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K. Rudnieva,
Forensic Expert of the Department of Forensic Chemistry and Physics of Criminalistics Laboratory at the KhRIFE Kharkiv, Ukraine
ORCID: https://orcid.org/0000–0002–7676–749X
E-mail: rudneva770@ukr.net

M. Zarubina,
Head of the Department of Forensic Chemistry and Physics at KhRIFE Kharkiv, Ukraine
ORCID: https://orcid.org/0000–0002–5855–3295
E-mail: 17171785@ukr.net

L. Nardid,
Senior Researcher of the Department of Forensic Chemistry and Physics of Criminalistics Laboratory at KhRIFE Kharkiv, Ukraine
ORCID: https://orcid.org/0000–0002–5855–3295
E-mail: nardidlily@gmail.com

P. Karnozhitsky,
PhD in Law, Senior Researcher, Associate Professor at the Department of National Technical University Kharkiv Polytechnic Institute Kharkiv, Ukraine
ORCID: https://orcid.org/0000–0002–5855–3295
E-mail: lab.vtii@gmail.com

O. Pashkova,
Forensic Expert of the Department of Forensic Chemistry and Physics of Criminalistics Laboratory at the KhRIFE Kharkiv, Ukraine
ORCID: https://orcid.org/0000–0002–5855–3295
E-mail: lab.vtii@gmail.com

INFLUENCE OF POLYMER CONTAINER MATERIAL ON THE RESULTS OF EXPERT RESEARCH ON PHTHALATE DETECTION

The article deals with the problem of determining the influence of capacity on the presence of phthalates – esters of phthalic acid (EFC) and its isomeric compounds in the studied objects. The work is relevant in connection with the lack of clear rules for the transportation of objects that are provided for forensic research on the content of phthalates, because the packaging of objects in improperly selected packaging may lead to the EFC falling into the object under study and false as a whole research results.

The purpose of the work was to establish the presence of phthalates in the bottled drinking water, which is being sold in the territory of Ukraine in a polymeric container made of polyethylene terephthalate (PETF, PET), as well as tap water and distilled water and ethyl alcohol, placed in the same bottles during

research. Establishing an EFC in facilities placed in PET packaging is a direct confirmation of existing experience with similar topics.

The determination of the content of the EFC was carried out by gas chromatography-mass spectrometry. It is shown that in the composition of any drinking water from those sold on the domestic market in PET packaging, there is an EFC. In this case, the sample of mineral water in glass containers, as well as distilled, tap water, ethyl alcohol used in this work, the EFC did Not contain within the limits of quantitative definition. After the expiry of these samples of distilled water and ethyl alcohol in the PET packaging, the presence of phthalates was detected.

The results of this work are supported by well-known global studies to determine the possibility of migration of the EFC from the packaging material to its liquid content. Considering the results obtained, we have provided recommendations for the packaging of liquid research objects when they are sent to a study to determine the content of the EFC.

Key words: esters of phthalic acid; gas chromatography; mass spectrometry; mineral water; polyethylene terephthalate.

Formulation of Research Problem. Forensic research on diethyl phthalate (DEP) and its isomers is linked to several areas of expertise that include the examination of foodstuffs and the study of chemicals and specialty chemicals. In the study of EFCs as objects of industrial raw materials, the expert is asked whether these compounds are present in the test objects in relatively pure form or as impurities.

Other common tasks include determining the presence and quantitative content of DEPs in industrial, industrial or drinking water.

Given the wide range of constituent polymeric materials used in the manufacture of containers for the transportation of liquids, the possibility of the effect of packaging on the results of chemical analysis of the contents should be considered by the expert in the overall assessment of the research results.

