimethylsilyl (TMS) derivatives, and the resulting solution was used for partition coefficient determination and GC/MS analysis. This procedure was performed in triplicate for each sample.

### *n*-Hexane/Acetonitrile Partition

Samples were prepared for distribution coefficient determination at 22ºC. A 2 mL flask was charged through a pipette with 0.5 mL of *n*-hexane and 0.5 mL of acetonitrile. 40 µL of solution after derivatization and 0.5 µL of *n*-butyl benzene were added to the flask. Subsequently, the flask was closed and intensively shaken for 5 minutes. After the phases were separated, the solution was left for half an hour to attain the state of equilibrium. Afterward, each phase was subjected to GC analysis.

Distribution coefficients were calculated from the ratio of the peak areas of the components by using Eq. (1):

$$K_p = (S_h^x / S_a^x) \cdot (S_a^b / S_h^b) \cdot K_p^b \quad (1)$$

...where $S_h^x$ and $S_a^x$ are the peak areas of component *x* on the chromatograms of the hexane and acetonitrile phases, respectively. Whereas $S_a^b$ and $S_h^b$ are the peak areas of *n*-butyl benzene on these chromatograms, $K_p^b = (2.35 \pm 0.23)$ is the partition coefficient for *n*-butyl benzene.

### Chromatographic Analyses

Gas chromatographic analyses were carried out using an HP 6890 gas chromatograph with electronic pressure control (EPC), flame ionization detector (FID), an MSD 5973 mass spectrometric detector (electron impact source and quadru-

pole analyzer), and an HP 7673 autosampler from Agilent Technologies, USA. This device was equipped with HP-5 columns (5% phenylmethylsiloxane) size 30 m length × 0.25 mm, i.e. coated with 0.25 μm film thickness and split/splitless injector. The oven temperature was programmed from 50ºC (for ethyl ether and methanol eluates) or 40ºC (for hexane eluates), increased to 300ºC at a rate of 3ºC·min$^{-1}$, and was maintained for 30 minutes. The injector worked in splitless mode for 5 min. Helium of 99.999% purity was used as a carrier gas at the flow rate of 1 mL· min$^{-1}$. The flow rates of FID gases were as follows: hydrogen 40 mL·min$^{-1}$, air 400 mL·min$^{-1}$, and nitrogen (make-up gas) 40 mL·min$^{-1}$. The injector and FID temperatures were 280 and 300ºC, respectively. The scan frequency was 2.64 times/s and the mass range scanned was 40-600 amu. The electron impact source temperature was 230ºC with electron energy of 70 eV. The quadrupole temperature was 150ºC, and the GC interface temperature was 280ºC. The instrument was tuned on perfluorotributylamine.

## Results and Discussion

Preliminary experiments show that analysis of underivatized ethyl ether and methanol eluates leads to detection of a relatively small number of poor chromatographic peaks with inadequate precision. Ethyl ether and methanol eluates contain mainly organic acids, alcohols, and phenols. Such compounds have a polar character and could partly undergo dissociation. In order to make gas chromatography detection of this kind of compounds possible, a derivatization procedure is necessary. Among several derivatization methods, trimethylsilylation was chosen in the present study. Putting the TMS group into the molecule of compound affects the decrease of its boiling point and polarity. Additionally, an increase in MS detection sensitivity after silylation is usually observed. TMS derivatives are easy to obtain and are thermally stable during gas chromatography analysis. It is important that trimethylsilylation progresses stechiometrically and no by-products arise. Available databases of MS spectra (NIST MS, Wiley) contain an abundance of silylated compounds.

As a derivatizing agent, a bis(trimethylsilyl)trifluoroacetamide containing 1% of trimethylchlorosilane was selected. BSTFA reacts fast and completely and it can act as its own solvent. However, preliminary experiments demonstrated that the underivatized forms of some compounds were observed in the chromatograms. This indicated that complete derivatization cannot be achieved using this reagent alone. Also, the use of pyridine was examined. The volume of 100 μL of BSTFA plus 40 μL of pyridine was added to the dry residues and derivatization was performed for 30 min at 70ºC. The analysis of derivatized eluates ensured that the procedure was completed and the use of pyridine resulted in at least two-fold peak area increasing.

Prior to the start of research the amount of information concerning composition of organic compounds contained in sewage was rather poor, due to the limited number of publications concerning this problem. Additionally, composition of wastewater depends on local factors, e.g. number and kind of production plants located in the area supported by the sewage treatment plant, and the organization of the wastewater treatment process. For this reason, the conducted research belongs to a group of prospective analyses. To ensure the correct identification of the compounds, three independent parameters i.e. mass spectra, chromatographic retention indexes, and partition coefficients, were used in the identification process.

Mass spectra recorded during analyses were compared to the spectra contained in the NIST MS database. On the basis of the obtained chromatograms of the samples and chromatograms recorded for the mixture of $C_8$-$C_{40}$ *n*-alkanes, linear temperature programmed retention index (*I*) values were calculated according to the equation given by Van den Dool and Kratz [22]. Determined retention indexes were compared to indices available in catalogues [17, 23-25] and in the database created at the Environmental Chemistry Chair of the University in Białystok. The values of partition coefficients determined from Eq. 1 were compared to $K_p$ values from literature [16, 26]. The differential parameter *j*, which is a combination of $K_p$ and retention indices [21], was used additionally for identification of components. The *j* parameter was calculated from Eq. 2:

$$j=0.001I - \log K_p \qquad (2)$$

Principles of the use of *j* parameter and its values determined for numerous groups of compounds are given in works [21, 16].

