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Chromatographic Determination of Triticonazole in Oat Samples by High Performance Liquid Chromatography-Ultraviolet Detection (HPLC-UV)

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Abstract

The chemical burden on ecosystems has been increased day by day because of the growing activities of agricultural sector. The chemicals that are used to protect plants and humans from different diseases in agricultural lands are known as pesticides. On the other hand, these chemicals can be caused a wide range of side effects on health and environment. The proposed study aimed to determine triticonazole belonging to the fungicide class by high pressure liquid chromatography equipped with ultraviolet detection system (HPLC-UV). Hence, suitable chromatographic conditions were applied to detect triticonazole at a certain retention time. 5 different validation parameters including limit of detection (LOD), limit of quantification (LOQ), percent relative standard deviation (RSD%), linear range (LR) and coefficient of linear regression (R^2) were investigated. A comprehensive validation of the developed method was achieved by all these values. The selectivity of the method for the analyte was ensured by selecting the wavelength of maximum absorption (263 nm), which is peculiar to the analyte. Very low LOD/LOQ values of presented method were recorded as 0.87 and 2.90 µg kg^{-1} under the proposed conditions, respectively. Triticonazole was extracted from oat samples by using acetonitrile and the presence of the triticonazole was investigated by analytical measurements. No detectable analytical signal was obtained for triticonazole in the samples at the retention time interval. The accuracy and applicability of the developed method to the real samples was verified with recovery experiments and this yielded satisfactory percent recoveries. Matrix matching calibration strategy was used to improve quantification accuracy for triticonazole. The results were obtained in the range of 92.9 - 101.4 % for different spiked concentrations of samples within linear range.

1. Introduction

Pesticide is described as different kind of chemicals such as insecticides, herbicides, fungicides, and rodenticides that are used to wipe out targeted pests [1], [2] and they are also useful to avoid or control the occurence of detrimental organisms [3]. They play a significant role in different cultivation steps to protect agricultural areas against unwanted organisms to avoid their negative effects on crops [4]-[6]. Pesticides are used for not only growing crops, but also the protection of crops after harvesting [4], [7]. In addition, the usage of pesticides has also been considerable increased with the increased demand for food materials obtained by agricultural practices [8]. Although pesticides are highly effective against improving the crop yield and quality [9], they can cause side effects on human and environmental health [10]. Pesticide ruins can relocate to several environmental media by wind currents or leaching and be the reason for the pollution of aqua, air and soil [9]. Moreover, the challenges of storage and disposal of pesticides safely have also hazardous effects on environment [11]. Excess usage of pesticides causes pollution of soil and water and prevent the absorption of essential nutrients by plants. The bioaccumulative and high toxic characteristics of agricultural pesticides have also caused to damage the human

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being as they affect the functionality of endocrine and reproductive systems of living organisms [12]. However, the necessity of pesticide usage is known to fight various kind of pests in agricultural activities against some negations such as destroying crops and poor quality of the produced food [13], [14]. In summary, the usage of pesticides in modern agriculture is an important factor, having both benefits and drawbacks for human health and environmental quality [15].

Triazole fungicides are between azole pesticides that are the most widely preferred fungicides for agricultural activities such as growing various kind of fruits, vegetables and grain crops. They are called as endocrine disruptors that are considerable hazardous to the environment and the mankind because of their some chemical and physical properties [13] and they have been considered as the critical monitoring targets of environmental endocrine disruptors in many countries [16]. Exposed to the triazoles is easily occurred by nutrition, breathing and skin contact in daily life as they are used for many times in a growing season that catalyze their accumulation in products and transfer between environmental medias such as air, soil and water [17]. [(RS)-5-(4-chlorobenzylidene)-2,2-di- methyl-1-(1H-1,2,4- triazol-1-ylmethyl) cyclopentanol] is named as triticonazole and this broad-spectrum molecule has been widely preferred for the treatments of foliar and seed. In addition, the functionality of this molecule is based on interfering with biosynthesis and inhibiting steroid demethylation against phytopathogen [18]. Triticonazole hinders the progress of fungi mycelium both of over and inside of seeds and soil in seed applications. According to the literature data, triticonazole have various toxicological impacts on mammals [19]. Triticonazole could create toxicity effects for human health because this compound could also pass through blood brain barrier to deactivate a few cytochrome P450 enzymes (CYP) and cause skin irritation, carcinogenicity, and gentle distruption on endocrine system. Thus, developing a rapid, sensitive and a reliable method is necessary not only to determine triticonazole residues in real matrices, but also to determine this chemical for effective assessment of the risks to living organisms and environments [19], [20].

