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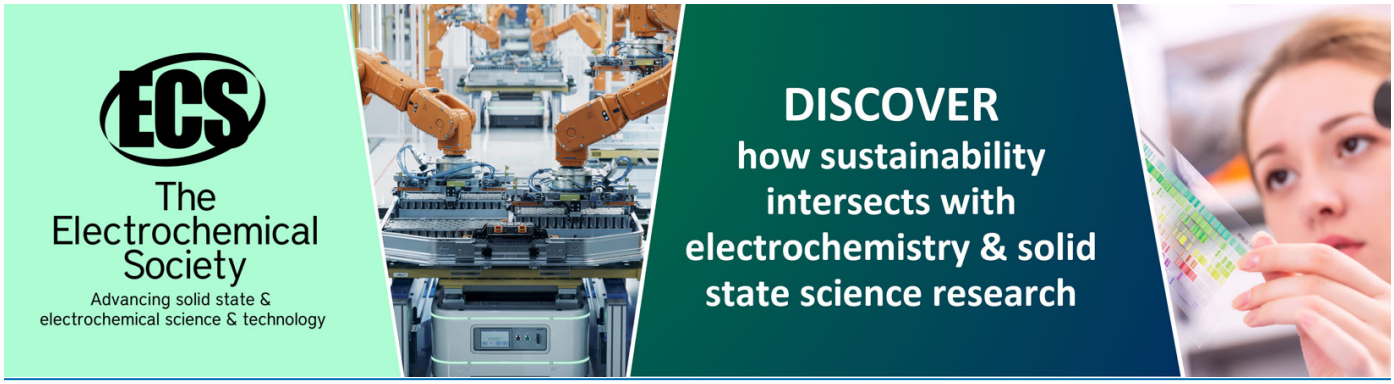


Control of pollution of the air of urbanized places at use of pesticides on the basis of triazolinthione

M S Grechina and A A Ivchenkova

Federal Scientific Center of Hygiene named after F. F. Erisman of Rospotrebnadzor, Moscow, 141014 Russian Federation

E-mail: mgsea@mail.ru

Abstract. An analytical solution to control the active ingredient in the air, a pesticide of the chemical class of triazolinthione – prothioconazole, which is a systemic fungicide of a new generation that has a protective, eradicating and curative effect, the article presents. The method is based on HPLC with an ultraviolet detector (detection wavelength 213 nm), involves the air samples on high-density paper filter with an aspiration rate of 5 l/min. Extraction of prothioconazole from the filters is performed with acetonitrile. To concentrate the extract from the filters, solid-phase extraction using octadecyl silane-based cartridges is used. Was noted that the concentration cannot be performed directly without diluting the aliquot of the extract with water in a ratio of 1:9. Due to the special properties of this active ingredient, its propensity for degradation, the amino acid cysteine is used to stabilize the aqueous solutions obtained. The linearity of the calibration characteristic was confirmed in the concentration range 0.05-0.5 μg/ml (correlation coefficient more than 0.999). The lower limit of the quantitation of prothioconazole in the air environment is 0.0025 mg/m3 when aspirating 80 L of air, which is 8 times lower than the established value of approximate safe level of influence prothioconazole in the air of urbanized places (0.02 mg/m^3). The total measurement error doesn't exceed 16%.

1. Introduction

Prothioconazole (RS)-2-(2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl-2,4-dihydro-1,2,4-triazol-3-thione) belongs to a relatively new group of active substances of the chemical class of triazolinethions, which is open by structural variation of the azole heterocycle [1]. The substance was first obtained from azole by its interaction with butyllithium and then sulfur [2]

Prothioconazole is a systemic fungicide of a new generation that has a protective, eradicating and prolonged curative effect [3]. According to studies conducted by the ISPA (Institute of Science of Food Production, Italy), the use of formulations containing prothioconazole effectively inhibits the development of rust, powdery mildew, septoria, fusarium spike [4], friability of stems and leaf patches on barley, wheat and other crops [5,6]. In this connection, preparations based on prothioconazole, are becoming increasingly common, while being used both individually and in a mixture with fungicides, insecticides and herbicides. At present, 10 formulations based on prothioconazole are allowed on the territory of the Russian Federation.

