

Original Research Article

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## Determination of Fosetyl Aluminium Fungicide Residues in Garlic using HPLC Method

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### ABSTRACT

#### Keywords

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A simple and inexpensive method was developed using liquid - liquid extraction, together with high performance chromatographic method for determination of fosetyl aluminium residues in garlic. The evaluated parameters include the extracts by distilled water and methanol solvents. The method was validated using garlic samples spiked with fosetyl aluminium at different fortification levels (0.03, 0.1 and 0.3 µg/g). Average recoveries (using each concentration three replicates) ranged 88-97 %, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.03-2.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01µg/g and 0.03µg/g respectively. Finally the garlic residue samples were analyzed by HPLC.

### Introduction

Fosetyl aluminum is a fungicide used worldwide to control diseases caused by members of the Peronosporales, especially root and crown rots caused by various Phytophthora species and foliar diseases caused by some downy mildews (Barrett S.R *et al.*, 2003). Although the mode of action of fosetyl-Al still remains controversial, it is probable that its breakdown product, the phosphonate anion, produces a direct antifungal effect on disease control, inhibiting growth and sporulation of a pathogen such as Phytophthora (Giordano *et al.*, 1995).

Fosetyl-Al is available in commercial formulations as wet powders (WP) and wet dispersible granules (WG). It is commonly found in combination with other pesticides such as mancozeb, cymoxanil, and folpet. Chemically, fosetyl-Al is the aluminium salt of the phosphonic acid monoethyl ester. According to IUPAC rules, the term "phosphonate" denotes the ionic form of the phosphonic acid and, for that reason; it is also used for denoting its salts and esters (Hernández *et al.*, 2003). Fosetyl- aluminum, is a molecule which has a low molecular

mass, ionic structure, and lacks UV absorption or fluorescence (Hooijschuur *et al.*, 2001). For these reasons, it is rather difficult to determine fosetyl- aluminum by conventional (reversed-phase) liquid chromatography, hence it has traditionally been separated by high performance ion chromatography (Moller *et al.*, 2003), gas chromatography after derivatisation, (Ouimette *et al.*, 1989) or micro column liquid chromatography (Pelegri *et al.*, 1993) and measured by using conductivity detection or flame photometric detection in environmental samples and food.

## Materials and Methods

### Standards, reagents and samples

Instrument :

The analytical standard of fosetyl aluminum (98.8%) was obtained from Sigma Aldrich. HPLC grade methanol and water was purchased from rankem and garlic was purchased from local market.

### Standard stock solutions

Mobile phase B :

The fosetyl aluminum stock solutions was individually prepared in acetonitrile at a concentration level 500 µg/g 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

Representative 100.0 gram portions of garlic fortified with 0.1 mL of working standard stock solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

### Extraction procedure of garlic

The homogenized sample (10 g) was taken in a centrifuge tube and to it 30 mL water was added. The mixture was vortexed for 30 s and then placed in a microwave for 1 min. The sample was allowed to cool to room temperature and then centrifuged for 5 minutes at 5000 rpm. 2 mL of this supernatant was transferred into an Eppendorf tube and centrifuged at 10000 rpm for 5 min. The clear supernatant was filtered through 0.2 µm membrane filter and subsequently injected to HPLC for estimation of the residues

## Instrumentation

### HPLC-PDA Separation parameters

Shimadzu 2010 HPLC system connected with LC Solution software system.

Detector : Photodiode Array (PDA)

Column used : PhenomenexLuna-C18 (150 mm x 4.6 mm x 5µ)

Mobile Phase A: 50% of 5 mm ammonium formate in Methanol

Mobile Phase B 50% of 5 mm ammonium formate in Milli Q Water

Flow : 0.8 mL per minute

Injection volume : 20 µL

Oven Temperature: 30°C

Retention time in minutes

(Approximately) : 4.5 minutes

### 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 (Tentu Nageswara Rao *et al.*, 2015; Nageswara Rao *et al.*, 2012). The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.01 and 0.1 µg/g. Linearity was determined by different known

concentrations (0.03, 0.1, 0.3, 0.6, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD µg/g) 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 µg/g) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

## **Results and Discussion**

### **Specificity**

Aliquots of fosetyl aluminum, control sample solution, extracted solvents and mobile phase solvents were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (Figure 1 and 2). Furthermore, the retention time of fosetyl aluminum was 4.5 min (Approximately).