The chromatograms registered during the analysis of the *n*-hexane and acetonitrile phase after *n*-hexane/acetonitrile partition of ether and methanol eluates are presented in Figs. 1-2. Table 1 contains the list of 123 volatile organic compounds extracted from wastewater and values of the parameters used for analyte identification. The average compound concentration in crude and treated sewage is also provided in this Table. Since the large number of substances was detected and qualitative analysis was the main aim of this work, the peak area summations of total ion current (TIC) were used as semi-quantitative assessment of the content of particular substances. Concentrations of phthalates are not included in Table 1 because some quantities of these compounds were registered during blank analysis. Concentrations of phthalic acid esters in the subject wastewater were determined using another method described in [27].

In most cases, the identification on the basis of MS spectra and *I* values allowed us to carry out reliable identification of individual components. However, in some cases the determination of the partition coefficient values and *j* parameters proved to be crucial in the identification process. Such a situation occurred, for example, in the cases of compounds 44 and 89 from Table 1.

As a result of computer comparison of the registered spectrum of compound number 44 (*I*=1295) with the database, a list of compounds was obtained. TMS esters of ethylmalonic acid and glycerol were at the beginning of the list.

Table 1. Identification results of trace organic compounds extracted from municipal wastewaters.

| No. | Identification result | $I_{exp}$ | $I_{lit}$ | $K_p$ | $j$ | Content, [%] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Influent | Effluent |
| 1 | Methoxybenzene | 920 | 917 | 0.16 | 1.72 | 0.29±0.27 | 0.93±0.47 |
| 2 | 6-Methyl-3-heptanone | 945 | 940 | - | - | 0.20±0.39 | 0.07±0.13 |
| 3 | Benzaldehyde | 965 | 961 | - | - | 0.01±0.01 | - |
| 4 | 4-Octanone | 969 | 975 | - | - | - | 0.07±0.13 |
| 5 | 1,3,5-Trimethylbenzene | 987 | 994 | - | - | - | 0.03±0.03 |
| 6 | Ethylene glycol, di-TMS | 992 | 989 | 2.66 | 0.57 | 0.43±0.12 | 2.10±0.93 |
| 7 | 1,2-Propanediol, di-TMS | 1012 | 1011 | 3.36 | 0.49 | 0.02±0.03 | 0.27±0.50 |
| 8 | D-Limonene | 1021 | 1020 | - | - | 0.01±0.02 | 0.10±0.20 |
| 9 | 2,3-Butanediol, di-TMS | 1051 | 1040 | 6.99 | 0.21 | 0.01±0.03 | - |
| 10 | Phenol, mono-TMS | 1054 | 1055 | 1.44 | 0.90 | 0.09±0.10 | - |
| 11 | Cadaverine, tetra-TMS | 1061 | 1035 (DB1) | - | - | 0.74±0.48 | 2.30±1.36 |
| 12 | Lactic acid, di-TMS | 1071 | 1066 | 1.53 | 0.89 | 0.87±0.78 | 3.03±0.80 |
| 13 | Hexanoic acid, mono-TMS | 1077 | 1075 | 2.90 | 0.61 | 0.85±1.23 | 0.33±0.20 |
| 14 | Glycolic acid, di-TMS | 1080 | 1080 | 4.05 | 0.47 | 0.28±0.36 | 0.23±0.17 |
| 15 | 1,3-Butanediol, di-TMS | 1087 | 1089 | 7.59 | 0.21 | 0.33±0.39 | 1.30±1.10 |
| 16 | Pyruvic acid, di-TMS | 1097 | 1090 | 1.75 | 0.85 | 0.06±0.06 | 0.93±0.70 |
| 17 | *n*-Undecane | 1103 | 1100 | - | - | - | 0.03±0.03 |
| 18 | Aromatic hydrocarbon C11H16 | 1109 | - | - | - | 0.10±0.14 | - |
| 19 | 2-Octanol | 1111 | 1115 | 0.41 | 1.50 | 0.22±0.33 | 0.87±1.20 |
| 20 | Glyoxylic acid enole, di-TMS | 1137 | 1135 | - | - | 0.07±0.11 | 0.50±0.43 |
| 21 | 2-Hydroxybutanoic acid, di-TMS | 1138 | 1136 | - | - | 0.02±0.03 | - |
| 22 | Oxalic acid, di-TMS | 1143 | 1145 | 0.35 | 1.60 | 0.28±0.25 | 1.00±0.67 |
| 23 | Dipropylacetic acid, mono-TMS | 1149 | 1151 | 3.20 | 0.64 | 0.56±0.76 | - |
| 24 | 3-Hydroxybutyric acid, di-TMS | 1162 | 1167 | 1.90 | 0.88 | 0.02±0.02 | - |
| 25 | Benzyl acetate | 1165 | 1163 | - | - | 0.01±0.01 | - |
| 26 | Heptanoic acid, mono-TMS | 1170 | 1165 | 3.30 | 0.68 | 1.94±2.48 | 0.23±0.13 |
| 27 | 3-Hydroxyisobutyric acid, di-TMS | 1171 | 1165 | - | - | 0.01±0.01 | 0.10±0.07 |
| 28 | 2-Hydroxyisobutyric acid, di-TMS | 1173 | 1171 | 1.91 | 0.89 | 0.02±0.04 | 0.17±0.20 |
| 29 | *p*-Methylacetophenone | 1183 | 1182 | - | - | 0.01±0.01 | 0.07±0.17 |
| 30 | 1-Octanol, mono-TMS | 1186 | 1182 | - | - | 0.15±0.25 | 0.10±0.13 |
| 37 | Cyclohexanecarboxylic acid, mono-TMS | 1201 | 1198 | 2.10 | 0.88 | 0.32±0.51 | 0.17±0.37 |
| 32 | Propanedioic acid, mono-TMS | 1215 | 1216 | 0.43 | 1.57 | 0.01±0.01 | 0.30±0.47 |
| 33 | Isoborneol, mono-TMS | 1223 | 1224 | - | - | 0.07±0.01 | 0.50±1.03 |
| 34 | Borneol, mono-TMS | 1226 | 1224 | - | - | 0.03±0.12 | 0.37±0.67 |
| 35 | Methylpropanedioic acid, di-TMS | 1229 | 1225 | 0.51 | 1.52 | 0.02±0.03 | 0.53±0.67 |
| 36 | 3-Hydroxyvaleric acid, di-TMS | 1241 | 1245 | 2.51 | 0.84 | 0.07±0.13 | 0.07±0.07 |
| 39 | Benzoic acid, mono-TMS | 1247 | 1247 | 0.78 | 1.35 | 0.68±0.62 | 0.50±0.70 |
| 40 | Urea,N,N', di-TMS | 1249 | 1249 | - | - | 0.01±0.01 | 0.73±0.33 |
| 41 | 4-Hydroxyisovaleric acid, di-TMS | 1258 | 1260 | - | - | 0.01±0.01 | - |