In the environment, prothioconazole metabolizes to a more stable compound, prothioconazole-desthio [7]. This metabolite influences the formation of powerful shoots, a well-developed root system, increased bushiness, drought resistance, and high-quality grain parameters, and provides

reliable protection of the culture against many types of diseases and harmful insects at the initial stages of its growth [8, 9].

The established value of ADI of prothioconazole is 0.05 mg/kg of body weight, approximate safe level of prothioconazole in air of urbanized places is 0.02 mg/m^3. The existing official methods for measuring the concentration of prothioconazole in the air provide a detection lower limit of 0.05 mg/m^3.

The main aim of current work was to develop a method for measuring the concentrations of prothioconazole in the air of urbanized place using high-performance liquid chromatography with an ultraviolet detector to monitor air pollution when a pesticide is used in agricultural production.

2. Materials and Methods

An analytical standard sample of prothioconazole (99.8% main component content); water and acetonitrile qualifications for HPLC, orthophosphoric acid (85%), glacial acetic; methanol, ethanol 95% and L-cysteine was used.

Identification and quantification of prothioconazole was performed on an Agilent 1200 liquid chromatograph equipped with a diode array detector, a binary pump, a vacuum degasser, a thermostatically controlled column compartment and a standard autosampler. Chromatographic steel column 150 mm long, inner diameter 4.6 mm, containing sorbent C18, 5μm granulation (ZORBAX Eclipse XDB-C18 (Cat. No. 993967-902); room temperature; isocratic elution – mobile phase: 0.02 M orthophosphoric acid – acetonitrile (40:60 v/v); the flow rate of the eluent is 1.0 cm^3/min; the volume of the injected sample is 30 μl.

The stock solution of prothioconazole with a concentration of 100 μg/ml and with a concentration of 10 μg/ml were prepared in acetonitrile. Working solutions for calibration with concentrations of prothioconazole 0.05; 0.1, 0.2, 0.35 and 0.5 μg/ml were prepared in the mobile phase and used freshly prepared.

High-density paper filters, diameter 7.0 cm were used for sampling; SPE cartridges of Waters Sep Pak C18 Classic (Cat. No. WAT051910) were used for sample preparation.

Sampling of the air is made by air aspiration through a high-density paper filter with a volume flow of 5 l/min. Extraction of the substance from the filters was carried out with acetonitrile (twice in 10 ml portions). Half of the extract from the filter (10 ml) was diluted with water in a ratio of 1:9. 0.02 ml of glacial acetic acid and 0.005 g of L-cysteine were added to the solution, after mixing solution passed through pre-conditioned with 2 ml of methanol, following 5 ml of 0.05% acetic acid SPE Sep Pak C18 cartridge. After applying the sample, the cartridge was washed with 5 cm^3 of a mixture of acetonitrile-0.05% acetic acid (2:8, by volume) to remove the interfering components. The substance was eluted from a cartridge 2 ml of the same mixture in a volume ratio of 9:1.

Statistical analysis included the determination of the mean value and the relative standard deviation (RSD) from the results of a study of model air samples with theintroducing of prothioconazole.