Analysis of recent research and publications. Polymeric materials include several polymers that differ in their composition and structure, which results in differences in their performance and applications. One of these polymers includes polyethylene terephthalate (PET, PETE), which is used in the packaging of food, beverages, technical liquids and the like. DEP have become widespread as plasticizers for various types of polymeric materials, mainly polyvinyl chloride (PVC), and polymers used for food packaging. It should be noted that some of the phthalates contained in the polymers are not chemically bound to the molecules of the material itself, that is, they are prone to migration to another contact medium. In the production and bottling of drinking water, phthalates can pass through the process of contact with polymer parts, containers, etc., directly into the water itself.

Thus, the paper shows that phthalates are capable of migration from the PET material to its contents, with the rate of saturation of the EFC content being significantly dependent on temperature, and the

concentration itself is gradually set at a constant maximum equilibrium level, after slightly more than 2 months of exposure[1]. The Research was performed on di-2-ethylhexylphthalate. You can also see an increase in phthalate content throughout the study period (up to 4 months). [2] Some differences between the results can be explained by the conditions of the study and the raw material base. When ingested with food, water, cosmetics, etc., phthalates have a special accumulation potential and pose a potential threat and danger to human health, causing various diseases, such as the endocrine system, allergies, etc.[3] The paper states that the use of secondary PET polymers is one of the most important sources of phthalates entering drinking water.

It should be noted that the basic requirements of the allowable amount of migration (SCM) of chemicals released from polymeric materials in contact with foodstuffs and methods of their determination are set by sanitary norms in accordance with the legislation of Ukraine.

Previous studies have shown a correlation between the consumption of beverages and food stored in polymer containers and the appearance of phthalates in humans. [4]

The main DEPs, metabolites of which are found in vital products, include the following: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), diisobutyl phthalate (DiBP), butylbenzyl phthalate (BBP), ethyl dibutyl (BBP) (DEHP).

Concentration of phthalates in the contact medium increases under the influence of solar radiation and temperature rise[5] The transition of several basic phthalates from polymeric containers to water [6] was also confirmed. It should be noted that the speed of this transition also depends on the temperature and time of storage of water.The article emphasizes that the content of phthalates in mineral water stored in polymer (PET)

[1] *Farhoodi M. at al.* Effect of environmental conditions on the migration of di(2-ethylhexyl) phthalate from pet bottles into yogurt drinks: influence of time, temperature, and food simulant. The Arabian Journal for Science and Engineering. 2008. Vol 33. P 281–287.

[2] *Zaki G. at al.* Concentrations of several phthalates contaminants in Egyptian bottled water: Effects of storage conditions and estimate of human exposure. Science of the Total Environment. 2018. Vol 618. P. 142–150.

[3] *Sax L.* Polyethylene Terephthalate May Yield Endocrine Disruptors. Environmental Health Perspectives. 2010. Vol 118. P. 445–448.

[4] *Dong R.H. at.al.* Association between Phthalate Exposure and the Use of Plastic Containers in Shanghai Adults. Biomedical Environmental Scinces. 2017. Vol 30. P. 727–736.

[5] *Schmida P. at.al.* Does the reuse of PET bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? Water research. 2008. Vol 42. P. 5054–5060.

[6] *Luo Q.at. al.* Migration and potential risk of trace phthalates in bottled water: A global situation. Water research. 2018. Vol 147. P. 362–367.

containers is almost 20 times higher than for the water stored in glass bottles.[1]

The authors state that gas chromatography with mass-selective detection (GC-MS) is one of the most acceptable methods for detecting DEPs in water. The article also demonstrates the use of the GC-MS method for detecting DEPs in wine, using deuterated DEPs as internal standards. [2] The GC-MS method was found to be suitable for the detection of phthalates in vegetable oil.[3] The GC-MS method has been determined to be the most common in the study of phthalates in consumer products.[4]

It should be noted that not only were the chromatographic methods suitable for the analysis of phthalates in drinking water and beverages, a rapid method for the detection of phthalates using electrochemical sensors was also proposed.[5] The paper shows the possibility of investigating several DEPs using liquid chromatography with a UV detector.[6] In this case, instead of conventional methods of extraction with organic solvents can be used methods of solid-phase microextraction[7].Given the presence of a wide range of existing literary sources devoted to meticulous research into the ways of getting DEP to water, the possibility of migration from the composition of polymeric containers, and the lack of appropriate recommendations for the expert treatment of objects of study in this direction, Ye advisable to conduct their own experimental experiments tasks:

– establishing the possibility of using relatively simple sampling technique for research objects, including the extraction and concentration stage;

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[1] *Montuori P. at.al.* Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. Food Additives and Contaminants. 2008. Vol 25. P. 511–518.

