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IMPROVEMENT OF ANALYTICAL DETERMINATION OF CYPROCONAZOLE AND PROPICONAZOLE IN FRUIT CROPS

Goal. *Development and unification of the method of simultaneous determination of cyproconazole and propiconazole in fruits (apples, peaches, grapes) by gas-liquid chromatography (GC).* **Methods.** *The active substances were determined by gas-liquid chromatography (GC) using a gas chromatograph «Perkin Elmer 8410» with an NPD detector. Propiconazole and cyproconazole were identified by the retention time of the compounds, quantification by the calibration dependence of the chromatographic peak area of the analyte on its concentration in the calibration solution by correlation and regression analyzes.* **Results.** *The choice of the method of determination is limited by the physicochemical properties of the active substance and the characteristics of the object under study (matrix). The optimal conditions for determination were selected using a chemical-analytical monitoring algorithm and a system for multiquantitative determination of pesticides in matrices. According to them, the matrices are analyzed in stages: extraction, purification, qualitative and quantitative determination. Cyproconazole and propiconazole are low-polar compounds (μ of their cis- and trans-isomers are 4.43; 4.71 and 3.80; 4.20 D, respectively), so extraction was performed with chloroform (ε 5.1). Purification of extracts from coextractive substances of matrices was performed by TLC. Compounds were identified by retention time, and quantification (C, μg/ml) was performed on the areas of chromatographic peaks (S, mm²) in the range of linear detection (2—10 ng) according to the linear regression equations:* $Sc = 11.991\ C — 6.096$ *(*$R^2 = 0.998$*);* $Sp = 13.721\ C - 4.221$ *(*$R^2 = 0{,}994$*). The average value of measurement is 81.0—87.8%; standard deviation 2.21—5.38%; confidence interval (P 0.95; n = 15) 1.94—4.72%.* **Conclusions.** *Optimal selective conditions for the analysis of triazole derivatives by chromatographic methods provide simultaneous determination of test compounds and quality control of fruit crops on the content of residual amounts* *of cyproconazole and propiconazole at the level of hygienic standards (MAL 0.05—0.10 mg/kg).*

analytical control; gas-liquid chromatography; triazoles

In Ukraine, the system of protection of fruit crops is based mainly on the repeated use of pesticides during the entire growing season. A significant part is made up of fungicides, the range of which is constantly expanding due to mixed formulations, where each component has a certain biological effect. To protect fruit crops from various types of fungal diseases, compounds of various chemical classes with different mechanisms of action on target objects (copper-containing pesticides, dithiocarbamic acid derivatives, pyridine derivatives, etc.) are used. Today, triazole derivatives are widespread — compounds of contact-systemic action, which are transported through the vascular system of plants mainly acropetally (along the xylem) with a solution of mineral salts, and to a lesser extent basipetally (along the phloem) with an assimilation flow; block the synthesis of ergosterol in the membranes of pathogen cells, thereby slowing down the development of diseases. Combined preparations, the components of which are representatives of this class — propiconazole and cyproconazole, are used to expand the spectrum of action in order to avoid secondary infection, increase the protective effect. In terms of toxicity, cyproconazole (LD_{50} oral for rats 1020—1330 mg/kg) is a moderately dangerous, propiconazole (LD_{50} oral for rats — 3046 mg/kg) is slightly dangerous compound. The use of such a complex of active ingredients necessitates the control of their residues in the crop (fruits), especially when they are used in baby and diet food.

To control fungicide residues in the fruits at the level of hygienic standards, highly sensitive physical and chemical methods of analysis are used: thin-layer (TLC), gas-liquid (GLC), high-performance liquid chromatography (HPLC). The advantage of these methods is the combination of two processes: the separation of a mixture of substances and their quantitative determination [1].

The purpose of the research in this work was to develop and unify the methodology for the simultaneous determination of cyproconazole and propiconazole in fruits (apples, peaches, grapes) by gas-liquid chromatography (GLC).

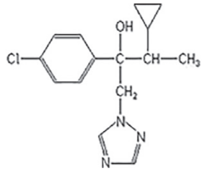
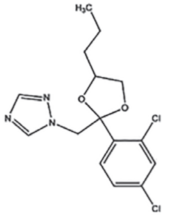
Objects and methods of research. A fungicide containing triazoles in a ratio of 1 : 3 (80 g/l cyproconazole + 250 g/l propiconazole). Characteristics and physico-chemical properties of the studied active substances are presented in the table.

When developing the determination procedure, analytical standards for cyproconazole and propiconazole were used. The stock standard solution of each active ingredient contained 100 μg of the compound in 1 ml of the appropriate organic solvent. Sequential dilution of the initial solutions was used to prepare working calibration solutions with an analyte mass concentration of 5.0; 4.0; 3.0; 2.0; 1.0 μg/ml and a control solution with an analyte mass concentration of 3.5 μg/ml. For gas-liquid chromatography (GLC) studies, a Perkin Elmer 8410 gas chromatograph with an NPD detector selective for nitrogen and phosphorus was used.