Table 1. Continued.

| No. | Identification result | $I_{exp}$ | $I_{lit}$ | $K_p$ | $j$ | Content, [%] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Influent | Effluent |
| 42 | Octanoic acid, mono-TMS | 1269 | 1268 | 3.51 | 0.72 | 2.39±3.86 | 1.43±1.13 |
| 43 | Phosphoric acid, tri-TMS | 1290 | 1290 | 2.10 | 0.97 | 1.05±1.34 | 7.95±9.35 |
| 44 | Glycerol, tri-TMS | 1295 | 1298 | 8.45 | 0.37 | 5.94±5.00 | 29.82±22.06 |
| 45 | *n*-Tridecane | 1299 | 1300 | 36.29 | -0.26 | 0.52±0.92 | 0.23±0.50 |
| 46 | Malic acid, mono-TMS | 1315 | 1317 | - | - | 0.01±0.00 | - |
| 47 | Succinic acid, di-TMS | 1324 | 1322 | - | - | 0.23±0.23 | 0.67±0.37 |
| 48 | Glyceric acid, tri-TMS | 1346 | 1345 | - | - | 0.51±0.63 | 0.23±0.17 |
| 49 | Nonanoic acid, mono-TMS | 1366 | 1365 | 5.10 | 0.66 | 1.14±1.60 | 1.66±1.00 |
| 50 | 3-Methyltridecane | 1377 | 1371 | - | - | - | 0.07±0.17 |
| 51 | *iso*-Tetradecane | 1392 | 1395 | - | - | - | 0.10±0.20 |
| 52 | *n*-Tetradecane | 1399 | 1400 | - | - | 0.09±0.11 | 0.87±1.20 |
| 53 | Hydrocinnamic acid, di-TMS | 1418 | 1416 | - | - | 0.95±1.51 | 0.07±0.10 |
| 54 | 2-Methylpentanedioic acid, di-TMS | 1427 | 1432 | 0.75 | 1.55 | 0.05±0.03 | 0.77±0.60 |
| 55 | Decanoic acid, mono-TMS | 1463 | 1450 | 6.52 | 0.65 | 1.54±2.59 | 0.73±0.50 |
| 56 | 2-Hydroxyoctanoic, di-TMS | 1469 | 1464 | 5.44 | 0.73 | 0.06±0.03 | 0.47±0.17 |
| 57 | *iso*-Pentadecane | 1496 | 1492 | - | - | - | 0.10±0.20 |
| 58 | *n*-Pentadecane | 1499 | 1500 | - | - | 0.04±0.06 | 0.57±0.63 |
| 59 | 2,6-di-tertbutyl-*p*-cresol (BHT) | 1511 | 1512 | 9.00 | 0.56 | 0.27±0.32 | 9.38±18.50 |
| 60 | Salicylic acid, mono-TMS | 1518 | 1524 | 0.99 | 1.52 | 0.29±0.32 | 0.40±0.47 |
| 61 | Methyl di-isopentylphosphate | 1521 | 1514 | - | - | 0.64±0.99 | - |
| 62 | Anisic acid, mono-TMS | 1524 | 1529 | 0.65 | 1.71 | 0.01±0.02 | 0.07±0.13 |
| 63 | Proline, mono-TMS | 1532 | 1536 | - | - | 0.37±0.13 | 1.00±0.80 |
| 64 | Vanillin, mono-TMS | 1537 | 1532 | - | - | 0.09±0.08 | 0.13±0.17 |
| 65 | Cinnamic acid, mono-TMS | 1545 | 1541 | - | - | 0.06±0.10 | - |
| 66 | 1-Dodecanol, mono-TMS | 1575 | 1572 | - | - | 0.01±0.02 | - |
| 67 | *n*-Hexadecene | 1593 | 1592 | - | - | - | 0.27±0.50 |
| 68 | *n*-Hexadecane | 1599 | 1600 | - | - | 0.05±0.05 | 0.47±0.40 |
| 69 | Ibuprofen, mono-TMS | 1626 | 1615(DB1) | - | - | 0.22±0.33 | - |
| 70 | 4-Hydroxybenzoic acid, di-TMS | 1634 | 1636 | - | - | 0.01±0.00 | - |
| 71 | *a*-Arabinofuranose, tetra-TMS | 1648 | 1645 | 12.5 | 0.55 | 0.06±0.08 | 0.10±0.20 |
| 72 | *b*-Arabinofuranose, tetra-TMS | 1650 | 1645 | 11.5 | 0.59 | 0.08±0.14 | 0.13±0.27 |
| 73 | Decanoic acid, mono-TMS | 1658 | 1657 | 10.8 | 0.62 | 2.32±3.81 | 0.70±0.57 |
| 74 | *a*-Ribofuranose, tetra-TMS | 1664 | 1657 | - | - | 0.01±0.02 | 0.50±1.00 |
| 75 | 2-Hydroxyphenylacetic acid, di-TMS | 1699 | 1697 | 0.95 | 1.72 | 0.05±0.05 | 0.20±0.30 |
| 76 | Fucose, tetra-TMS | 1702 | 1700 | 14.9 | 0.53 | 0.05±0.08 | - |
| 77 | Phthalic acid, di-TMS | 1704 | 1708 | - | - | 0.04±0.08 | 0.03±0.03 |
| 78 | D-Xylose, tetra-TMS | 1740 | 1740 | 13.4 | 0.61 | 0.05±0.05 | 0.17±0.20 |
| 79 | Tridecanoic acid, mono-TMS | 1770 | 1755 | 12.0 | 0.69 | 0.01±0.01 | 0.17±0.33 |
| 80 | Vanillic acid, di-TMS | 1776 | 1778 | - | - | 0.06±0.09 | 0.10±0.20 |