[2] *Carrillo J.D. at.al.* Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography–mass spectrometry Use of deuterated phthalates as internal standards. Journal of Chromatography. 2008. Vol 1181. P. 125–130.

[3] *Pereira J. at.al.* Determination of phthalates in olive oil from European market.Food control. 2019 Vol. 98 P. 54–60.

[4] *González-Sálamo J. at.al.*Analytical methods for the determination of phthalates in food. Current Opinion in Food Science. Vol 22 P. 122–136.

[5] *Zia A.I. at.al.* Rapid and molecular selective electrochemical sensing of phthalates in aqueous solution. Biosensors and Bioelectronics. 2015. Vol 67. P. 342–349; *Zia A.I. at.al.* Technique for rapid detection of phthalates in water and beverages. Journal of Food Engineering. 2013.Vol 2016. P. 515–523

[6] *Salazar-Beltrán D. at.al.* Determination of phthalate acid esters plasticizers in polyethylene terephthalate bottles and its correlation with some physicochemical properties. Polymer Testing. 2018. Vol.68, P. 87–94.

[7] *He J. at.al.* Preparation and evaluation of molecularly imprinted solid-phase micro-extraction fibers for selective extraction of phthalates in an aqueous sample. Analytica Chimica Acta. 2010.Vol. 674. P. 53–58.

– accelerated research using a chromatographic column;

– confirmation of the fact of migration of DEPs to the composition of water and organic solvents during their storage in PET containers;

– making recommendations on the packaging of objects to eliminate their contamination with DEPs.

Article Purpose. The purpose of this work is to determine the presence of phthalates in the composition of bottled drinking water, which is sold in the territory of Ukraine, namely in polymeric containers made of polyethylene terephthalate (PET, PETE), as well as tap, distilled water and ethyl alcohol, placed in similar bottles for carrying experimental research.

Main content presentation. Analysis of the literature sources of existing DEP research methods and their removal from objects, water, provided the following approach to sample preparation for their further chromatographic research. 100 ml of the test sample of water were extracted three times with chloroform the *puriss. p.a.* brand in portions of 10 ml.

The resulting extract was transferred to a porcelain cup and evaporated to dryness, then re-dissolved in chloroform and quantitatively transferred to a 2 ml glass chromatographic vial. The resulting solution was stored at 4° C. Immediately before gas chromatographic analysis, the sample vial was kept at room temperature for 1 hour.

Gas chromatographic studies were performed using a Shimadzu GCMS-QP2020 gas chromatograph-mass spectrometer under the following conditions:

– column manufactured by Shimadzu (length – 30 m, inner diameter – 0.25 mm, film thickness of the stationary phase – 0.25 microns, type of stationary phase – HP-5);

– initial column temperature: 60°C;

– isotherm: 1 min;

– heating rate: 20 ° C / min;

– final temperature of the column is 260° C;

– duration of analysis: 40 minutes;

– sample volume: 1 μl;

– flow separation: 1: 2;

– scan mode – full ion current (Scan) and individual ions (SIM);

– Mass Spectrum Database – NIST 17 and Wiley (11th Edition).

