

ANALYTICAL CONTROL OF ACTIVE substances of the class of imidazolinones in herbicide formulations

Goal. The choice of a chromatographic method and the development of a method for the analytical determination of imazethapyr, imazamox, imazapyr in herbicide preparative forms in the joint presence. **Methods.** Active substances were analyzed by thin layer chromatography. The linear range of the dependence of the area of chromatographic zones on the amount of active substance was assessed by a mathematical-statistical method. **Results.** Determination of pesticides by thin-layer chromatography includes the main stages: classification of pesticides by polarity; extraction of active substances from the analyzed sample; chromatographic separation, detection and quantification. The decisive factor in the analysis stages is the dipole moment (μ , D), which characterizes the polarity of the compounds. Imazethapyr, imazamox and imazapyr are polar compounds ($\mu \geq 6$, D) with the corresponding values: 6.1; 6.4 and 6.8 D. Extraction of active substances is carried out with ethanol. Qualitative determination — in a thin layer of adsorbent silica gel in the mobile phase: a mixture of ethanol with acetic acid in a ratio of 4 : 1.5. Identify compounds at the wavelength of the spectrum λ 254 nm and using the developing reagent silver ammonia. The calibration dependence of the area of the chromatographic zone of the compound on its amount is linear in the detection range of 0.30–1.30 μ g and is described by the regression equations for: imazethapyr $S = 12.345 C + 0.7778$, $R^2 = 0.99$; imazapyr $S = 9.3671 C + 1.081$, $R^2 = 0.99$; imazamox $S = 7.6234 C + 1.4462$, $R^2 = 0.98$. The equation is used to quantify the active ingredients in the chromatogram. **Conclusions.** The use of the method of thin layer chromatography makes it possible to select selective conditions for the analysis of imazethapyr, imazamox, and imazapyr in the presence of herbicides in preparative forms. The developed technique is an express method of control and provides determination of active substances in the

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course of one analysis with high accuracy (relative error is less than 5% at $n = 5$, $P = 0.95$). The analytical support of the developed and patented method allows for analytical control of active substances of the imidazolinone class in the formulation of pesticides by analytical and toxicological laboratories, testing services without the use of expensive devices.

analytical control; preparative form; thin layer chromatography; imidazolinones

An integral element of modern technologies for growing crops is the strategy of herbicide application, which involves the introduction of combined preparations based on two or more active substances. Thus, recently herbicides based on imidazolinone derivatives are widely used, of which about 32 are currently registered in Ukraine [1]. Imidazolinones are compounds of systemic action that inhibit the activity of acetolactate synthetase, an enzyme of acetoxylic acid, disrupt the synthesis of protein, DNA and slow down the growth of plant cells. Typical representatives of this class are: imazamox, imazapyr and imazethapyr [2]. The use of such preparations in agriculture, sets the task of their simultaneous determination in different matrices.

The expansion of the range of preparations continues to stimulate the development and application of

methods of analytical determination. Thus, in Ukraine, in the analytical chemistry of pesticides there are two main areas: analysis of residual amounts in different matrices and control of formulations (active substances of pesticides) [3]. The first is used to control residues in agricultural products and environmental facilities and is based on the need to develop separate methods for each pesticide and each matrix [4–10] and methods for the analysis of multicomponent pesticides, which ensures their simultaneous determination in one batch [11]. Regarding the control of formulations, there are currently virtually no methods for determining the active substances in combined pesticides and a systematic approach to this problem. Analytical control of preparations is coordinated by the international organization CIPAC, which provides development and validation of analytical methods for the determination of new preparations and their active substances. They can be identified only by high-performance liquid chromatography-mass spectrometry (HPLC/MS/MS) based on the relevant bases of active substances. But there are whole classes of pesticides that are not certified by CIPAC methods for the control of formulations (for example imidazolinones, some sulfonylureas, etc.) [12, 13]. Therefore, the task is to determine imazethapyr, imazapyr and imazamox in preparative forms in the course of one analysis.

The aim of the study was to choose the chromatographic method and to develop a method for the analytical determination of imazethapyr, imazamox and imazapyr in preparative forms of herbicides in the combined presence.

Materials and methods of research.

