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Development of an Analytical Method for the Determination of Cymoxanil in Potato Flour Samples by High Performance Liquid Chromatography

Tuğçe UNUTKAN GÖSTERİŞLİ *[1,2]

Abstract

In this study, a high performance liquid chromatography-ultraviolet detection method was introduced for the detection of cymoxanil. The experimental parameters of the procedure were thoroughly evaluated. Cymoxanil was eluted by ammonium formate buffer (50.0 mM, pH 4.0):acetonitrile (55:45 v/v) as mobile phase. Under the optimal conditions, the limits of detection and quantification (LOD and LOQ) were found as 7.4 – 24.8 µg kg^{-1}, which allows trace determination of pesticides in food samples. Proposed method was then implemented for the determination of selected fungicide in potato flour samples with acceptable % recoveries in the range of 81.4–112.9%, while the values of relative standard deviation (RSD) were below 10% showing a satisfactory applicability for cymoxanil in such complex real samples matrix.

Keywords: Cymoxanil, potato flour, high performance liquid chromatography, fungidice

1. INTRODUCTION

Pesticides are defined as toxic chemical compounds for living organisms because of their environmental persistence and bioaccumulation [1]. Agricultural pesticide residues are one of the critical environmental contaminants that adversely impact the human health and food safety [2, 3]. Among diverse groups of foods, fruits and vegetables are known to contain more pesticide residues that contaminate water, air, and soil with their high toxicity as a result of agricultural activities [1]. Accordingly, pesticides, even at trace concentrations, are harmful to both the environment and human health, including cancer, asthma, diabetes, parkinson's disease, cognitive effects, and unknown diseases [4-6].

Cymoxanil was introduced in 1977 as an agrochemical fungicide to control related pathogens in fruit, vegetables, and plants. In practice, it is mainly used to treat seeds and protect crops from fungus attacks [7, 8]. However, long-term consumption of cymoxanil can have adverse effects on the liver, body immunity and gastrointestinal system and can even cause cancer [9]. The limit in order to reduce risks regarding the high consumption of cymoxanil residues in drinking water was determined to be 0.38 ppm [10]. Taking these into account, it is urgent to develop a sensitive analytical

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method for the determination of cymoxanil residues in food products for public health.

There are various analytical methods for assessing cymoxanil, such as high-performance liquid chromatography (HPLC) [11], liquid chromatography tandem mass spectrometry (LC-MS/MS) [12], gas chromatography (GC) [13], and voltammetry [14]. However, some methods have some limitations, including high costs, complicated sample pretreatments such as derivatization steps for GC, and high requirements for skilled technicians. In addition, HPLC could provide accurate and reliable determination results for cymoxanil [15, 16]. Considering the limitations of GC methods, there has been an increasing demand for immediate, simple, and effective methods to monitor the presence of cymoxanil in food samples.

In this study, a sensitive and suitable method for the determination of cymoxanil was established in potato flour samples. Several parameters affecting chromatographic elution were investigated to obtain the best experimental results.

2. MATERIALS AND METHODS

2.1. Instrumentation and Chromatographic Conditions

A Shimadzu LC-20AT HPLC system, consisted of a delivery pump, SPD-20A UV-Vis detector and a SIL-20A HT autosampler was used in the experiment. A Phenomenex-Aqua C18 column (250× 4.6 mm i.d., 5µm particle size) was used for chromatographic separation at ambient temperature. The isocratic mobile phase consisted of ammonium formate buffer (50.0 mM, pH 4.0) and acetonitrile in the ratio of 55:45 (v/v), at a constant flow rate of 1.2 mL min^{-1} with 30 µL injection volume and UV detection at 254 nm. The pH measurements were performed with a Hanna HI 2211 pH/Orp meter. The overall analysis time was 7.0 min.

2.2. Reagents and Chemicals

The standards for the analyzed fungicide, cymoxanil was supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All substances were analytical grade. Acetonitrile, formic acid, ammonium hydroxide (25%) were supplied from Merck, Germany. Deionized water was supplied from the Elga PureLab Flex 3 Ultrapure, London, UK. Cymoxanil calibration and working solutions were gravimetrically prepared by appropriate dilution of stock solutions with acetonitrile to obtain different concentrations and stored at +4 °C in a refrigerator until use.

2.3. Sample Preparations

Potato flour sample was purchased from a local market in İstanbul, Türkiye. Firstly, each potato flour sample was thoroughly mixed and homogenized.

An aliquot (10 g) of potato flour samples was weighed inside a 50 mL-flask and filled up to a final volume with acetonitrile. The solution was vigorously mixed in an ultrasound bath for 30 min. Acetonitrile extract was passed through a 0.45 µm syringe filter to prevent any blockage that may occur in the analytical column and spiked at 50 µg L^{-1}-5.0 mg L^{-1} within the linear calibration range. Finally, matrix-matched standard solutions were placed into 2.0 mL vials prior to chromatographic analysis.

3. RESULTS AND DISCUSSION

3.1. Chromatographic Separation of Cymoxanil

In this study, primarily instrumental parameters such as column, mobile phase composition, and flow rate were optimized to determine optimum chromatographic conditions and symmetric/sharp peaks.

Several commercially obtained chromatographic columns, including a Zorbax C18 column (250 mm, 4.6 mm, 5 µm), Zorbax C8 column (250 mm, 4.6 mm, 5 µm) and a Phenomenex-Aqua C18 column (250

mm, 4.6 mm, 5 µm) were initially tested to obtain a well-separated signal with good shape. The eluted peak belonging to cymoxanil was more tailed in the first two columns compared to the Phenomenex-Aqua column. The most efficient and satisfactory separation for cymoxanil was achieved with the Phenomenex-Aqua C18 column using an ammonium formate buffer (50.0 mM) adjusted to pH 4.0 with ammonia and acetonitrile in a ratio of 55:45 v/v mobile phase. The most appropriate flow rate for the mobile phase was 1.2 mL min^{-1}. Upon using these parameters, the retention time of cymoxanil was 4.85 min.