Qualitative and quantitative chromatogram processing was performed using software and databases, as well as EFC samples prepared as chloroform solutions. The following samples were subject to the whole research:

– 1 sample of mineral water in a glass bottle (object # 1);

– 5 samples of mineral water in PET container (objects №2-6, object №2 – the same mark as sample №1);

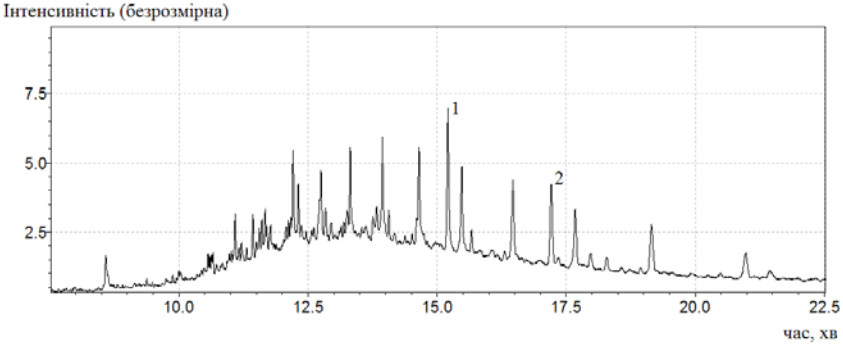
– 1 sample of tap water selected from the general water supply network (cold water) in 1-2 minutes. after opening the water tap;

– 1 sample of distilled water;

– 1 sample of ethyl alcohol aged for 1 week in PET containers;

– 1 sample of distilled water, aged for 1 week in PET container.

For the original samples of ethyl alcohol and chloroform, blank experiments were conducted which consisted of evaporating 30 ml of the sample. Subsequently, the evaporated samples were brought to 1 ml with a suitable solvent and quantitatively transferred into a 2 ml vial. Subsequently, these ethanol and chloroform samples were stored and investigated in the same manner as other samples.



At the same time, 30 ml of chloroform was evaporated in an evaporation cup and quantitatively transferred to a 2 ml vial, and then examined as well as extraction. At the same time, 30 ml of chloroform was evaporated in an evaporation cup and quantitatively transferred to a 2 ml vial, after which the same was investigated as the extraction.

Composition of chloroform from one batch showed peaks of phthalic and isophthalic acid esters (Fig. 1).

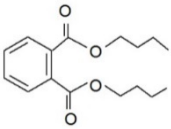
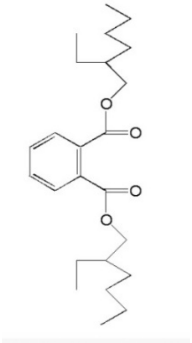
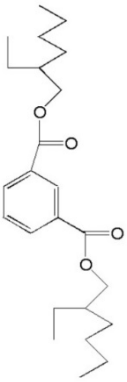
It should be noted that several peaks of several components, including hydrocarbons, were observed in the composition of all chloroform samples, but their presence did not interfere with the establishment of phthalates.

Fig. 1. Full-ion chromatogram, where 1 is the peak of 2-ethylhexylphthalate, 2 – the peak of 2-ethylisoxylphthalate.

After chromatography on the respective extracts, the presence of a significant portion of the extracts of dibutyl phthalate, 2-diethylhexylphthalate, 2-diethylhexylisophthalate which formulas are shown in table 1.

Table 1.

Compounds of phthalic and isophthalic acids as part of the studied

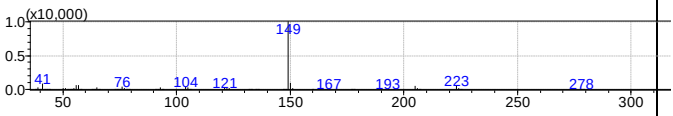
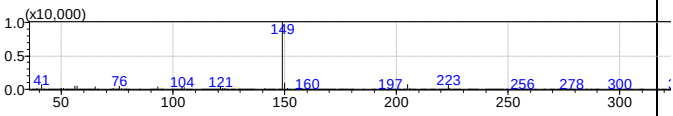
Назва	дибутилфталат	2–діетилгексилфталат	2–діетилгексилізофталат
Формула			