Propiconazole and cyproconazole were identified by the retention time of the compounds, quantitative determination — by the calibration dependence of the area of the chromatographic peak of the analyte on its concentration in the calibration solution using correlation and regression analyses.

Research results. The choice of a method determination of a pesticide is limited, first of all, by the physicochemical properties of the pesticide and the peculiarity of the object (matrix) under study. The optimal conditions for the determination were selected using conceptual developments (an algorithm for chemical-analytical monitoring and a method for system of multimedia determination of pesticides in matrices), the essence and structure of which are presented in a number of scientific publications [2, 3]. According to these developments, matrices are analyzed according to the stages: extraction (extraction), purification, qualitative and quantitative determination, which makes it possible, under selective conditions, to determine cyproconazole and propiconazole simultaneously in the process of one analysis.

1. Characteristics of active substances

Parameters	Active substance	
	Cyproconazole	**Propiconazole**
Name (IUPAC)	(2RS, 3RS, 2SR, 3SR)-1-(1H-1,2,4- triazol-1-yl)-2-(4-chlorophenyl)-3-cyclopropylbutan-2-ol	(2RS,4RS;2SR,4SR)-1,2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl-1-H-1,2,4-triazole
Structural formula		
Empirical formula	$C_{15}H_{18}ClN_3O$	$C_{15}H_{17}N_3O_2Cl_2$
Molecular mass	291.78	342.22
Solubility in water, mg/l (20°C)	93	150
Solubility in organic solvents, mg/l (20°C)	$3.6 \times 10^5 - 1.3 \times 10^3$	1.6×10^3

Compounds, depending on their physicochemical properties, are characterized by polarity according to magnitude of the dipole moment of the molecule (μ, D). Cyproconazole and propiconazole are low-polar compounds (μ of their cis- and trans-isomers — 4.43; 4.71 and 3.80; 4.20 D, respectively), therefore, extraction from the test sample was carried out with chloroform (ε 5.1), which is the best extractant, from the standpoint of the empirical rule «like in like».

The obtained extracts were purified from coextractive substances of the matrices by TLC by partition chromatography in a thin layer of the adsorbent under conditions of ascending one-dimensional elution in a saturation chamber. The active substances, depending on the value of μ, move at different speeds in a thin adsorbent layer and are localized at different distances from the start line in the form of separate chromatographic zones of cis- and trans-isomers with the corresponding values of Rf (Table 2). Zones of localization of cyproconazole and propiconazole (2 × 1 cm) are removed with a scalpel and transferred to a measuring tube, where 1 ml of the corresponding organic solvent is added. Aliquots are added to the chromatograph column.

The result of the research largely depends on the choice of chromatographic process conditions: the type of detector; the mobile and stationary

2. Parameters of the chromatographic determination cyproconazole and propiconazole

gas liquid chromatography (GLC) method	
Detector type	NPD
Type of chromatographic column	Glass packed
Column size	2 m × 3 mm
Carrier	Chromosorb W
Stationary phase	5% OV-17+1.95% OV-210
Carrier gas (nitrogen) velocity	30 + 2 ml/min
Hydrogen speed	20 + 2 ml/min
Air speed	60 ml/min
Column oven temperature	230 ± 2°C
Detector temperature	250 ± 2°C
Evaporator temperature	250 ± 2°C
Linear detection range	2 − 10 ng
Minimum amount of detection	2 ng
Retention time	Cyproconazole: 6.38 ± 0.18 min Propiconazole: 8.04 ± 0.15 min
thin layer chromatography (TLC) method	
Plate «Sorbfil»	Silicagel adsorbent STH-1A
Mobile phase	Hexane — ethanol (3 : 0.7, v/v)
Developing reagent	Bromophenol blue with bleaching of the chromatogram 0.05% citrate acid solution
Value Rf (isomers)	Cyproconazole (0.35±0.05 & 0.40±0.05) Propiconazole (0.55±0.05 & 0.60±0.05)

phases; the temperature regime of the detector, column, evaporator. When determining these active substances, a flame ionization detector is used, modified to determine substances whose molecules contain nitrogen and phosphorus atoms (NPD). In this detector, the source of ionization is a hydrogen flame. Organic substances in the flame of a hydrogen burner are ionized, resulting in an ionization current, the strength of which is proportional to the number of charged particles and is recorded by the device in the form of chromatographic peaks with the corresponding retention time and areas. The optimal, selective chromatographic determinations conditions of cyproconazole and propiconazole are presented in table 2.

It should be noted that when using gas-liquid chromatography, the isomers of cyproconazole and propiconazole are not identified by separate peaks as individual compounds, because they have the same retention time; only the actual active substances are separated (Fig.). Therefore, TLC can be used as an additional detection option cyproconazole and propiconazole to confirm the results of the determination by GLC, but in this case, the qualitative and quantitative assessment is carried out by the sum of the areas of isomers.