Selection of the most appropriate analytical determination method is based on physical and chemical characteristics of the related analytes because each of them has different properties of solubility and volatility owing to chemical structure of them [21]. In the literature, several traditional chromatographic separation methods have been used for determination of trace triazole pesticides including gas chromatography (GC) [22], supercritical fluid chromatography (SFC) [23], high pressure liquid chromatography coupled with ultraviolet detection (HPLC- UV) [24] and ultra performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [25]. In addition, many studies for monitoring pesticide residues in different mediums were also performed by various instrumental systems in Türkiye including GC-MS [26] and GC-MS/MS [27], LC-MS/MS [28]. In order for a compound to be determined by HPLC, it should be low-volatile and thermally unstable [29]. Thus, HPLC is applied to polar, thermolabile and nonvolatile compounds easily on the contrary to GC and is highly preferred for pesticide residue analysis. Low resolution obtained on HPLC can be enhanced by coupling selective detection devices to the system for reliable results. Ultraviolet (UV) spectroscopy is commonly opted for the analysis of pesticide residues. Although this technique has low selectivity, it is preferred for monitoring targets owing to low cost, simplicity and wide range of application [30].

Herein, the determination of triticonazole by HPLC-UV in oat samples was planned. High accuracy and precision for triticonazole in related matrix was aimed by the established method presented. The novelty of this work relies in the optimization and validation of an efficient, interference-free, rapid and simple method for the determination of triticonazole that is one of the more heavily used triazole fungicides in agriculture and is predicted to increase usage in the environment. Moreover, low detection limits have been achieved by using a single sample preparation step followed by high pressure liquid chromatography equipped with ultraviolet determination.

2. Material and Method

2.1. Instrumentation and Chromatographic Conditions

HPLC-UV is one of the analytical techniques for detecting the residues of triazole fungicides. Various analytical approaches [31], [32] were presented for the elution of triazoles in the literature. In this study, chromatographic separation and quantification of triticonazole was carried out by Shimadzu LC-20AT HPLC system coupled with UV detector. A Phenomenex-Aqua C18 (250 mm, 4.6 mm, 5 µm) analytical column was used to elute analyte by passing through with the help of a mobile phase that was composed of acetonitrile and ammonium formate buffer (50.0 mM, pH 4.0). Elution was achieved by the isocratic elution mode with a ratio of 70:30 (v/v). The flow rate of mobile phase was 1.2 mL min^{-1}, the injection volume of sample was 20 µL and the

operation wavelength of the UV detection system was 263 nm. The retention time of triticonazole and the total run time were 4.6 and 6.5 min, respectively. pH adjustments were carried out by a Hanna Instruments Edge® Multiparameter– HI2020 pH meter. An OHAUS Pioneer PA214C precision scale (0.10 mg) was used for weighing processes. Sample agitation processes were performed by a HAPA M−100 model ultrasonicator and a Kermanlar mechanical shaker.

2.2. Reagents and Chemicals

High purity standard of triticonazole (131983-72-7) was purchased from Sigma Aldrich (St. Louis, Missouri, USA) and 1000 mg L^{-1} of stock standard solution of triticonazole was prepared by dissolving in acetonitrile (Merck- Darmstadt, Germany). Analytical grade substances such as acetonitrile, formic acid, ammonium hydroxide (25%) were also supplied from Merck (Darmstadt, Germany). Preparation of different concentrations of triticonazole standard solutions was gravimetrically carried out.

