ANALYTICAL SUPPORT OF HYGIENIC CONTROL OF OXATHIAPIPROLIN RESIDUAL AMOUNTS IN ENVIRONMENTAL OBJECTS AND POTATOES

Introduction. Providing of analytical control over the application of pesticides in environmental objects and agricultural products – is a mandatory component of preregistration studies of new compounds. The most common method for the control of pesticides application in the Ukraine is high performance liquid chromatography.

The aim of the study – to develop analytical methods for oxathiapiprolin determination in the air, water, soil and potatoes for hygiene monitoring of compliance with hygienic standards in the environment and agricultural raw materials.

Research Methods. Oxathiapiprolin – is a compound from new piperidinyl-thiazole-isoxazolines chemical class. For investigation the HPLC method was used.

Results and Discussion. Optimal conditions of oxathiapiprolin chromatography were established: mobile phase – acetonitrile mixture + bidistilled water (75+25), UV detector wavelength – 260 nm, thermostat column temperature – 30 °C. Retention time – 4.6±0.1 minutes. Linear detection range is 0.5–10.0 mg/ml. Dependence of calibration area (S) peaks of the substance on its concentration (ρ) described by linear regression equation: S_{oxathiapiprolin}=272.4+36315.7×ρ.

The best results are obtained when concentrating the air on a filter paper “blue ribbon” followed by extraction with acetone. When choosing extractants to oxathiapiprolin determination in water, the best result obtained with ethylacetate; soil – with acetone, potatoes – with a mixture of acetonitrile + 0.5 % aqueous phosphoric acid, followed by purification of extracts by adsorption.

Conclusions. Thus, developed analytical methods meet modern requirements, are selective and allow to control oxathiapiprolin content in water at detection limit level – 0.002 mg/dm³, air of working zone – 0.2 mg/m³, atmosphere air – 0.003 mg/m³, soil – 0.03 mg/kg, potato – 0.003 mg/kg.

KEY WORDS: oxathiapiprolin; liquid chromatography; air; water; soil; potatoes.
(log $K_{ow}$, 20 °C): 3.6. Solubility in organic solvents, g/l (20 °C): acetonitrile – 129.9; ethanol – 13.5; acetone – 162.8; ethylacetate – 33.9; dichloromethane – 352.9; o-xylene – 5.8; n-octanol – 0.03; n-hexane – 0.01 [1].

For investigation the HPLC method was used.

RESULTS AND DISCUSSION. On the first stage of the study we conducted a series of experiments to establish optimal conditions for oxathiapiprolin chromatography, given in Table 1. In the selection of the mobile phase, a series of studies in acetonitrile+bidistilled water systems in various combinations (85+15, 75+25 and 65+35, vol.+vol.) were conducted. To determine the optimal wavelength detection, we studied wavelength range from 220 to 300 nm.

On the next stage of research calibration curve was constructed (Fig. 1), range in which there is a linear relationship between peak area and oxathiapiprolin concentration was determined. Calibration curve was constructed in accordance with international standard [7].

Also, we conducted a series of laboratory experiments on the selection of extractants for the removal of the substance from the air, water, soil, potatoes samples and determining of the most effective way of extracts samples purification. At the stage of extractants selection the following organic solvents have been used – methanol, acetonitrile, acetone, chloroform, hexane, ethylacetate, dichloromethane, and combinations thereof.

As a result of the studies optimal conditions for extraction and purification, which provide selective removal of the test compound from matrices without impurities that interfere with chromatographic determination were set.

Air samples were taken on various sorption materials; the best results are obtained when concentrating the air on a filter paper “blue ribbon”. After oxathiapiprolin concentration from air on sorp-

Table 1 – Conditions of oxathiapiprolin chromatography

| Characteristics of the determination method | Oxathiapiprolin |
|---------------------------------------------|-----------------|
| Chromatograph                               | Liquid Chromatograph “Shymadzu” |
| Detector                                    | UV detector     |
| Column                                      | steel chromatographic (250×4.6) mm filled with Nucleosyl C18 (100–5) |
| Precolumn                                   | steel chromatographic (4×3) mm filled with Nucleosyl C18 (100–5) |
| Mobile phase                                | mixture of acetonitrile + bidistilled water (75+25, vol.+vol) |
| Mobile phase flow rate, ml/min              | 1.0             |
| UV detector wavelength, nm                  | 260             |
| Temperature of thermostat column, °C        | 30              |
| Volume of liquid chromatograph loop injector, ml | 20              |
| Retention time under these conditions, minutes | 4.6±          |
| Linear range of detection, μg/ml            | 0.5–10.0        |

The dependence of the oxathiapiprolin chromatographic peak areas (c.u.) on its concentration in the calibration solution (μg/ml) is given by the equation:

$$S_{oxathiapiprolin} = 272.4 + 36315.7 \times p$$
tion materials, the best extraction of material from sorbent was carried out with acetone.