Characteristics and physicochemical properties of the studied active substances are given in the table.

Chromatographic analysis of the active substance mixture was performed by thin layer chromatography

(TLC) using SORBFIL plates with a thin layer of adsorbent (with the addition of a fluorescent UV indicator) applied to an aluminum substrate. Identification of imazamox, imazapyr and imazethapyr was performed by Rf, and their quantification was determined by the formalized dependence of the area of chromatographic zones on the amount of active substance using correlation and regression analyzes.

Research results. Today in the analytical chemistry of pesticides the main physicochemical methods of analysis of organic compounds are chromatographic methods. When choosing a method of analysis, in addition to analytical tasks to be solved, there is the question of availability and availability of devices, their price range. For analytical control of pesticide formulations, especially with low content of active substances in formulations, the most acceptable method is thin layer chromatography (TLC), as a variant of liquid chromatography (LC), which provides selective detection of test compounds under optimal conditions of separation, qualitative and quantitative determination; has high sensitivity, is express and easy to perform.

Determination of pesticides according to the algorithm of chemical-analytical monitoring includes a number of main stages: classification

of pesticides by polarity; extraction of active substances from the analyzed sample; chromatographic separation, detection and quantification [14].

The choice of analysis method is limited by the physicochemical properties of the pesticide. The test compounds belong to the same chemical class and have very similar physicochemical properties (Table), which complicates their determination in the combined presence in the matrix. According to long-term research of the Laboratory of Analytical Chemistry of Pesticides, the properties of pesticides should be characterized using an integral indicator — dipole moment (μ , Debye), which characterizes the polarity of compounds and is decisive in the next stages of analysis. According to the three-stage classification, imazethapyr, imazamox and imazapyr are polar compounds ($\mu \geq 6$, Debye) with dipole moments of 6.1; 6.4 and 6.8 Debye respectively.

One of the common methods of extracting pesticides from different matrices is liquid extraction. It is a universal, express and fast method that does not lead to the destruction of components [3].

The extraction processes are closely related to the solubility process of the compounds. As the polarity of pesticides increases, their solubility in polar solvents with $\epsilon > 20$

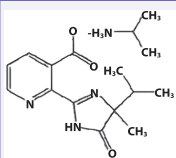
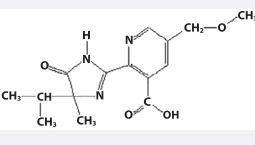
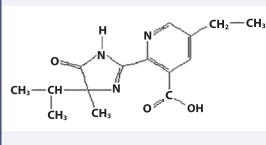
increases: acetone, ethanol, methanol. The best extractant will be one whose dielectric constant (ϵ) is close to the dipole moment of the compound. Therefore, the extraction of active substances from the studied preparations is carried out with ethanol (taking into account the solubility of active substances), which provides the highest percentage of extraction.

Chromatographic separation and detection of test compounds is also an important step in the determination, which includes the selection of the mobile phase and the developing reagent; setting the minimum amount of substance to be detected and the linear range of detection. In the analysis of formulations, in addition to the test compound, the extracts contain a significant amount of impurities that can form foreign zones of localization and complicate the chromatographic separation and determination of active substances. Analysis of the mixture of active substances was performed in a thin layer of adsorbent silica gel in the saturated mobile phase (mixture of ethanol with acetic acid in a volume ratio of 4: 1.5) with a dielectric constant $\epsilon = 19.4$. The elution ability of this phase determines the different speed of movement of the investigated compounds in the adsorbent, in proportion to the value of μ , and the formation of zones of their localization with the corresponding values of Rf: for imazethapyr 0.62; imazamox 0.51; imazapyr 0.45.

Compounds are identified in two ways: 1 — instrumental detection; 2 — using appropriate developing (chromogenic) reagents depending on the available reactive groups of elements and sensitivity. The first method is based on the properties of the studied compounds to form luminescent complexes at wavelengths of the spectrum λ 254 nm, which expands the possibility of identifying compounds. In the manifestation of thin-layer chromatograms with a solution of silver ammonia in the areas of localization of compounds, dark spots of reduced silver are formed.