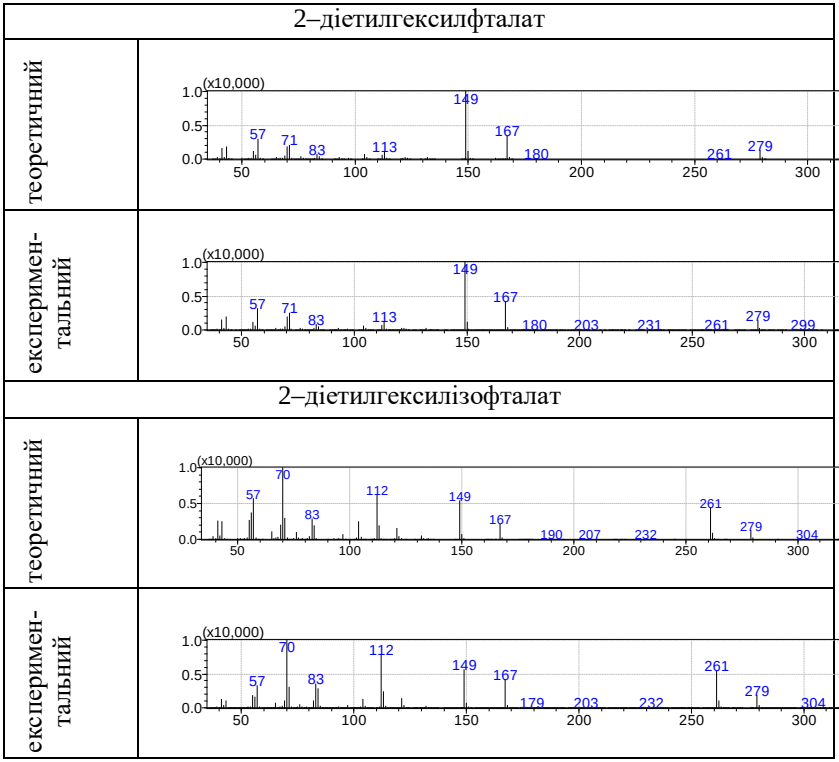
It should be noted that the mass spectra of the isomeric phthalates are similar to each other, therefore, to determine their specific accessory, samples of the corresponding compounds from the collection of the KhRIFE laboratory were additionally used.

The mass spectra of the corresponding phthalates are shown in table 2.

Table 2

Mass spectra of the corresponding phthalates (library spectra and obtained in this paper experimentally).

Назва компоненту/мас-спектр (бібліотека NIST 17)	
дибутилфталат	
теоретичний	
експериментальний	



It should be noted that the peaks of the mass spectra of these phthalates are not characterized by a pronounced peak of the molecular ion, but have a sufficiently characteristic value of m / z equal to 149.

Availability of an intense peak of the corresponding m / z can be considered as an indirect sign of the presence of phthalates in the sample. The quantitative and qualitative results of the phthalate content study are shown in Table 3.

Table 3

The results of the study of the content of phthalates in different samples.

sample	dibutyl phthalate	2-ethylhexyl phthalate	2-ethylhexyl isophthalate
Chloroform (blank sample)	–	–	–
Ethanol	3, 2	11, 4	21, 8
Distilled water	–	–	–

Tap water	0, 074	0, 012	0, 002
Sam. № 1 (glass)	0, 06	0, 08	0, 003
About. № 2 (polymer)	1, 6	3, 41	17, 1
About. № 3 (polymer)	1, 1	7, 3	8, 1
About. № 4 (polymer)	0, 095	6, 6	18, 3
About. № 5 (polymer)	0, 92	0, 075	0, 86

Quantitative calculation was performed on separate characteristic ions (m / z), with an additional two ions as confirmatory. In case of violation of the ratio of these ions to the characteristic calculation is not done because of the overlap of the same ions from the spectrum of other components. Examples of full-ion current chromatograms for each of the installed phthalates are shown in Figs. 2, 3, 4.