When choosing extractants to oxathiapiprolin determination in water, the best result obtained with ethylacetate. Best oxathiapiprolin extraction from soil samples was carried out with acetone, potatoes—with a mixture of acetonitrile + 0.5 % aqueous phosphoric acid, followed by purification of extracts by means of adsorption chromatography (Table 2).

The obtained extracts of air and water samples after extraction and purification were dried with anhydrous sodium sulfate 20–25 g by infusion in a conical flask of 250 ml volume for 30 minutes. The extract was filtered through a filter “red tape” in the pear-shaped flask for solvent distillation with a capacity 250 ml, sodium sulfate and filter were washed with 20 ml of extractant. Eluates from soil and potato samples were collected in pear-shaped flask for solvent distillation (50–100 ml). The combined extracts and eluates was evaporated on a rotary evaporator at water bath temperature no higher than 40 °C to volume 0.2–0.3 ml. The solvent residue was evaporated on air. The dry residue was quantitatively transferred to a graded test tube with a 10 ml capacity using acetonitrile. The final volume of the extract of air, water and soil samples was 2 ml potatoes – 1 ml.

Chromatograms of various samples extracts with oxathiapiprolin introduction to the water on level – 0.005 mg/dm³, air working zone – 0.5 mg/m³, atmosphere air – 0.008 mg/m³, soil – 0.1 mg/kg, potatoes – 0.1 mg/kg are shown in Fig. 2.

Metrological characteristics of oxathiapiprolin determination in environmental objects and agricultural raw materials by HPLC are given in Table 3.

According to the data given in the Table 3, the average oxathiapiprolin determination value in the study of various matrices was not less than 70 %, corresponding to modern requirements for guidance [8].

**Table 2 – Conditions of water, air, soil and potatoes sample preparation for oxathiapiprolin determination using chromatographic methods**

| Studied object                     | Sample, g/ml | Sample preparation phase | Extraction purification |
|------------------------------------|--------------|--------------------------|-------------------------|
| Air of working zone (“blue ribbon” filter) | 4.0 l acetone, 2×30 ml, each time during 30 minutes | not necessary |
| Atmosphere air (“blue ribbon” filter) | 250.0 l ethylacetate, 3×50 ml, each time during 1–2 minutes | not necessary |
| Water                              | 400.0 g acetone, 2×50 ml, each time during 30 minutes | solid phase extraction (glass column (300×8) mm with floryzyl PR), elution hexane + acetone (7+3, vol.+vol.) 1×25 ml |
| Soil                               | 20.0 g acetone, 2×50 ml, each time during 30 minutes | solid phase extraction (glass column (300×8) mm with floryzyl PR), elution hexane + acetone (7+3, vol.+vol.) 1×20 ml |
| Potato                             | 50.0 g acetonitrile + 0.5 % aqueous solution of phosphoric acid (2+1, vol.+vol.) during 30 minutes + 50 g NaCl during 30 minutes | solid phase extraction (glass column (300×8) mm with floryzyl PR), elution hexane + acetone (7+3, vol.+vol.) 1×40 ml |

**Table 3 – Metrological characteristic of methods for oxathiapiprolin determination in environmental objects and agricultural raw materials by HPLC**

| Studied object       | LOQ*, mg/dm³, mg/kg | DL*, mg/dm³, mg/kg | Measurement range, mg/dm³, mg/kg | Mean value of determination, R, % | Standard deviation (n=12), S, % | Confidence interval, (P=0.95), ± % |
|----------------------|---------------------|-------------------|---------------------------------|----------------------------------|--------------------------------|---------------------------------|
| Air of working zone  | 0.5                 | 0.2               | 0.5–5.0                         | 91.2                             | 1.3                            | 3.5                             |
| Atmosphere air       | 0.008               | 0.003             | 0.008–0.08                      | 91.0                             | 1.3                            | 3.5                             |
| Water                | 0.005               | 0.002             | 0.005–0.05                      | 89.2                             | 4.2                            | 2.7                             |
| Soil                 | 0.1                 | 0.03              | 0.1–1.2                         | 81.1                             | 5.0                            | 3.2                             |
| Potato               | 0.01                | 0.003             | 0.01–0.12                       | 75.3                             | 5.2                            | 3.3                             |

Note. * – LOQ – limit of quantification, DL – limit of detection.