Table 1. Continued.

| No. | Identification result | $I_{exp}$ | $I_{lit}$ | $K_p$ | $j$ | Content, [%] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Influent | Effluent |
| 81 | *a*-Xylopyranose, tetra-TMS | 1779 | 1781 | 13.8 | 0.64 | 0.05±0.04 | - |
| 82 | *n*-Octadecene | 1793 | 1792 | - | - | - | 0.07±0.13 |
| 83 | *b*-Xylopyranose, tetra-TMS | 1795 | 1796 | 15.0 | 0.62 | 0.05±0.04 | - |
| 84 | N,N [312,237(44),73(42),194,147] | 1802 | - | - | - | 0.03±0.04 | 1.63±1.86 |
| 85 | Phytane | 1808 | 1811 | - | - | 0.01±0.01 | 0.10±0.13 |
| 86 | Azelaic acid, di-TMS | 1810 | 1806 | 1.56 | 1.62 | 0.31±0.43 | 0.27±0.30 |
| 87 | Caffeine | 1837 | 1838 | - | - | 0.23±0.29 | - |
| 88 | *a*-Fructofuranose, penta-TMS | 1839 | 1841 | - | - | 0.02±0.04 | 0.37±0.57 |
| 89 | Shikimic acid, tetra-TMS | 1844 | 1846 | 6.40 | 1.03 | 1.22±0.48 | 0.63±0.83 |
| 90 | Tetradecanoic acid, mono-TMS | 1854 | 1850 | 18.0 | 0.60 | 2.54±3.58 | 0.57±0.50 |
| 91 | *b*-Fructofuranose, penta-TMS | 1855 | 1852 | 15.0 | 0.68 | 0.02±0.03 | - |
| 92 | Inositol, hexa-TMS, isomer 1 | 1871 | 1874 | 45.0 | 0.22 | 1.01±1.52 | 0.83±1.66 |
| 93 | *a*-Galactofuranose, penta-TMS | 1890 | 1895 | 21.8 | 0.55 | 0.02±0.04 | 1.80±3.13 |
| 94 | Sebacic acid, di-TMS | 1901 | 1905 | 1.95 | 1.61 | - | 1.46±1.26 |
| 95 | *a*-Glucopyranose, penta-TMS | 1933 | 1933 | 29.4 | 0.46 | 1.37±1.80 | 1.63±2.06 |
| 96 | *b*-Mannopyranose, penta-TMS | 1946 | 1944 | 21.0 | 0.62 | 0.09±0.07 | - |
| 97 | Pentadecanoic acid, mono-TMS | 1953 | 1950 | 20.5 | 0.64 | 0.83±1.24 | 0.30±0.13 |
| 98 | Di-n-butyl phthalate | 1961 | 1956 | 0.30 | 2.48 | ND | ND |
| 99 | 1-Hexadecanol, mono-TMS | 1967 | 1969 | - | - | 0.02±0.02 | 0.07±0.13 |
| 100 | Mannitol | 1973 | 1975 | - | - | 0.14±0.27 | 1.30±0.93 |
| 101 | Inositol, hexa-TMS, isomer 2 | 1998 | 1999 | 51.5 | 0.29 | 0.01±0.03 | - |
| 102 | 6-Hexadecenoic acid, mono-TMS | 2029 | 2020 | - | - | 0.70±1.09 | 0.23±0.17 |
| 103 | *b*-Glucopyranose, penta-TMS | 2032 | 2033 | 31.0 | 0.54 | 1.44±1.82 | 0.50±0.43 |
| 104 | Hexadecanoic acid, mono-TMS | 2052 | 2050 | 28.0 | 0.61 | 16.11±21.77 | 2.10±0.97 |
| 105 | Naproxen, TMS | 2083 | 2045(DB1) | - | - | 0.28±0.48 | - |
| 106 | N,N [116,73(88),117(81),57,71] | 2112 | - | - | - | 0.62±0.76 | - |
| 107 | myo-Inositol, hexa-TMS | 2128 | 2134 | 57.7 | 0.37 | 0.05±0.04 | 0.10±0.10 |
| 108 | Heptadecanoic acid, mono-TMS | 2150 | 2153 | 30.0 | 0.67 | 0.53±0.84 | 0.17±0.23 |
| 109 | Linoleic acid, mono-TMS | 2215 | 2208 | 23.0 | 0.85 | 1.37±2.28 | 0.07±0.13 |
| 110 | Oleic acid, mono-TMS | 2221 | 2219 | 22.5 | 0.87 | 9.18±14.15 | 0.47±0.47 |
| 111 | 4-Hydroxyhippuric acid, di-TMS | 2233 | 2239 | - | - | 1.46±2.40 | 0.13±0.27 |
| 112 | Octadecanoic acid, mono-TMS | 2249 | 2255 | 35.5 | 0.70 | 10.83±15.8 | 1.36±1.30 |
| 113 | Dehydroabietic acid, mono-TMS | 2386 | 2373 | - | - | 1.48±1.97 | 0.47±0.83 |
| 114 | Pentadecanedioic acid, di-TMS | 2399 | 2400 | 7.12 | 1.55 | 0.43±0.63 | 0.47±0.60 |
| 115 | N,N [73,183(83),361(55),217,199] | 2537 | - | - | - | 0.01±0.01 | 0.07±0.10 |
| 116 | Di-2-etylhylhexylphthalate | 2546 | 2540 | 1.47 | 2.38 | ND | ND |
| 117 | NN [445,446(37),73(24),273,447] | 2595 | - | - | - | 6.89±8.43 | 4.26±7.79 |
| 118 | NN [73,117(61),116(50),121,57] | 2643 | - | - | - | 1.30±2.59 | - |
| 119 | Squalen | 2828 | 2828 | - | - | 0.30±0.37 | - |