Under certain selective assay conditions, the minimum detection rate of each compound is 0.30 μg ; linear detection range 0.30—1.30 μg (Fig. 1). Graduation dependences of the area of the chromatographic zone (S , mm^2) of the active substance on its amount in the calibration solution (C , μg) are described by linear regression equations ($P = 0.95$ and $n = 5$) for:

Characteristics of active substances

Parameters	Active substance		
	imazapyr	imazamox	imazethapyr
IUPAC name	2 - (4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid	(RS) -2- (4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) -5-methoxymethyl nicotinic acid	(RS) -5-ethyl-2- (4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid
Structural formula			
Empirical formula	$\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_3$	$\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_4$	$\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_3$
Molecular Weight	261.3	305.3	289.3
Solubility in water (25°C, g/l)	11,3	(pH 5) 116, (pH 7) >626	1,4
Solubility in organic solvents	methanol — 105; acetone — 3.9; hexane — 9.5×10^{-3} ; toluene — 1.8	methanol — 67.0; acetone — 29.3; hexane — 7×10^{-3} ; toluene — 2.2	methanol — 105; acetone — 48.2; hexane — 0.9; toluene — 5
Preparations	Arsenal New, RK; Grader, RK Vitalite, RK; Euro — Lightning, RK; Euro — Land, RK; Captor, RK	Pulsar 40, RK; Paradox, RK	Euro — Lang, r.k.; Emerald, RK; Pari, v.r.k.; Picador, RK; Picket SL, RK; Prado, RK; Sapphire, v.r.k.; Sickle, v.r.k.; Tapir, v.r.k.; Jupiter, v.r.k.

imazethapyr

$$S = 12.345 C + 0.7778, R^2 = 0.99;$$

imazapyr

$$S = 9.3671 C + 1.081, R^2 = 0.99;$$

imazamox

$$S = 7.6234 C + 1.4662, R^2 = 0.98.$$

Coefficients of determination R^2 , which show the share of variability of the performance indicator (S) from the factorial (C) acquire the appropriate values. Regression equations are used to quantify (μg) the active substances on the chromatogram.

The solution of the set analytical problem made it possible to develop an express method (block diagram) for the determination of imazapyr, imazamox and imazethapyr in preparative forms of herbicides in their combined presence (Fig. 2).

Established selective conditions for the analysis of imidazolinones were the fundamental basis of the patent №120888 (Ukraine) [15]. The research was conducted within the framework of PSR 12 Scientific bases of modern technologies of forecasting and management of phytosanitary condition of agrocenoses (Plant protection); № SR 0116U003542, 0119U001156.

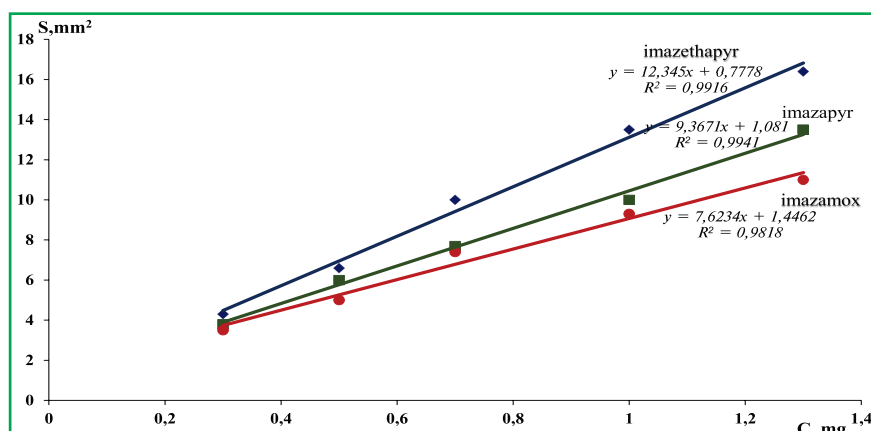


Fig. 1. Dependence of the areas of the chromatographic zones of imazethapyr, imazapyr and imazamox from their number (calibration solutions)

CONCLUSIONS

The use of thin layer chromatography makes it possible to select selective conditions for the analysis of imazethapyr, imazamox and imazapyr in the combined presence of herbicides in formulations. The developed technique is an express method of control and provides determination of active substances in the process of one analysis with high accuracy (relative error, less than 5% at $n = 5$, $P = 0.95$).