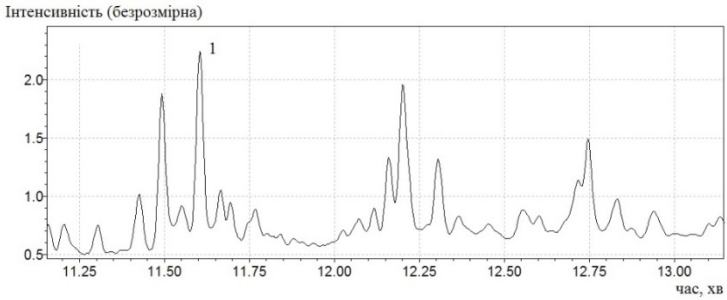


Fig. 2. Example of a water sample chromatogram (object № 2) where 1-dibutyl phthalate.

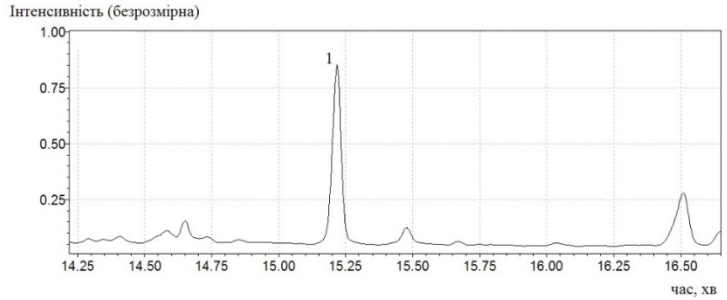


Fig. 3. Example of a water sample chromatogram (object № 3) wherein 1 is 2-diethylhexylphthalate.

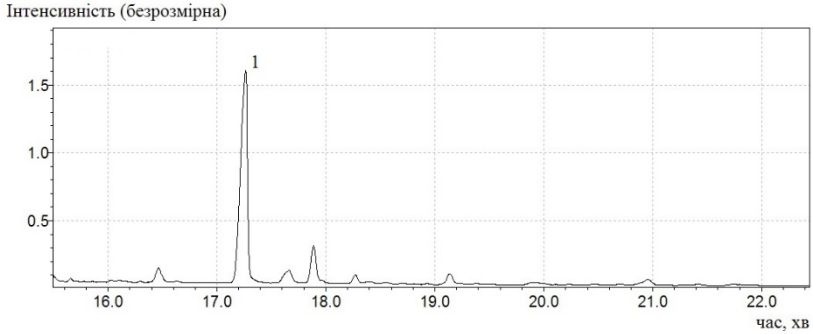


Fig. 4. An example of a chromatogram of a water sample of object № 2 is polymer (determination of 2-diethylhexylisophthalate).

Material composition of the container (bottles) was determined by IR spectrometry[1, 2], in the middle region of the spectrum, using disturbed full internal reflection on a ZnSe crystal. The absorption spectra were recorded on a NICOLET 380 spectrometer with the SMART PERFORMER prefix manufactured by ThermoElectronCorporation, USA, under standard IR conditions – the spectra were recorded in the wavenumber range from 650 to 4000 cm^{-1}.

The parameters of the analysis:

– measurement area: 650–4000 cm^{-1};

– resolution: 4;

– recording speed: 0, 6329;

– strengthening: 8;

– number of scans: 32.

According to the results of the study, it was found that all samples of the container are made of polyethylene terephthalate. Absorption bands were observed in the spectra of the container material in the main ranges: 723 cm^{-1}, 873 cm^{-1}, 1016 cm^{-1}, 1100 – 1110 cm^{-1}, 1240 cm^{-1}, 1340 cm^{-1}, 1410 cm^{-1}, 1720 cm^{-1}. This set of absorption bands is characteristic of polyethylene terephthalate (PET, PETE).