Table 1. Continued.

| No. | Identification result | $I_{exp}$ | $I_{lit}$ | $K_p$ | $j$ | Content, [%] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Influent | Effluent |
| 120 | NN [117,116(93),57(70),71,89] | 2909 | - | - | - | 0.01±0.02 | - |
| 121 | Dihydrocholesterol | 3057 | 3057 | - | - | 0.17±0.34 | - |
| 122 | Cholestan-3-one (coprostan-3-one) | 3078 | 3078 | - | - | 4.42±6.22 | 0.30±0.47 |
| 123 | Cholesterol, TMS | 3146 | 3150 | - | - | 6.43±9.15 | 0.87±1.00 |

$I_{exp}$ – experimental retention index on the HP-5 phase (5% phenylmethylsiloxane)
$I_{lit}$ – retention index from literature [23-25]
$K_p$ – partition coefficient in *n*-hexane/acetonitrile system
$j$ – group identification parameter
DB1 – retention index on the DB-1 phase (methylsiloxane)
ND – not determined.

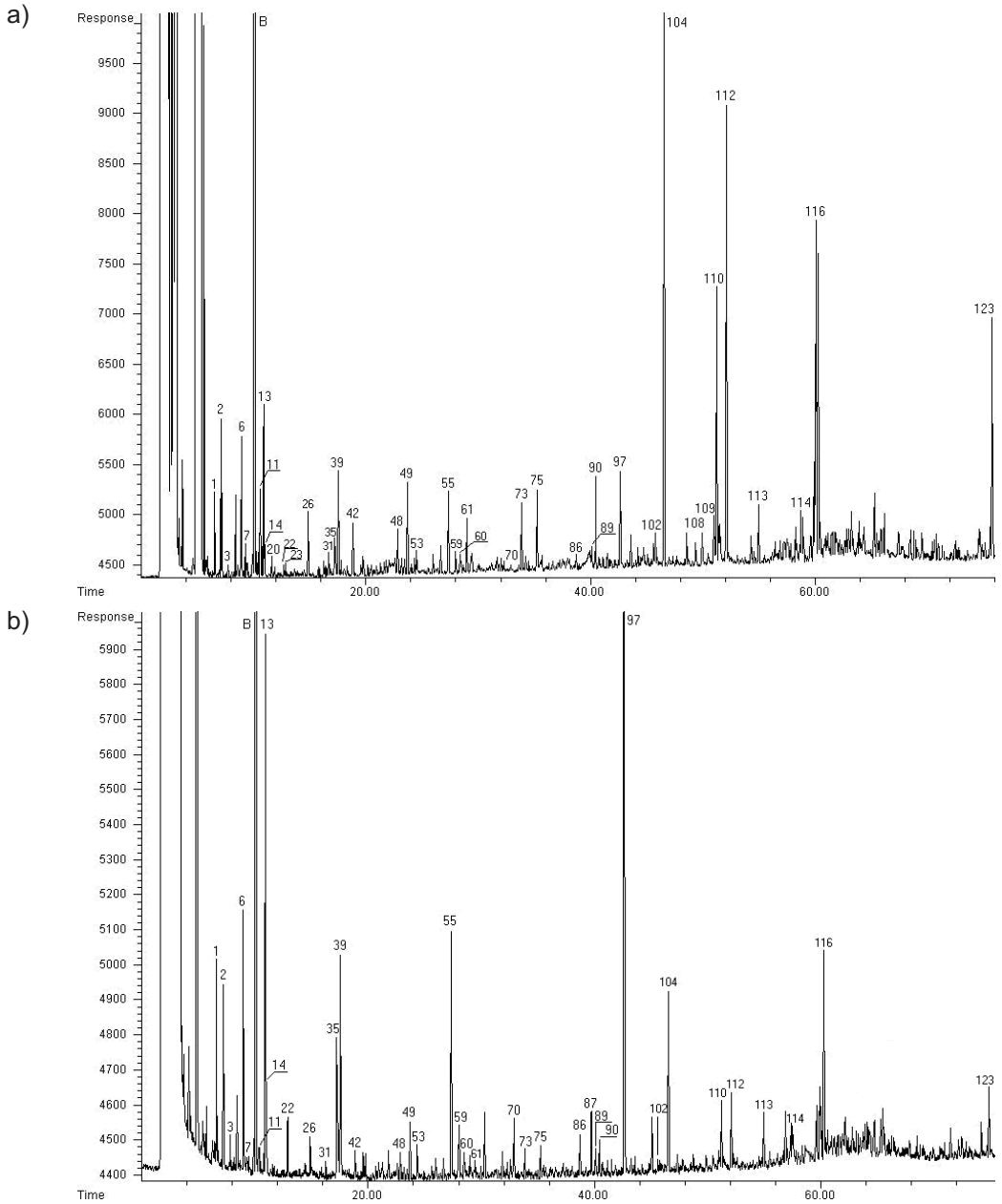


Fig. 1. Chromatograms of diethyl ether eluate of influent wastewater sample: a) *n*-hexane phase, b) acetonitrile phase. Peak numbers refer to Table 1; B – butylbenzene (internal standard).

The choice between those compounds appeared impossible even when using the values of retention indices that are similar: 1289±5 for ethylmalonic acid and 1298±5 for glycerol. However, the partitioning of compound 44 in the *n*-hexane/acetonitrile system ($K_p$=8.45, $j$=0.37) is characteristic of polyols ($K_p$ and $j$ values for ethylmalonic acid equal to 0.81 and 1.38, respectively [16]).

Difficulties have also arisen in the identification of compound 89, because the mass spectra of fructose and shikimic acid, given as a result of identification based on the mass spectrum, are very similar. The set of main peaks in their spectra (*m/z* 73, 204, 147, 217) is identical and they only slightly differ in intensity. The literature values of retention indexes of these compounds differ only by four units (fructose $I$=1842±5, shikimic acid $I$=1846±5). At the same time, the $K_p$ and $j$ values for these compounds differ greatly [16] and allowed us to make a choice in favour of the shikimic acid.

18 organic compounds were identified in hexane eluates. They were 2,6-ditertbutylhydroxytoluene (BHT), dihydrocholesterol and coprostan-3-one, and a group of aliphatic and aromatic hydrocarbons. Mostly aliphatic and aromatic acids, alcohols and polyalcohols, caffeine and drugs: naproxen and ibuprofen were eluted from the SPE column using ether. In methanol eluate carbohydrates, inositol, proline and a considerable group of aliphatic acids were found, which failed to be completely eluted using diethyl ether.

Compounds detected in sewage can be divided into three groups. The first group consists of compounds present