2.3. Sample Preparations

Two brand of oat samples were supplied from a supermarket chain, İstanbul, Türkiye. Firstly, the oat samples were pounded in a mortar to pulverize to increase surface area. Then, 2.50 g of each pounded oat samples was weighed on an analytical balance and transferred into a sample tube and completed to final weight of 40 g with acetonitrile. In order to achieve an efficient mixing process, a mixing time of 10 minutes with an ultrasonicator and a mixing time of 15 minutes with a mechanical shaker were applied, respectively. Particulate-free samples were obtained by performing filtration process with a Whatman Qualitative Filter Papers (diameter: 12.5 cm, pore size: 11 µ) and then with a syringe filter (0.45 µm). The prepared samples were spiked within the linear range of the method and analyzed under their related optimum conditions.

3. Results and Discussion

All measurements were made on the basis of triple measurements and standard deviations were calculated to evaluate the precision and repeatability of the presented method. Calibration plot was obtained by using the average of peak areas of each measurement. Recovery percentages of triticonazole were calculated to check the both accuracy/applicability of the presented method.

3.1. Analytical Figures of Merit

Operational and chromatographic conditions mentioned in Section 2.1 were applied and the total run time required for triticonazole elution was completed within 6.5 mins. The signal of triticonazole was acquired within 4.6 min of the total run time. Linear regression analysis was carried out within 3.23-53619 µg kg^{-1}. Calibration plot were developed by the peak areas of the analyte obtained at each concentration value versus increasing concentrations of the analyte. As illustrated in Table 1, an excellent linearity was achieved for the analyte (R^2=1.000) over a wide concentration range. The values used to evaluate the analytical performance of the detection system such as limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of lowest concentration of triticonazole, gives a signal to noise ratio (S/N) ≥3 and ≥10, respectively, as below and given in Table 1:

$$LOD = 3 * SD/Slope \quad (1)$$

$$LOQ = 10 * SD/Slope \quad (2)$$

Table 1. Analytical performance of HPLC-UV system and comparison to other methods in the literature

Method	LOD, µg kg^{-1}	LOQ, µg kg^{-1}	RSD%	Linear Range, µg kg^{-1}	R^2
HPLC-UV	0.87	2.90	12.5	3.23-53619 (y=76.526x+528.8)	1.000
LC-MS/MS [33]	1.54	5.15	-	-	0.999
LC-MS/MS [34]	3.00 (ng g^{-1})	10.00 (ng g^{-1})	4.09	2.50-200 (ng g^{-1})	0.997
DLLME/GC-FID [35]	11 (µg L^{-1})	36 (µg L^{-1})	-	50-80000 (µg L^{-1})	0.993

The standard deviation value (SD) was calculated by measuring the lowest concentration of the calibration plot seven times and was used to obtain the LOD and LOQ values of the system as 0.87 and 2.90 µg kg^{-1}, respectively. The RSD% value, calculated from the results obtained, represents the precision and indicated satisfactory repeatability.

Figure 1 shows overlay chromatograms obtained for triticonazole in the linear range under the optimum conditions.

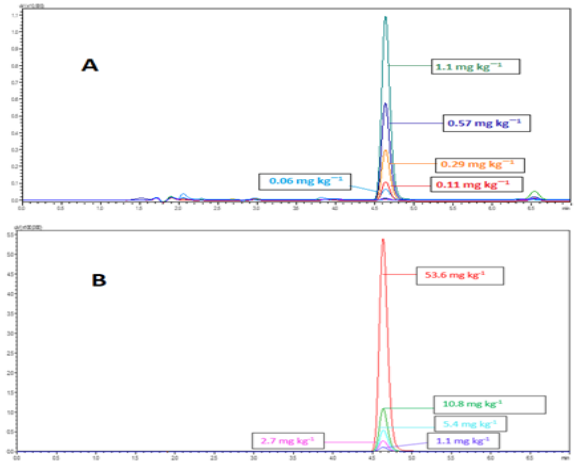

Figure 1. Overlay chromatograms of triticonazole in the linear range under the optimum conditions: A) The linear range of 3.23 µg kg^{-1}-1.1 mg kg^{-1}. B) The linear range of 1.1-53.6 mg kg^{-1}.