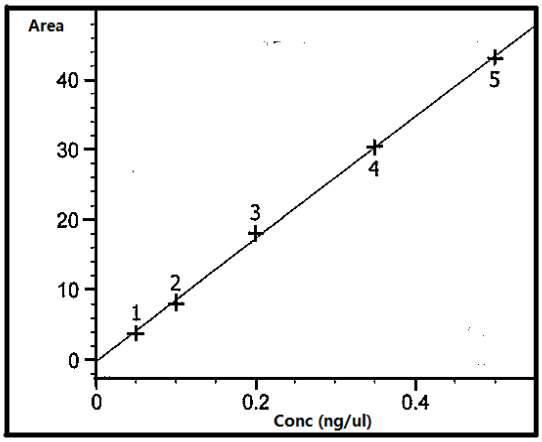
3. Results

A preliminary evaluation of the aggregate state of prothioconazole in the air was performed using computational methods [8] based on the saturated vapor pressure of the substance at a specific temperature (less than 4×10^{-7} Pa at 20 °C) and molecular weight (344.3). The calculated value of the natural volatility of prothioconazole (0.6×10^{-10} mg/m^3) is significantly lower than the approximate safe level of influence of prothioconazole in the air of urbanized places (0.02 mg/m^3), which makes it possible to make a conclusion about the hygienic significance of the presence of the substance in the air of urbanized place as an aerosol. Therefore, when sampling, it is possible to use air aspirating through high-density paper filter, with an increase in the collection time to 16 min and a volumetric flow rate of up to 5 L/min. Acetonitrile was used for extraction.

Several wavelengths of the maximum absorption (213, 230, 280 nm) are observed in the absorption spectrum of prothioconazole in the UV area of a range. At the same time, the greatest response is

observed at 213 (which is chosen as the main one) and 280 nm. To improve the reliability of substance identification, the analysis was performed at 2 wavelengths.

The correlation between the injected amount of substance and detector response was linear ranging from 0.05 µg/ml to 0.5 µg/ml (correlation coefficient is 0.99956), signal-to-noise ratio at the detection limit of 23:1. On this figure 1 we can see calibration characteristic in the concentration range 0.05-0.5 µg/cm^3.

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prothioconazole at exp. RT: 5.800
DAD1 A, Sig=213,4 Ref=360,100
Correlation:            0.99956
Residual Std. Dev.:     0.55873
Formula: y = mx + b
      m:      87.17963
      b:      -1.43927e-1
      x: Amount[ng/ul]
      y: Area
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Figure 1. Calibration characteristics.

Under the selected chromatographic conditions, prothioconazole forms a clear symmetrical peak at a retention time of 5.7–5.9 minutes. The figure 2 and figure 3 show chromatograms of the calibration solutions with the concentration of prothioconazole 0.05 µg/cm^3 and 0.05 µg/cm^3.

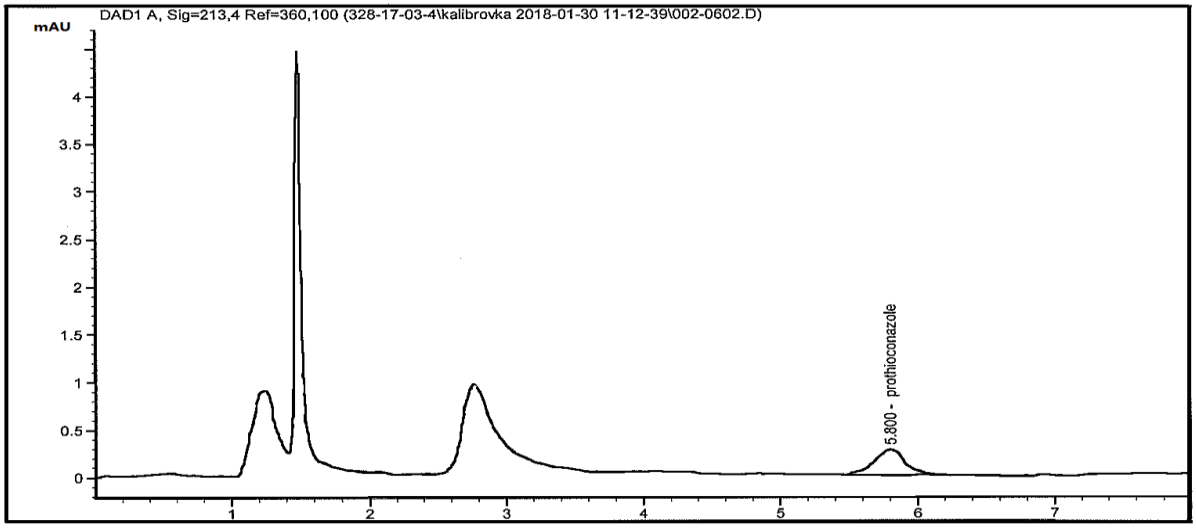

Figure 2. Chromatogram of the calibration solution with the concentration of prothioconazole 0.05 µg/cm^3.