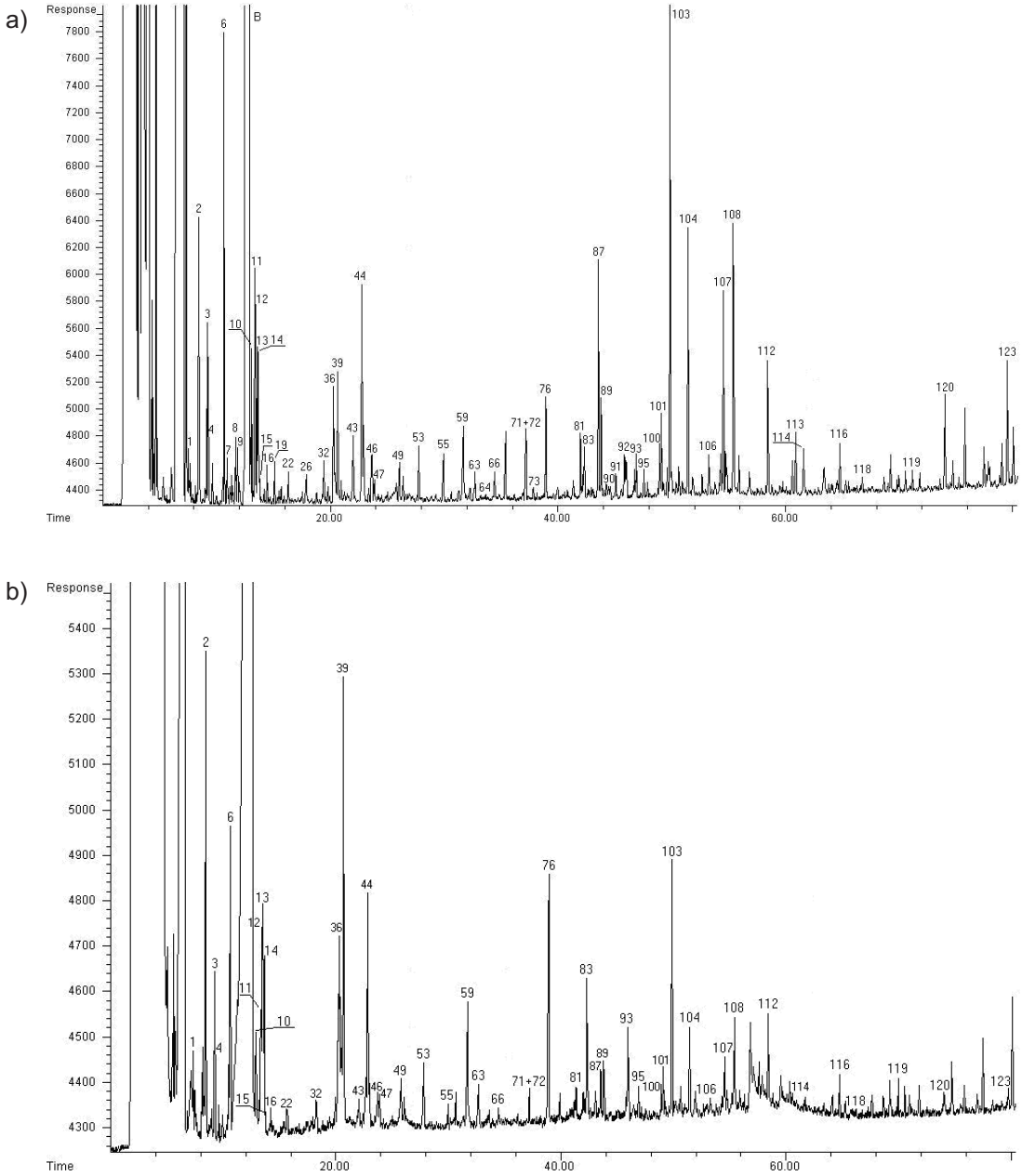


Fig. 2. Chromatograms of methanol eluate of influent wastewater sample: a) *n*-hexane phase, b) acetonitrile phase. Peak numbers refer to Table 1; B – butylbenzene (internal standard).

both in crude and treated sewage. These substances are durable enough not to undergo decomposition in a purification process ($C_{14}$-$C_{16}$ alkanes) or belong to readily decomposing compounds, whereas their additional source is probably the metabolism of organisms forming activated sludge (acids, among others: lactic, decanoic, succinic). The second group comprises compounds present only in crude sewage, i.e. readily decomposing substances (aromatic acids, sterols, caffeine). The third group includes substances absent in crude but present in treated sewage: malonic and decanedioic acids, urea, and terpene compounds: limonene, borneol, isoborneol. These compounds originate from activated sludge or (bio)chemical transformation of other substances in the process of purification. Their source can also be the leaching of a given substance retained earlier in the activated sludge.

The majority of compounds detected in municipal wastewater from the wastewater treatment plant in Białystok are substances of natural origin, originating mainly in decomposition of vegetable and animal food remains as well as human excrement, and they usually do not pose any treat to the environment and human health. Although some of these compounds (e.g. carboxylic acids) are not classified as important pollutants, they can contribute to the leaching and in consequence bioavailability of toxic compounds like polychlorinated biphenyls (PCBs) and heavy metals present in the bottom sediments, by changes in their solubilities in water or organic matter [28]. One of the natural compounds detected in the subject wastewater samples was cholesterol. Cholesterol has previously been identified as an endocrine disrupting compound (EDC) [29].

Some substances detected in sludges are known for their harmful influence on human health and the natural environment. Three compounds: phenol, di-*n*-butyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEHP) detected in municipal wastewater are included on an EPA-established list of priority pollutants. Four chemicals: phenol, DBP, DEHP, and benzaldehyde, are on the EPA's list of target compounds. Phthalalic acid esters are counted as endocrine disrupters and they alter the normal functioning of the endocrine system and cause important reproductive and developmental alterations, such as feminization and decreased fertility [30].