[1] Kalinina L. S. Kachestvennyj analiz polimerov [Qualitative analysis of polymers]. Moskva : Himija, 1975. S. 193–195; Kupcov A. H., Zhizhin G. N. Fur'e-spektry kombinacionnogo rasseivanija infrakrasnogo pogloshhenija polimerov [Fourier spectra of Raman scattering of infrared polymer absorption]: spravochnik. Moskva : Fizmat lit, 2001. S. 167–169 [in Russian].

An example of the IR spectrum of the container material of object # 2 is shown in Fig. 5.

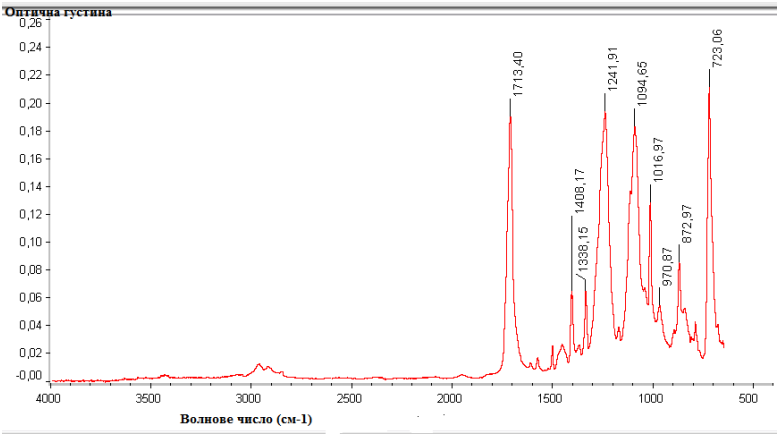


Fig. 5. Example of the IR spectrum of PET container material

The results of the analysis in Table 3 indicate the presence of DEPs in all water samples that were packed in PET containers.

In addition, in the samples of water and organic solvent (ethyl alcohol), which did not initially contain DEP (within the definition), after exposure in PET containers, which previously contained objects №2 – 6, the content of DEP, high quality composition which were somewhat like the water content (objects № 2-6).

Conclusions. Because of the conducted researches the cumulative correspondence of the obtained experimental data on determination of DEP content in the composition of water contained in PET container was established, world experience on such problem, which indicates the fact of DEP transition from the packaging material to its content.

In addition to water, phthalates can be saturated with organic solvents, ethyl alcohol. This demonstrates the acceptability of the results of research on DEP in water and the general methodology for phthalates in domestic, including forensic, practice.The following guidelines should be noted as recommendations for the packing of research sites for the expert study of chemical products and specialty chemicals for phthalates establishment at any level (qualitative and quantitative), the following should be noted:

– container material may be made of any polymer that does not contain phthalates. One indication of the composition of the container material is the marking on its outer surface;

– packaging that is most suitable for the packaging of objects is metal or glass containers (metal canisters or glass containers, bottles, etc.). Note that the lids and gaskets that can be used are usually polymeric, so they must also be made of any non-PET material;

– if there is enough object, it is advisable to rinse the inner surface of the study object (2-3 times) before packing, to avoid the possible presence of EFC residues in the object.

References

Carrillo, J.D. at. al. (2008). Determination of phthalates in wine by head space solid-phase microextraction followed by gas chromatography–mass spectrometry Use of deuterated phthalatesas internal standards. *Journal of Chromatography*. Vol. 1181. 125–130.

Dong, R.H. at. al. (2017). Association between Phthalate Exposure and the Use of Plastic Containers in Shanghai Adults. *Biomedical Environmental Scinces*. Vol. 30. 727–736.

Farhoodi M. at al. (2008). Effect of environmental conditions on the migration of di
(2-ethylhexyl) phthalate from pet bottles into yogurt drinks: influence of time, temperature, and food simulant. *The Arabian Journal for Science and Engineering*. Vol. 33. 81–287.