### **Linearity**

50.61 mg of fosetyl aluminum reference standard was taken into 50 mL volumetric flask and dissolved in HPLC water, sonicated and made upto the mark with the same solvent. The concentration of the stock solution was 1000 µg/mL. From this stock solution prepared by different known concentrations of standard solutions (0.03, 0.1, 0.3, 0.6, 1.0 and 2.0 µg/mL) were prepared into different 10 mL volumetric flasks and made upto the mark with acetone. The serial dilution details were presented in table 1. These standard solutions were directly injected into a HPLC. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The calibration details were given in table 2. The peak areas obtained from

different concentrations of standards were used to calculate linear regression equation. This was  $Y=7497.65 X + 3.58$  with correlation coefficient of 1.0000 respectively. A calibration curve showed in (Figure 3).

### **Accuracy and precision**

Recovery studies were carried out at 0.03, 0.1 and 0.3 µg/g fortification levels for fosetyl aluminum in garlic. The recovery data and relative standard deviation values obtained by this method are summarized in table 2.

### **Detection and quantification limits**

The limit of quantification was determined to be 0.03 µg/g. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (88-97%, RSD<2%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram (SANCO Guidelines, 2009). The limit of detection was determined to be 0.03 µg/g at a level of approximately three times the background of control injection around the retention time of the peak of interest.

### **Storage stability**

A storage stability study was conducted at refrigerator condition ( $5 \pm 3^\circ\text{C}$ ) and Ambient temperature ( $25 \pm 5^\circ\text{C}$ ) of 0.6 µg/g level fortified fruit samples were stored for a period of 30 days at this temperature and analysed for the content of fosetyl aluminum before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 3% for fosetyl aluminum showing no significant loss of residues on storage. The results are presented in table 3 and 4.

**Table.1** Serial dilutions of linearity standard solutions

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
1000	0.100	10	10.0
10	2.000	10	2.0
10	1.00	10	1.0
10	0.6	10	0.6
10	0.3	10	0.3
10	0.1	10	0.1
1.0	0.3	10	0.03

**Table.2** Recoveries of the fosetyl aluminum from fortified garlic control sample (n=3)

Fortified Concentration (mg/kg)	Replication	Recovery (%)
		Garlic
0.03	R1	87.23
	R2	88.11
	R3	88.74
	<b>Mean ± S.d.</b>	<b>88.03 ± 0.76</b>
0.1	R1	92.54
	R2	91.84
	R3	93.65
	<b>Mean ± S.d.</b>	<b>92.68 ± 0.91</b>
0.3	R1	96.86
	R2	97.64
	R3	96.57
	<b>Mean ± S.d.</b>	<b>97.02 ± 0.55</b>

These numbers were calculated from four (3) replicate analyses of given sample (fosetyl aluminum) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

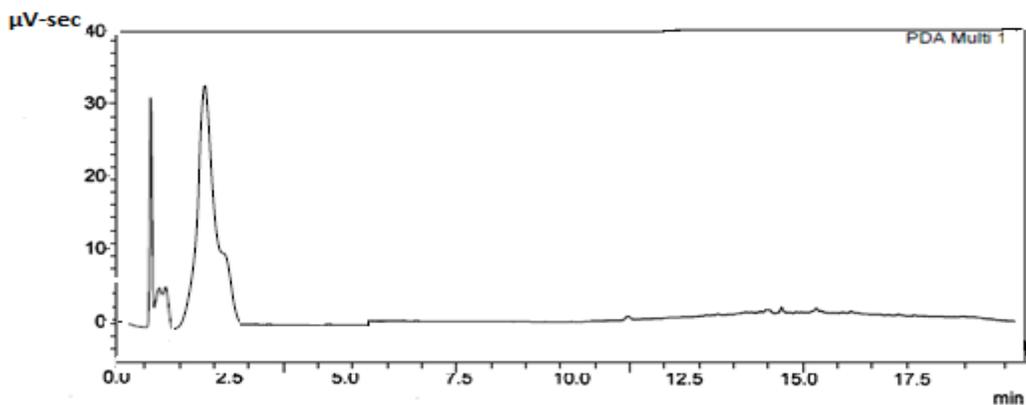
**Table.3** Storage stability details at refrigerator condition ( $5 \pm 3^{\circ}\text{C}$ )

Fortification Concentration in $\mu\text{g/g}$	Storage Period in Days	Recovery in %
0.3	0	98
		96
		95
		97
		98
		97
	Average	<b>96.83</b>
		<b>1.17</b>
		<b>1.21</b>
	30	95
		96
		94
		95
		93
		94
Average	<b>94.50</b>	
	<b>1.05</b>	
	<b>1.11</b>	