Other potentially harmful substances to human and water organisms detected in the examined sewage are: 2,6-ditertbutylhydroxytoluene, phosphoric acid, and drug remnants. BHT as a strongly lipophilic and persistent compound undergoes bioaccumulation in tissues and organs of water organisms. Even a small amount of this compound getting into the environment can lead to its substantial accumulation in the fatty tissue of fish. Butylated hydroxytoluene has exhibited contradictory actions depending on the study and on experimental animal studies. Cancer growth has shown to be inhibited in some studies, and increased in others [31, 32], since its toxicological implication is in permanent revision. Although detailed knowledge about the ecotoxicological effect of pharmaceutical substances is still incomplete, these contaminants must be classified as environmentally relevant. Phosphoric acid introduced into the environment causes eutrophication of water reservoirs.

Overall compound removal along the wastewater treatment train was 93%, but potentially harmful compounds like BHT and phosphoric acid were removed at a rate of less than 50%.

## Conclusion

The conducted research shows that many organic pollutants are present in municipal wastewater, some of them harmful to the environment and also to humans. Target compound monitoring with dedicated analytical methods can be insufficient to asses the quality of effluents introduced into the environment. Screening of unknown organic compounds provides significant information about the composition of sewage and can be useful for maintaining the suitable purity of surface water.

## Acknowledgements

The authors would like to thank Barbara Grudzień and the management of the wastewater treatment plant in Białystok for their help in taking samples.

## References

1. KOT-WASIK A., DĘBSKA J., NAMIEŚNIK J. Analytical techniques in studies of the environmental fate of pharmaceuticals and personal-care products. Trends Anal. Chem. **26**, 557, **2007**.
2. AL-RIFAI J.H., GABELISH C.L., SCHÄFER A.I. Occurrence of pharmaceutically active and non-steroidal estrogenic compounds in three different wastewater recycling schemes in Australia. Chemosphere **69**, 803, **2007**.
3. AMY G., DREWES J. Soil aquifer treatment (SAT) as a natural and sustainable wastewater reclamation/reuse technology: fate of wastewater effluent organic matter (EfOM) and trace organic compounds. Environ. Monit. Assess. **129**, 19, **2007**.
4. CESPEDES R., LACORTE S., GINEBREDA A., BARCELÓ D. Chemical monitoring and occurrence of alkylphenols, alkylphenol etoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the Ter River basin (Catalonia, N. E. Spain). Anal. Bioanal. Chem. **385**, 992, **2006**.
5. GATIDOU G., THOMAIDIS N.S., STASINAKIS A.S., LEKKAS T.D. Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry. J. Chromatogr. A **1138**, 32, **2007**.
6. GRUNG M., LICHTENHALER R., AHEL M., TOLLEFSEN K.-E., LANGFORD K., THOMAS K.V. Effect-directed analysis of organic toxicants in wastewater effluent from Zagreb, Croatia. Chemosphere **67**, 108, **2007**.
7. VULLIET E., BAUGROS J.-B., FLAMENT-WATON M.-M., GRENIER-LOUSTALOT M.-F. Analytical methods for the determination of selected steroid sex hormones and corticosteroids in wastewater. Anal. Bioanal. Chem. **387**, 2143, **2007**.