3.2. Real Sample Application

In order to ensure accuracy and applicability of the presented method, real sample application was carried out by two different oat samples. Matrix matching strategy was preferred to minimize effect of matrix interferences caused by matrix components. The method mentioned in Section 2.3 were applied to both of the oat samples belonging to different brands. The samples were prepared by spiking different concentrations of triticonazole within the linear range into the sample matrix prior to HPLC-UV detection. Measurements of unspiked sample solutions were also performed and no detectable analytical signal was observed for triticonazole in the samples at the retention time interval.

The percentage recovery results obtained from the spiked samples are given in Table 2. The results demonstrated the capability of the presented method for accurate quantification of trace levels of triticonazole in different oat samples and were found in the range of 92.9 and 101.4%. Overlay chromatograms of 1.0 mg kg^{-1} triticonazole standard solution and two different oat samples at spiked concentration of 1.0 mg kg^{-1} are demonstrated in Figure 2. As shown in Figure 2, matrix effect can overestimate or underestimate the results. Since there was no signal for the unspiked analysis, the high peak height/area recorded for the sample suggested overestimation or false positive for the analyte. It was therefore imperative to use matrix matching to mitigate the matrix effects and obtain accurate quantification results for the analyte.

Table 2. Percent recoveries of triticonazole in real samples

Samples	Spiked concentration, µg kg^{-1}	Recovery%
Oat-Brand A	58	95.1 ± 0.6
	110	96.9 ± 0.1
	275	101.4 ± 0.5
	549	101.0 ± 0.2
	1121	99.5 ± 0.3
	2826	100.3 ± 0.1
Oat-Brand B	54	92.9 ± 0.3
	110	96.3 ± 0.5
	268	99.7 ± 0.4
	552	100.5 ± 0.3
	1075	100.7 ± 0.2
	2895	100.2 ± 0.6

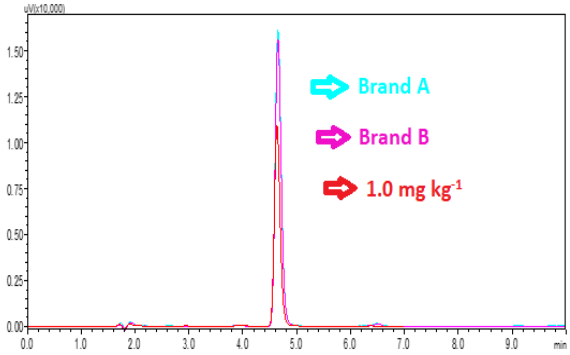

Figure 2. Overlay chromatograms of 1.0 mg kg^{-1} triticonazole standard solution and two different oat samples at spiked concentration of 1.0 mg kg^{-1}.

4. Conclusion and Suggestions

The proposed study has been performed to develop a simple and cheap determination method for the sensitive determination of triticonazole by HPLC-UV and to confirm the accuracy/applicability of the method by recovery studies. Very low detection/quantification limits (0.87 and 2.90 µg kg^{-1}, respectively) and quite wide linear range (3.23-53619 µg kg^{-1}) have been achieved for the analyte. Two different oat samples from different brands were used for real sample applications and satisfying recovery percentages (92.9 - 101.4%) for different spiked concentrations within linear range of the developed

method were obtained. The determination of triticonazole was carried out in a short analysis time period. As a result, the presented method was ensured various remarkable profits such speediness and easiness to detect triticonazole in oat samples.

Statement of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of BEU Fen Bilimleri Dergisi in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that BEU Fen Bilimleri Dergisi and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than BEU Fen Bilimleri Dergisi.

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