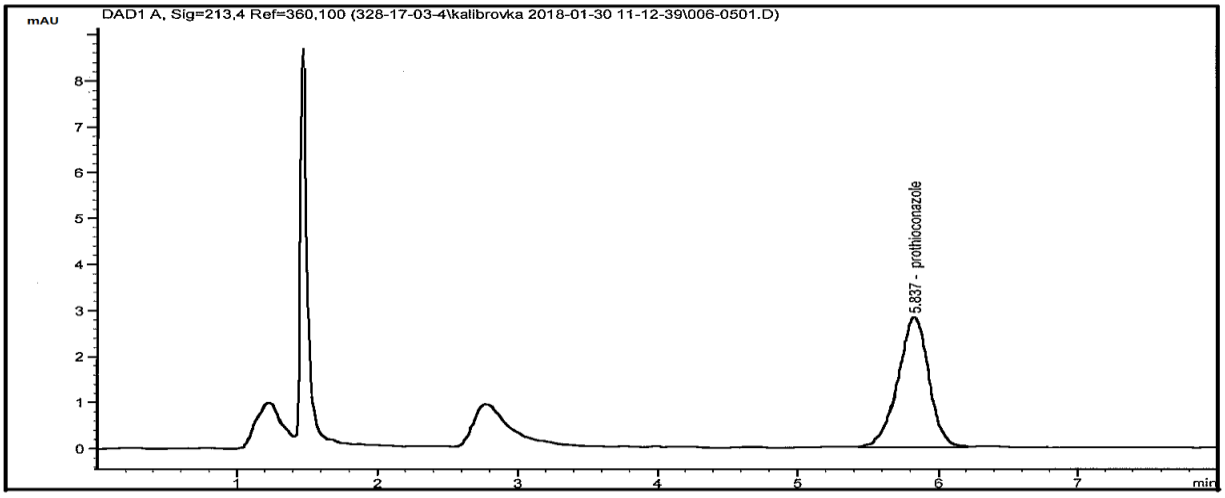

Figure 3. Chromatogram of the calibration solution with the concentration of prothioconazole 0.5 μg/cm^3.

The lower limit of the quantitation in the injected volume of the sample is 1.5 ng. The lower limit of the quantitation in the air is 0.0025 mg/m^3 (the total volume of the sampled air is 80 L). It has been shown that solutions of prothioconazole in acetonitrile with a concentration of 100 μg /ml and 10 μg/ml can be stored in a freezer at a temperature of -18 °C for a month.

The determination of the recovery prothioconazole from the analytical samples was carried out experimentally in 4 levels. To prepare model air samples, to high-density paper filters, the substance was applied over the entire measurement range (0.2–2 μg) using a solution of prothioconazole in acetonitrile at a concentration of 10 μg/ml with varying aliquots. The established range of recovery of prothioconazole from filters is 87.3–111.8% (mean 95.1%). The relative standard deviation of repeatability is 1.77–6.02%. It has been experimentally established that the exposed filters can be stored in the freezer (temperature -15÷-20 °C) for not more than 10 days.

The total error of the procedure for measuring the concentrations of prothioconazole in atmospheric air is 16%.

4. Discussion

The development of a method for measuring the concentrations of prothioconazole in the air of urbanized places included justification of the conditions for sampling, sample preparation and subsequent chromatographic analysis. Sampling of the air was carried out by aspiration through a high-density paper filter. With an increase in the collection time of up to 16 minutes and a volumetric flow rate of up to 5 l/min, no breakthrough of the substance was noted.

Prothioconazole is poorly soluble in water, soluble well in ethyl acetate, acetone, acetonitrile, simplest alcohols, weakly in lower limit hydrocarbons, low photolytic resistance to sunlight, average photochemical half-life is 47.7 hours [10].