8. ESCALAS A., GUADAYOL J.M., CORTINA M., RIVERA J., CAIXACH J. Time and space patterns of volatile organic compounds in a sewage treatment plant. Water Res. **37**, 3913, **2003**.
9. CHENG W.H., HSU S.K., CHOU M.S. Volatile organic compounds emissions from wastewater treatment plants in Taiwan: Legal regulations and costs of control. J. Environ. Manage. **88**, 1485, **2008**.
10. KÜCHLER T., BRZEZINSKI H. Application of GC-MS/MS for the analysis of PCDD/Fs in sewage effluents. Chemosphere **40**, 213, **2000**.
11. FUKAZAWA H., MATSUSHITA H., TERAO Y. Identification of co-mutagenic chlorinated harmans in final effluent from a sewage treatment plant. Mutat. Res. **491**, 65, **2001**.
12. YOU J., LAO W., WANG G. Analysis of organic pollutants in sewage by supercritical fluid extraction. Chromatographia **49**, 399 **1999**.
13. GAO H., ZHAO T., KONG X., HU Z. Analysis of unknown organic pollutants in sewage by solid-phase extraction combined with gas chromatography-mass spectrometry. J. Chromatogr. Sci. **42**, 91, **2004**.
14. DING W.-H., FUJITA Y., AESCHIMANN R., REINHARD M. Identification of organic residues in tertiary effluents by GC/EI-MS, GC/CI-MS and GC/TSQ-MS. Fresen. J. Anal. Chem. **354**, 48, **1996**.
15. PAXEUS N., SCHRÖDER H.F. Screening for non-regulated organic compounds in municipal wastewater in Göteborg, Sweden. Water Sci. Technol. **33**, 9, **1996**.
16. ISIDOROV V.A., KOTOWSKA U., VINOGOROVA V.T. GC identification of organic compounds based on partition coefficients of their TMS derivatives in a hexane-acetonitrile system and retention indices, Anal. Sci. **21**, 483, **2005**.
17. BABUSHOK V.I., LINSTROM P.J., REED J.J., ZENKEVICH I.G., BROWN R.L., MALLARD W.G., STEIN S.E. Development of a database of gas chromatographic retention properties of organic compounds. J. Chromatogr. A **1157**, 414, **2007**.
18. D'ACAMPORA ZELLNER B., BICCHI C., DUGO P., RUBIOLO P., DUGO G., MONDELLO L. Linear retention indices in gas chromatographic analysis: a review. Flavour Frag. J. **23**, 297, **2008**.
19. BRACK W. Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? Anal. Bioanal. Chem. **377**, 397, **2003**.
20. BEREZKIN V.G., LOSHILOVA V.D., PANKOV A.G., YAGODOVSKII V.D. Chromatographic-Partitioning Method; Nauka, Moscow, **1976** [In Russian].
21. ZENKEVICH I.G., VASILEV A.V. Comparative-evaluation of informational value of supplementary data in gas-chromatographic identification – new possibilities for the use of distribution coefficients in hexane-acetonitrile system. J. Anal. Chem. **48**, (3), 335, **1993** [In Russian].
22. VAN DEN DOOL H., KRATZ P. A generalization of retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. **11**, 463, **1963**.
23. DE ZEEUW R. A., FRANKE J. P., MAURER H. H., PFLEGER K. Gas chromatographic retention indices of toxicologically relevant substances on packed or capillary columns with dimethylsillicone stationary phases; 2nd ed.; VCH Verlag: Weinheim, **1992**.
24. Standard GC retention index library, Sadtler Research Laboratory: Philadelphia, **1986**.
25. NIST Chemistry WebBook. http://webbook.nist.gov/chemistry/.
26. AHMED H., POOLE C.F. Model for the distribution of neutral organic compounds between *n*-hexane and acetonitrile. J.Chromatogr. A **1104**, 82, **2006**.
27. KOTOWSKA U., GARBOWSKA K., ISIDOROV V.A. Distribution coefficients of phthalates between absorption fiber and water and its using in quantitative analysis. Anal. Chim. Acta **560**, 110, **2006**.
28. AGUILAR C.P., PERUZZOLO M., DI LUCCIO M., DALLAGO R.M., FILHO I.N. Qualitative study of organic compounds in wastewaters of a swine slaughterhouse. Environ. Monit. Assess. **116**, 103, **2006**.
29. PARK K.J., MÜLLER C.T., MARKMAN S., SWINSCOW-HALL O., PASCOE D., BUCHANAN K.L. Detection of endocrine disrupting chemicals in aerial invertebrates at sewage treatment works. Chemosphere **77**, 1459, **2009**.
30. LATINI G., DEL VECCHIO A., MASSARO M., VERROTTI A., DE FELICE C. Phthalate exposure and male infertility. Toxicology **226**, 90, **2006**.
31. TRYPHONAS H., LACROIX F., LOK E.,. JEE P. D, CLAYSON B., HAYWARD S., MILLER D., MEHTA R. The effect of butylated hydroxytoluene on selected immune surveillance parameters in rats bearing enzyme-altered hepatic preneoplastic lesions. Food Chem. Toxicol. **37**, 671, **1999**.
32. SAFER A.M., AL-NUGHAMISH A.J. Hepatotoxicity induced by the anti-oxidant food additive, butylated hydroxytoluene (BHT), in rats: An electron microscopical study. Histol. Histopathol. **14**, 391, **1999**.