RESEARCH ARTICLE

DETERMINATION OF AZOXYSTROBIN FUNGICIDE RESIDUES IN DIFFERENT AQUATIC TOX MEDIUM FOLLOWED BY HPLC – UV METHOD


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ABSTRACT

A simple, and sensitive high performance liquid chromatography (HPLC) was developed for the determination of azoxystrobin residues in different aquatic tox mediums. The tox mediums were those which provide nutrients and help the growth of different aquatic organisms for their survival and multiplication. The constituent of different mediums includes blended water for fish, M4 Medium for Daphnia magna, and OECD TG 201 medium for Algae. The method was validated using in aquatic tox samples spiked with azoxystrobin at different concentration levels (0.05 and 0.5 mg/L). Average recoveries (using each concentration six replicates) ranged 91-99%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.05-5.0 mg/L and limit of detection (LOD) and limit of quantification (LOQ) were 0.02 mg/L and 0.05 mg/L respectively. The proposed method can be applied successfully for the determination of azoxystrobin residues in different aquatic solutions.

INTRODUCTION

The chemical compounds are used to control pests and diseases in agriculture crop protection, they are intended to eradicate weeds, to kill pests and to control vectors of dangerous diseases of man and animals are called pesticides. In the modern agriculture practices use of agro chemicals became an integral part for good yield to meet the demands of growing population. The major question is the extent of their judicial use. Fungicides are a group of chemicals which are used primarily to control spoilage of crops through fungal attack. Fungicides can be divided into protectant and specific types. Protectants are the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the germination of fungal spores. Specific type fungicides are so called because they act on one specific chemical reaction in the fungus. Strobilurin fungicides are one of the Specific type fungicides (De Fraine et al., 2002). Their invention was inspired by a group of fungicidically active natural products. The outstanding benefits they deliver are currently being utilized in a wide range of crops throughout the world. First launched in 1996, strobilurins (now include the world's biggest selling fungicide, azoxystrobin. By 2002 there will be six strobilurin active ingredients commercially available for agricultural use (European Commission (EC), SEC 2007). Azoxystrobin is broad spectrum fungicide of the class of synthetic compounds called β- methoxyacrylates. These chemicals are derived from naturally occurring strobilurins, highly effective phytotoxic compounds produced by species of mushrooms found naturally in Czech forest(Hayes W. J. 1991). The mode of action of azoxystrobin is to prevent the respiration of fungicide to the disruption of electron transport chain, preventing ATP synthesis, azoxystrobin has the broadest spectrum of any antifungal treatment, and it is effective against all four groups of fungi – the Ascomycota, Deuteromycota, Basidiomycota and Oomycota. It is therefore able to prevent or cure powdery mildew, downy mildew, rust and rice blast diseases.(Giza and U. Sztwiertnia (2003). The determination of azoxystrobin was reported in many substrates like fruits, Vegetables, water, soil and crops etc. In this particular study a HPLC-UV method was developed and validated for the determination of azoxystrobin in aquatic toxic medium according to regulatory guideline: SANCO, 1107/2009/EEC. The current study explains about a suitable method for the determination of azoxystrobin in aquatic tox mediums (Fish Medium (1:1.7 Reverse osmosis water and well water), Daphnia (M4 Medium) and Algae medium (OECD – TG201 Medium). So, the present research azoxystrobin which analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV).

EXPERIMENTAL

Standards, Reagents and samples

The analytical standards of Azoxystrobin (99.4%), was obtained from Sigma Aldrich. Acetonitrile was purchased from Rankem, New Delhi. Analytical grade solvent, ortho phosphoric acid, was supplied from Merck Limited, Mumbai.
Standard stock solutions
The fungicide standard stock solutions were individually prepared in acetonitrile at a concentration level 1000 mg/L and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample preparation
The samples were allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Test medium
Test medium is a constitute of different macro nutrients, salts and vitamins. This helps in the survival of different organisms during exposure of different compounds.

Blended water: A mixture of well water and reverse osmosis water in the ratio of 1:1.7 liters. This provides enough nutrients for the survival of fish during test item exposure.

M4 Medium: It is a combination of Trace elements, Marco nutrients and vitamins. The composition was given in Table 1. OECD TG 201 medium: This helps in the growth of green alga as it provides the required nutrients and useful salts which helps in their growth and multiplication (28). The composition was given in Table 2.

20X AAP Medium: This medium with different constitutes of nutrients will help in the growth and survival of alga during test item exposure (29). The composition details were given in Table 2.

Chromatographic separation parameters
The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (PhenomenexLuna-C18) Column temperature was maintained at 30°C. The injected sample volume was 10µL. Mobile Phases A and B was Acetonitrile and 0.1% ortho phosphoric acid (65:35 (v/v)). The flow-rate used was kept at 1.0 mL/min. A detector wavelength was 220 nm. The external standard method of Calibration was used for this analysis.

Method validation
Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 mg/L. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0 , 2.0 and 5.0 mg/L) were prepared by diluting the stock solution. The limit of detection (LOD, mg/L) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, mg/L) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity
Specificity of the method was checked by injecting acetonitrile, 0.1% orthophosphoric acid, standard, and extracts of media control. From the specificity of the method, it was concluded that there was no significant intereference observed to interfere with the analysis of azoxystrobin residues shown in Fig.2. Furthermore, the retention time of Azoxy steward was about 6.4 min.

Linearity
Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0 , 2.0 and 5.0 mg/L) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area.
The limit of detection was determined to be 0.02 mg/L at a level of approximately three times the background of control injection around the retention time of the peak of interest.

**Table 3. Recoveries of the Azoxystrobin tested from fortified different mediums**

<table>
<thead>
<tr>
<th>Fortification Concentration Azoxystrobin in mg/L</th>
<th>Recovery (%)</th>
<th>Blended water</th>
<th>OECD TG 201</th>
<th>M4</th>
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**CONCLUSION**

The mobile phase Acetonitrile and 0.1% ortho phosphoric acid showed good separation and the analysis time required for the chromatographic determination of the azoxystrobin fungicide is very short (around 12 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines. For azoxystrobin fungicide, the sensitivity of the method was good enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of azoxystrobin fungicide residues in different toxin medium samples (meant for fish, Daphnia and Algae).
Acknowledgement

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References


OECD Guideline for testing of chemicals (No.201, Adopted: 23rd March, 2006).

OECD Guideline for testing of chemicals (No.221, Adopted: 03rd March, 2006).

Regulatory Guideline on Plant Protection Products, SANCO, 1107/2009/EEC.


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