González-Sálamo, J. at. al. Analytical methods for the determination of phthalates in food. *Current Opinionin Food Science.* Vol. 22. 122–136.

He, J. at. al. (2010). Preparationandevaluation of molecularly imprinted solid-phase micro-extraction ibersforselectiveextraction of phthalates in anaqueou ssample. *Analytica Chimica Acta*. Vol.674. 53–58 [in English].

Kalinina, L.S. (1975). Kachestvennyj analiz polimerov. Moskva: Himija [in Russian].

Kupcov, A. H., Zhizhin, G. N. (2001). *Fur'e-spektry kombinacionnogo rasseivanija infrakrasnogo pogloshhenij apolimerov*: spravochnik. Moskva: Fizmatlit [in Russian].

Luo Q.at.al. (2018). Migration and potential risk of trace phthalates in bottled water: A global situation. *Water research.* Vol. 147. 362–367.

Montuori P. at. al. (2008). Assessing human exposure to phthalic acid and phthalateesters from mineral water stored in polyethyleneterephthalate and glass bottles. *Food Additives and Contaminants.* Vol. 25. 511–518.

Pereira, J. at.al. (2019). Determination of phthalates in olive oil from European market. *Food control.* Vol. 98. 54–60.

Salazar-Beltrán, D. at. al. (2018). Determination of phthalate acid esters plasticizers in polyethyleneterephthalate bottle sandits correlation with some physico chemical properties. *Polymer Testing.* Vol.68. 87–94.

Sanitarni Normi «Dopustima kil'kist' migracii (DKM) himichnih rechovin, jaki vidiljajut'sja z polimernih ta inshih materialiv, shho kontaktujut' z harchovimi produktami ta metodi ïh viznachennja SanPin 42–123–4240–86 [Chinnij vid 1986–12–31] [in Russian].

Sax, L. (2010). *PolyethyleneTerephthalate May Yield Endocrine Disruptors. Environmental Health Perspec*tives. 2010. Vol 118. P. 445–448.

Schmida, P. at. al. (2008). Does the reuse of PET bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? *Water research.* Vol 42. 5054–5060.

Spektral'na baza danih. URL: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi (data zvernennia 10.05.2019).

Zaki, G. at al. (2018). Concentrations of several phthalates contaminants in Egyptian bottled water: Effects of storage conditions and estimate of human exposure. *Science of the Total Environment*. Vol. 618. 142–150.

Zia, A.I. at. al. (2015). Rapid and molecular selective electrochemical sensing of phthalate sinaqueous solution. *Biosensors and Bioelec*tronics. 2015. Vol. 67. 342–349.

Zia, A.I. at.al. (2013). Technique for rapid detection of phthalates in water and beverages. *Journal of Food Engineering*. Vol. 2016. 515–523.

К. Є. Руднєва, М. В. Зарубіна, Л. В. Нардід,
П. В. Карножицький, О. М. Пашкова

ВПЛИВ МАТЕРІАЛУ ПОЛІМЕРНОЇ ТАРИ НА РЕЗУЛЬТАТИ ЕКСПЕРТНОГО ДОСЛІДЖЕННЯ З ВИЯВЛЕННЯ ФТАЛАТІВ

У статті розглянуто проблему визначення впливу матеріалу ємності на наявність фталатів – складних ефірів фталевої кислоти (ЕФК) та ізомерних її сполук у складі досліджуваних об'єктів.

Мета роботи – встановлення наявності фталатів у складі експериментальних зразків та у складі бутильованої питної води, яка реалізується на території України у полімерній тарі.

Визначення вмісту ЕФК проводилося методом газової хроматографії-мас-спектрометрії.

Враховуючи отримані результати, надано рекомендації з пакування рідких об'єктів дослідження при направленні їх на дослідження з метою встановлення вмісту ЕФК.

Ключові слова: складні ефіри фталевої кислоти; газова хроматографія-мас-спектрометрія; мінеральна вода; поліетилентерефталат.

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