Analytical support (implementation) of the developed and patented

method makes it possible to carry out analytical control of active substances of the imidazolinone class in pesticide formulations by analytical and toxicological laboratories, testing services without the use of expensive devices.

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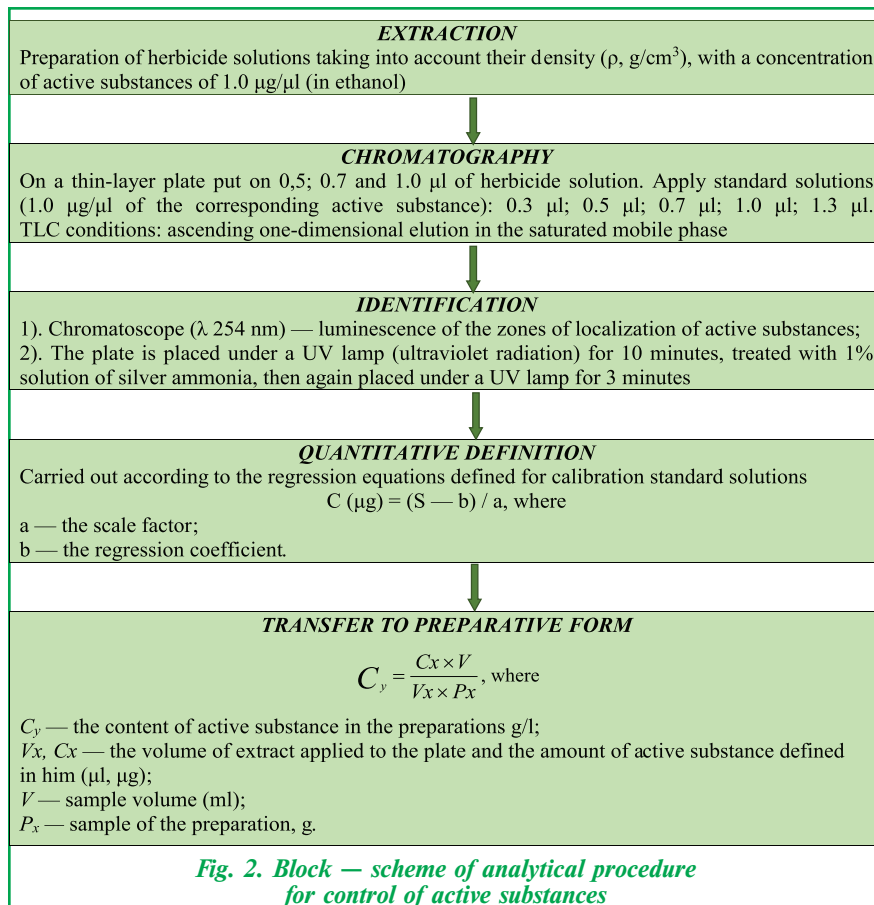


Fig. 2. Block — scheme of analytical procedure for control of active substances

peas by high performance liquid chromatography]. *Zb. Metodychni vkazivky z vyznachennia mikrokilkosti pestytsydiv v kharchovykh produktakh, kormakh ta navkolyshnomu seredovyshchi*. № 54. P. 89—106. (in Ukrainian).

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Аналітичний контроль діючих речовин класу імідазолінонів у препаративних формах гербіцидів

Мета. Вибір хроматографічного методу і розробка методики аналітичного визначення імазетапіру, імазамоксу та імазапіру в препаративних формах гербіцидів за сумісної присутності. **Методи.** Діючі речовини аналізували методом тонкошарової хроматографії. Лінійний діапазон залежності площі хроматографічних зон від кількості діючої речовини оцінювали математично-статистичним методом.