3.2. Analytical Figures of Merit

The performance of the developed method, such as the linear range (LR), correlation coefficient (R^2), limit of detection, and quantification (LOD and LOQ) was evaluated under the optimum conditions stated in Table 1. The calibration plot was established by plotting the peak areas against changing concentrations of cymoxanil in the range of 26.1 µg kg^{-1} – 50.1 mg kg^{-1}. The prepared chromatographic method takes 7.0 min with a 4.85 min retention time and exhibits an excellent level of linearity (R^2 = 1.000) in the linear concentration range, good separation (Figure 1) and very low LOD and LOQ values where preconcentration is not required. The LOQ was designated by determining the lowest concentration of cymoxanil that could be measured. The LOD (3xStd Dev/Slope) was 7.4 µg kg^{-1} (mass based) and the LOQ (10xStd Dev/Slope) was 24.8 µg kg^{-1} as shown in Table 2. The repeatability of retention time was also satisfactory.

Table 1 Optimum parameters of HPLC-UV system

Column	Phenomenex-Aqua C18 (250 mm, 4.6 mm, 5 µm)
Mobile Phase	Ammonium formate buffer (50.0 mM, pH 4.0) and acetonitrile (55:45, v/v)
Flow Rate	1.2 mL min^{-1}
Injection Volume	30 µL
Wavelength	254 nm

Table 2 Analytical performance values for HPLC-UV system

Method	HPLC-UV
LOD, µg kg^{-1}	7.4
LOQ, µg kg^{-1}	24.8
LR	26.1 µg kg^{-1} – 50.1 mg kg^{-1}
R^2	1.0000

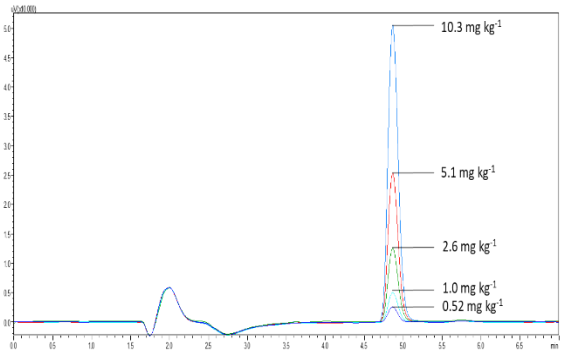

Figure 1 Overlay chromatograms of 0.52, 1.0, 2.6, 5.1 and 10.3 mg kg^{-1} standard solutions of cymoxanil

3.3. Recovery Studies

Two brands of potato flour were used to evaluate the performance of the method with real samples. Blank analysis indicated that they were free of the target analyte or didn't contain detectable amounts of the analyte. The method accuracy was checked with two spiked potato flour samples in the linear concentration range of 0.10-2.50 mg kg^{-1} with the results shown in Table 3. The matrix matching strategy was used to quantify cymoxanil in the spiked samples. Although the analyte had a different retention time in

the potato flour samples because of the diversity and complexity of the food samples matrix than the standard, both samples represented similar chromatographic behavior with each concentration point.

Matrix matching calibration, where all standards were prepared in the extract of another potato flour sample, was used. Hence, no problem was observed. There was no chromatographic interference from the matrix on the cymoxanil signal. Recovery values using the developed method were in the range of 81.4–112.9%. Chromatograms of 2.50 mg kg^{-1} potato flour samples are shown in Figure 2.

Table 3 Percent recovery results in the HPLC-UV system

Samples	Spiked concentration, mg L^{-1}	Recovery% ± Std Dev, %
Potato Flour-Brand A	0.10	81.4 ± 2.7
	0.20	98.9 ± 6.5
	0.50	93.1 ± 1.8
	1.00	96.5 ± 1.8
	2.50	96.9 ± 0.5
Potato Flour-Brand B	0.10	98.7 ± 1.9
	0.20	112.9 ± 2.5
	0.50	104.9 ± 3.6
	1.00	102.7 ± 0.9
	2.50	104.4 ± 0.3

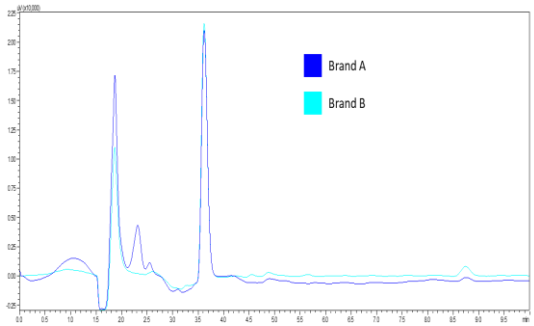

Figure 2 Overlay chromatograms of 2.5 mg kg^{-1} standard solutions of spiked potato flour samples

4. CONCLUSION

The present work has been undertaken to develop a suitable and sensitive method for the quantification of cymoxanil in potato flour samples. To achieve the best performance of the method, the effects of various experimental parameters were investigated. The mobile phase composition was optimized focusing on the efficient chromatographic separation and short analysis time period. The retention time value of cymoxanil was 4.85 min with a 7.0 min total run time. Therefore, the developed HPLC method was successfully performed to potato flour samples for recovery studies. The developed method showed remarkable features involving wide linear range, low LOD/LOQ values, and acceptable precision and satisfactory recovery results. As a result, the proposed method is rapid, and easy applicable to analyze cymoxanil in food samples.

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Authors' Contribution
The author contributed solely to the study.

The Declaration of Conflict of Interest/ Common Interest
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The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science 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

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