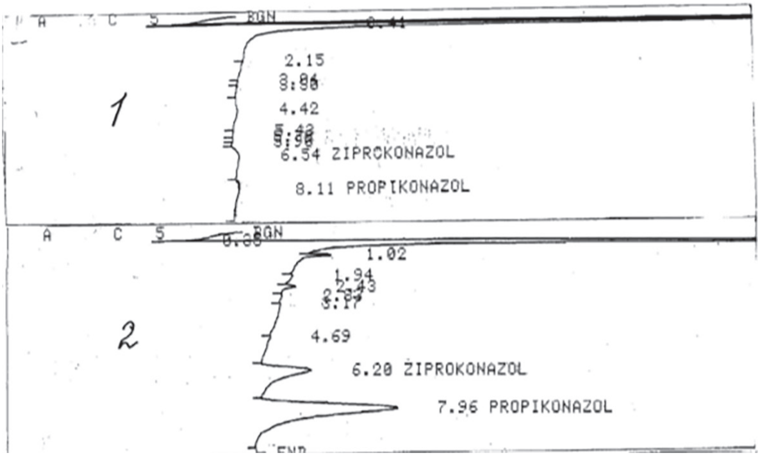


Fig. Chromatogram of model fruit samples (peaches):

1 — control sample;

2 — model sample with the introduction of 0.05 mg/kg cyproconazole + 0.16 mg/kg propiconazole

The calibration dependence of the area of the chromatographic peak of cyproconazole or propiconazole on the concentration in the calibration solution is described by the corresponding linear regression equations:

$Sc = 11.991\ C - 6.096\ (R^2 = 0.998)$,

$Sp = 13.721\ C - 4.221\ (R^2 = 0.994)$, where *Sc* and *Sp* — the peak area of cyproconazole or propiconazole respectively, mm^2; *C* — the concentration of cyproconazole/propiconazole in the calibration solution, μg/ml.

The equation is used to quantify cyproconazole and propiconazole in the analyzed samples.

The accuracy of the determination was confirmed by the «applied-detected» method when analyzing model samples into which a certain amount of the active substances was added (within the determination concentration range of 0.05—0.25 mg/kg), taking into account their ratio in the preparation. The chromatogram of the mixture is presented in the figure.

The average value of the determination is 81.0—87.8%; standard deviation 2.21—5.38%; confidence interval (P 0.95; n=15) 1.94—4.72%.

CONCLUSIONS

Optimal selective conditions for the analysis of triazole derivatives by chromatographic methods provide simultaneous determination of test compounds and quality control of fruit crops on the content of residual amounts of cyproconazole and propiconazole at the level of hygienic standards (MAL 0.05 — 0.10 mg/kg).

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Удосконалення аналітичного визначення залишкових кількостей ципроконазолу та пропіконазолу в урожаї плодових культур

Мета. *Розробка та уніфікація методики одночасного визначення ципроконазолу та пропіконазолу у плодах (яблука, персики, виноград) методом газорідинної хроматографії (ГРХ).* **Методи.** *Діючі речовини визначали методом газорідинної хроматографії (ГРХ) з використанням газового хроматографа «Perkin Elmer 8410» з NPD-детектором. Ідентифікували пропіконазол та ципроконазол за часом утримання сполук, кількісне визначення — за калібрувальною залежністю площі хроматографічного піку аналіту від його концентрації в калібрувальному розчині за допомогою кореляційного та регресійного аналізів.* **Результати.** *Вибір методу визначення обмежується фізико-хімічними властивостями діючої речовини та особливістю досліджуваного об’єкта (матриці). Оптимальні умови визначення були обрані з використанням алгоритму хіміко-аналітичного моніторингу та системи мультикількісного визначення пестицидів у матрицях. Відповідно до них матриці аналізуються за етапами: екстрагування, очищення, якісне та кількісне визначення. Ципроконазол і пропіконазол є малополярними сполуками (μ їх цис- і транс-ізомерів — 4,43; 4,71 і 3,80; 4,20 Д відповідно), тому екстракцію проводили хлороформом (ε 5,1). Очистку екстрактів від коекстрактивних речовин матриць проводили методом ТШХ. Ідентифікували сполуки за часом утримання, а кількісне визначення (С, мкг/мл) проводили за площами хроматографічних піків (S, мм^2) у діапазоні лінійного детектування (2—10 нг) відповідно до рівнянь лінійної регресії: $S_ц = 11{,}991\ C - 6{,}096$ ($R^2 = 0{,}998$); $S_п = 13{,}721\ C - 4{,}221$ ($R^2 = 0{,}994$). Середнє значення вимірювання становить 81,0—87,8%; стандартне відхилення 2,21—5,38%; довірчий інтервал (Р 0,95; n=15) 1,94 —4,72%.* **Висновки.** *Оптимальні селективні умови аналізу похідних триазолу хроматографічними* *методами забезпечують одночасне визначення досліджуваних сполук та контроль якості урожаю плодових культур за вмістом залишкових кількостей ципроконазолу та пропіконазолу на рівні гігієнічних нормативів (МДР 0,05—0,10 мг/кг).*

аналітичний контроль; газорідинна хроматографія; триазоли

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