**CONCLUSIONS.** Thus, developed analytical methods meet modern requirements, are selective and allow to control oxathiapiprolin content in different objects and can be used for air of working zone, atmosphere air, water and soil safety control during the application of pesticides based on piperidinyl-thiazole-isoxazoline class compound.
Fig. 2. Chromatograms: a – calibration solutions with oxathiapiprolin concentrations 0.5 μg/ml; b – working zone air samples with the oxathiapiprolin introduction on level 0.5 mg/m³; c – atmosphere air samples with the oxathiapiprolin introduction on level 0.008 mg/m³; d – water samples with the oxathiapiprolin introduction on level 0.005 mg/m³; e – soil samples with the oxathiapiprolin introduction on level 0.1 mg/kg; g – potato samples with the oxathiapiprolin introduction on level 0.01 mg/kg.

LIST OF LITERATURE
1. Public release summary on the evaluation of the new active oxathiapiprolin in the product DuPont Zorvec Enicide Fungicide / APVMA Product Number 68375. – 2015. – 54 p.
2. Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein / R. J. Pasteris, M. A. Hanagan, J. J. Bisaha [et al.]. // Bioorg. Med. Chem. – 2016. – No. 24(3). – P. 354–361.
3. Determination of oxathiapiprolin concentration and dissipation in grapes and soil by ultrahigh-performance liquid chromatography–tandem mass spectrometry / Yu. Pingzhong, Jia Chunhong, Ercheng Zhao [et al.]. //
References

1. Public release summary on the evaluation of the new active oxathiapiprolin in the product DuPont Zorvec Enicade Fungicide. (2015).

2. Pasteris, R.J., Hanagan, M.A., Bisaha, J.J., Finkelstein, B.L., Hoffman, L.E., Gregory V. ... Berger, R.A. (2016). Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein. Bioorg. Med. Chem., 24 (3), 354-361.

3. Pingzhang, Yu., Chunhong, Jia, Ercheng, Zhao, Li, Chen, Hongju, He, Junjie, Jing, Min, He (2017). Determination of oxathiapiprolin concentration and dissipation in grapes and soil by ultrahigh-performance liquid chromatography–tandem mass spectrometry. Retrieved May 18, 2017, from http://onlinelibrary.wiley.com/doi/10.1002/jsfa.8178/full.

4. Jin Jang, Heejung Kim, Jung Ah Do... (2017). Establishment of an analytical method for determination of fungicide oxathiapiprolin in agricultural commodities using HPLC-UV detector. Journal of Food Hygiene and Safety, 31 (3), 186-193.

5. Antonenko, A.M., Bardov, V.H., Korshun, O.M., Karpenko, V.V., Korshun O.M., & Lipavska, A.O. (2012). Optimization of analytical control of residual quantities of herbicide active substances Master Power EA. Medychni perspektyvy – Medical Prospects, XVII (2), 78-83 [in Ukrainian].

6. Ravelo-Pérez, L.M., Hernández-Borges, J., Rodríguez-Delgado, M.Á. (2006). Pesticides analysis by liquid chromatography and capillary electrophoresis. Journal of Separation Science, 29 (17), 2557-2577.

7. Mezhdunarodnyi standart ISO 0 8466-1:1990 (Е). Kachestvo vody – Kalibrovka i otsenka analiticheskikh metodov opredeleniya rabochikh kharakteristik. Chast 1: Statisticheskaya obrabotka lineynoy kalibrovchnoy funktsii [Water quality – Calibration and evaluation of analytical methods for determining performance. Part 1: Statistical processing of a linear calibration function] [in Russian].

8. Resolution of the Ministry of Health of Ukraine No. 20 of April 20, 1999 “On the use of norms of accuracy and correctness of measurements when controlling the content of chemicals in food raw materials, foodstuffs and objects of the environment and the correspondence between the values of MDR and MPC and the boundaries of analytical determination of chemical Substances.”
Методи дослідження. Оксатіапипролін – сполука нового хімічного класу піперидиніл-тіазол-ізоксазолінів. Для дослідження використано метод високоекфективної рідинної хроматографії.

Результати й обговорення. Було встановлено оптимальні умови хроматографування оксатіапипроліну; рухома фаза – суміш ацетонітрил+бідистильована вода (75+25), довжина хвилі УФ-детектора – 260 нм, температура термостата колонки – 30 °С. Час утримування за данных умов становив (4,6±0,1) хв. Лінійний діапазон детектування – 0,5–10,0 мкг/мл. Градуовальна залежність площі (S) піків досліджуваної речовини від її концентрації (ρ) описана рівнянням лінійної регресії: $S_{оксатіапипроліну} = 272,4+36315,7\times ρ$.