Providing the necessary sensitivity of the measurement of prothioconazole in atmospheric air, in addition to increasing the volume of sampled air, required the procedure for concentrating the samples. Concentration of the sample by evaporation using a rotary vacuum evaporator and further dissolving the residue in a small volume of the desired solvent is not acceptable for this substance, which is characterized by low stability, is prone to degradation during sample preparation. A known effective method of extracting a substance from a solution is solid-phase extraction, which combines both the concentration and purification functions of the sample. In the framework of this work, Sep Pak C18 concentrating cartridges are used for sample preparation [11]. Satisfactory extraction of the prothioconazole on the sorbent was achieved by diluting an aliquot of the extract with water in a

volume ratio of 1:9. For the elution, a mixture of acetonitrile-0.05% acetic acid in a volume ratio of 9:1 was used.

Because of the very high lability of the substance being determined (it is shown that when the water samples are stored at a temperature below -18 °C, losses of up to 15% are noted), in the preparation of samples, the stabilization of the prothioconazole by introducing into the aqueous sample a new component, the amino acid L-cysteine $CH_2(SH)$-$CH(NH_2)$-COOH.

The molecular structure of prothioconazole involves the formation of two competitive tautomeric forms: thion ↔ thiol. Figure 4 shows two tautomeric forms of prothioconazole.

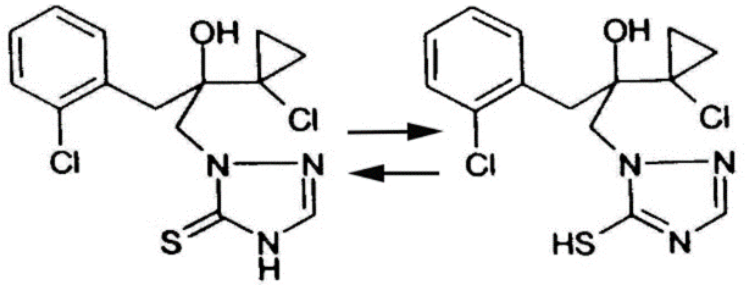

Figure 4. Tautomeric forms of prothioconazole: thion ↔ thiol.

When the equilibrium is shifted to the right, the predominant formation of thiol occurs. Further degradation to -S-CH3 or oxidation to the sulfonic acid -SO3H, followed by cleavage of the group, is accompanied by isolation of the prothioconazole-desthio. To achieve a shift of chemical equilibrium to the left, i.e. in the direction of the predominance of the original form of the active ingredient, the amino acid L-cysteine containing the thiol group -SH (based on 50 mg of the substance per 1 L solution) was introduced into the solution as a stabilizer. The use of cysteine for the chemical stabilization of various compounds and complexes (flavoring additives, pigmentary dyes, enzymes, nanoproducts) has been widely described in the literature [12, 13].

Due to the high photo lability of the prothioconazole, all sample preparation and analysis operations were performed with the exception of direct sunlight.

The created method was used in studies on the evaluation of exposure levels of a substance in the air in conditions of full-scale application of preparations based on proteoconazole under various agricultural applications: spraying of field crops, mechanized work 3 days after treatment, seed dressing (cereals, soybeans, corn) and planting (potato) material with subsequent or simultaneous planting, treatment of sunflower. The required active ingredient is not identified (less than the lower limit of quantification 0.0025 mg/m^3).

5. Conclusions

The conditions for the determination of prothioconazole in the air of urbanized places, including sampling for a high-density paper filter, extraction of the substance with acetonitrile, concentration of an aliquot of the extract from the filter, previously diluted with water in a volume ratio of 1:9, on Sep Pak C18 cartridges, are justified. To stabilize the substance in an aqueous solution, the amino acid L-cysteine was used. The developed method provides a lower limit of quantitative measurement of 0.0025 mg/m^3 (for aspirating 80 L of air), which is 8 times lower than the established value of the approximate safe level of influence of prothioconazole in the air of urbanized places (0.02 mg/m^3). The total error of the procedure for measuring the concentrations of prothioconazole in atmospheric air is not higher 16%.

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