Результати. Визначення пестицидів методом тонкошарової хроматографії включає основні етапи: класифікація пестицидів за полярністю; вилучення діючих речовин з аналізованої проби; хроматографічне розділення, детектування та кількісне визначення. Визначальним за аналізу є дипольний момент (μ , Д), що характеризує полярність сполук. Імазетапір, імазамокс та імазапір — полярні сполуки ($\mu \geq 6$, Д) з відповідними значеннями μ : 6,1; 6,4 та 6,8 Д. Вилучали діючі речовини етанолом. Якiсне визначення — у тонкому шарі адсорбенту силікагель в рухомій фазі: етанол + оцтова кислота у співвідношенні 4:1,5. Ідентифікували сполуки під хроматоскопом (λ 254 нм) та з використанням проявляючого реагенту розчину аміаку срібла. Градувальна залежність площі хроматографічної зони сполуки від її кількості є лінійною в діапазоні детектування 0,30—1,30 мкг і описується рівнянням регресії для: імазетапіру $S = 12,345 C + 0,7778$ ($R^2 = 0,99$); імазапіру $S = 9,3671 C + 1,081$ ($R^2 = 0,99$); імазамоксу $S = 7,6234 C + 1,4462$ ($R^2 = 0,98$). Рівняння використовували для кількісного визначення діючих речовин на хроматограмі. **Висновки.** Використання методу тонкошарової хроматографії дає змогу вибрати селективні умови аналізу імазетапіру, імазамоксу та імазапіру за сумісної присутності в препаративних формах гербіцидів. Розроблена методика є експресним методом контролю і забезпечує визначення діючих речовин в процесі одного аналізу з високою точністю (відносна похибка менше 5% при $n = 5$, $P = 0,95$). Аналітичне забезпечення розробленого та запатентованого методу дає змогу проводити аналітичний контроль діючих речовин класу імідазолінонів у формуляціях пестицидів аналітичними та токсикологічними лабораторіями, випробувальними службами без використання дорогих приладів.

аналітичний контроль; препаративна форма; тонкошарова хроматографія; імідазолінони

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Аналитический контроль действующих веществ класса имидазолинонов в препаративных формах гербицидов

Цель. Выбор хроматографического метода и разработка методики аналитического определения имазетапир, имазамокса, имазапир в препаративных формах гербицидов при совместном присутствии. **Методы.** Действующие вещества анализировали методом тонкослойной хроматографии. Линейный диапазон зависимости площади хроматографических зон от количества действующего вещества оценивали математическостатистическим методом. **Результаты.** Определение пестицидов методом тонкослойной хроматографии включает основные этапы: классификация пестицидов по полярности; извлечения действующих веществ из анализируемой пробы; хроматографическое разделение, детектирование и количественное определение. Определяющим при проведении анализа является дипольный момент (μ , Д), характеризующий полярность соединений. Имазетапир, имазамокс и имазапир — соединения полярные ($\mu \geq 6$, Д) с соответствующими значениями μ : 6,1; 6,4 и 6,8 Д. Извлечения действующих веществ проводят этанолом. Качественное определение — в тонком слое адсорбента силікагель в подвижной фазе: этанол + уксусная кислота в соотношении 4 : 1,5. Идентифицируют соединения под хроматоскопом (λ 254 нм) и с использованием проявляющего реагента раствора аммиака серебра. Градуировочная зависимость площади хроматографической зоны соединения от ее количества является линейной в диапазоне детектирования 0,30—1,30 мкг и описываются уравнениями регрессии для: имазетапир $S = 12,345 C + 0,7778$, $R^2 = 0,99$; имазапир $S = 9,3671 C + 1,081$, $R^2 = 0,99$; имазамокс $S = 7,6234 C + 1,4462$, $R^2 = 0,98$. Уравнение используют для количественного определения действующих веществ на хроматограмме. **Выводы.** Использование метода тонкослойной хроматографии позволяет выбрать селективные условия анализа имазетапир, имазамокс и имазапир при совместном присутствии в препаративных формах гербицидов. Разработанная методика является экспрессным методом контроля и обеспечивает определение действующих веществ в процессе одного анализа с высокой точностью (относительная погрешность менее 5% при $n = 5$, $P = 0,95$). Аналитическое обеспечение разработанного и запатентованного метода позволяет проводить аналитический контроль действующих веществ класса имидазолинонов в формуляции пестицидов аналитическими и токсикологическими лабораториями, испытательными службами без использования дорогостоящих приборов.

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