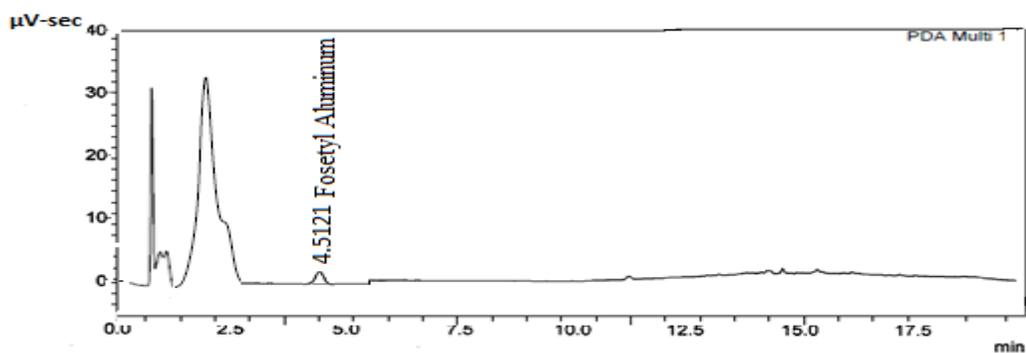
**Table.4** Storage stability details at ambient temperature ( $25 \pm 2^{\circ}\text{C}$ )

Fortification Concentration in $\mu\text{g/g}$	Storage Period in Days	Recovery in %
0.3	0	96
		95
		93
		95
		95
		94
	Average	94.67
		1.03
		1.09
	30	92
		93
		90
		91
		93
		92
Average	91.83	
	1.17	
	1.27	

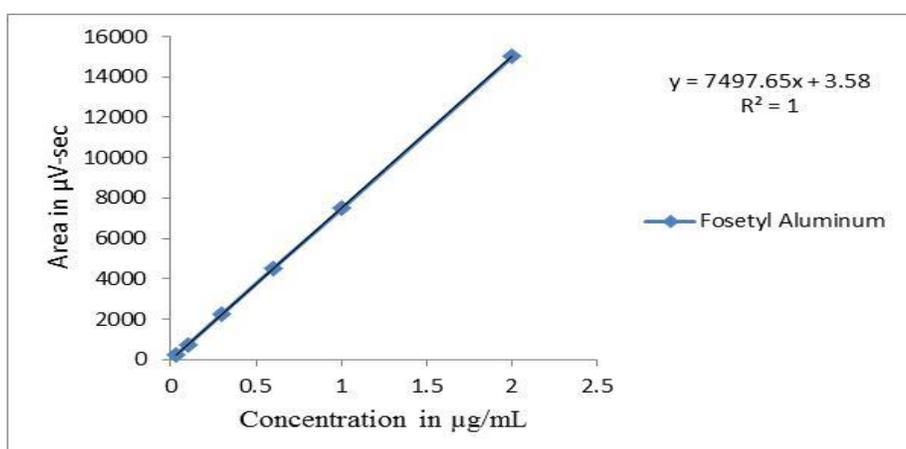
**Fig.1** Representative chromatogram at garlic control



**Fig.2** Representative chromatogram at fortification level of 0.01 µg/g



**Fig.3** Representative calibration curve of fosetyl aluminum



## Calculations

The concentration of acetaminophen in the samples analyzed by HPLC was determined directly from the standard curve.

$$Y = mx + c$$

Where,

Y = peak area of standard ( $\mu V \cdot \text{sec}$ )  
m = the slope of the line from the calibration curve  
x = concentration of injected sample (mg/L)  
c = 'y' intercept of the calibration curve

The recovered concentration or Dose concentration was calculated by using the formula:

$$\frac{\text{Recovered concentration or Dose concentration}}{\text{concentration}} = \frac{(x-c) \times D \times 100}{m \times P}$$

Where,

m = the slope of the line from the calibration curve  
x = sample area of injected sample ( $\mu V \cdot \text{sec}$ )  
c = 'y' intercept of the calibration curve  
D = Dilution Factor  
P = Purity of Test item

$$\% \text{ recovery} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

In conclusion, this paper describes a fast, simple sensitive analytical method based on HPLC-PDA to determine the fosetyl aluminum residues in garlic. The LLE extraction procedure is very simple and inexpensive method for determination of fosetyl aluminum residues in garlic. Satisfactory validation parameters such as

linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO) guidelines. Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the fosetyl aluminum residues in different commodities (fruit, juice, seed, oil, and water and soil samples).

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