Найкращі результати отримано при концентруванні повітря на паперовий фільтр "синя стрічка" з подальшою екстракцією ацетоном. При виборі екстрагентів для визначення оксатіапипроліну у воді найкращі результати одержано з етилацетатом, у ґрунті – з ацетоном, у картоплі – з сумішшю ацетонітрил+0,5 % водний розчин ортофосфорної кислоти. При концентруванні ґрунту та картоплі за допомогою адсорбційної хроматографії.

Висновок. Розроблені аналітичні методи відповідають сучасним вимогам, є селективними та дозволяють контролювати оксатіапипролін у воді на рівні межі виявлення – 0,002 мг/дм$^3$, у повітрі робочої зони – 0,002 мг/м$^3$, у атмосферному повітрі – 0,003 мг/м$^3$, у ґрунті – 0,03 мг/кг, в картоплі – 0,003 мг/кг.

КЛЮЧОВІ СЛОВА: оксатіапипролін; рідинна хроматографія; повітря; вода; ґрунт; картопля.

О. А. Новохацька, Д. С. Милохов, Е. П. Вавриневич, С. Т. Омельчук, А. Н. Антоненко
ИНСТИТУТ ГИГІЕНИ І ЭКОЛОГИИ НАЦІОНАЛЬНОГО МЕДИЦИНСЬКОГО УНІВЕРСИТЕТА ІМІЕНИ А. А. БОГОМОЛЬЦА, КИЄВ

АНАЛИТИЧЕСКОЕ ОБЕСПЕЧЕНИЕ ГИГИЕНИЧЕСКОГО КОНТРОЛЯ ОСТАТОЧНЫХ КОЛИЧЕСТВ ОКСАТИАПИПРОЛИНА В ОБЪЕКТАХ ОКРУЖАЮЩЕЙ СРЕДЫ И КАРТОФЕЛЕ

Вступление. Обеспечение аналитического контроля за использованием пестицидов в объектах окружающей среды и сельскохозяйственной продукции – обязательная составляющая предрегистрационных исследований новых веществ. Наиболее распространенным для контроля за применением пестицидов в Украине получи метод высокоэффективной жидкостной хроматографии.

Цель исследования – разработать аналитические методы определения оксатиапипролина в воздухе, воде, почве и картофеле для гигиенического контроля за соблюдением гигиенических нормативов в объектах окружающей среды и сельскохозяйственном сырье.

Методы исследования. Оксатиапипролин – соединение нового химического класса пиперидинил-тіазол-ізоксазолинов. Для исследования использован метод высокоэффективной жидкостной хроматографии.

Результаты и обсуждение. Были установлены оптимальные условия хроматографирования оксатиапипролина: подвижная фаза – смесь ацетонитрил+бідистильована вода (75+25), длина волны УФ-детектора – 260 нм, температура термостата колонки – 30 °С. Время удерживания в данных условиях составляло (4,6±0,1) мин. Линейный діапазон детектування – 0,5–10,0 мкг/мл. Градуировочная зависимость площади (S) пиков исследуемого вещества от его концентрации (ρ) описана уравнением линейной регрессии: $S_{оксатіапипроліну} = 272,4+36315,7\times ρ$.

Наилучшие результаты получены при концентрировании воздуха на бумажный фильтр "синяя лента" с дальнейшей экстракцией вещества ацетоном. При выборе экстрагентов для определения оксатиапипролина в воде наилучшие результаты получено с этилацетатом, в почве – с ацетоном, в картофеле – со смесью ацетонитрил+0,5 % водный раствор ортофосфорной кислоты с дальнейшим очищением экстрактов проб воздуха и картофеля с помощью адсорбционной хроматографии.

Вывод. Разработанные аналитические методы соответствуют современным требованиям, являются селективными и позволяют контролировать оксатиапипролин в воздухе на уровне предела обнаружения – 0,002 мг/дм$^3$, в воздухе рабочей зонь – 0,2 мг/м$^3$, в атмосферном воздухе – 0,003 мг/м$^3$, в почве – 0,03 мг/кг, в картофеле – 0,003 мг/кг.

КЛЮЧЕВЫЕ СЛОВА: оксатиапипролин; жидкостная хроматография; воздух; вода; почва; картофель.

Received 19.06.17

Address for correspondence: O. O. Novohatska, Hygiene and Ecology Institute of O. Bohomolets National Medical University, Myrslavska Street, 3, apt. 65, Kyiv, 02232, Ukraine, e-mail: alesya.novohacka@ukr.net.