

Research Method for Determining Ethephon Residues in Fruit (Bananas) on a High-Pressure Liquid Chromatograph with MS/MS Detector—LC/MS/MS When Using Ethephon as a Ripening Agent

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Abstract

Ethephon not only has the effect in the stimulation of defoliation, but also makes fruits ripen quickly and evenly. Ethephon pushes hard the process of fruit ripening from 1-2 days instead of 4-6 days as before. However, the residues of Ethephon in the fruits will cause health problems such as damage to the liver, kidneys. The method used to determine Ethephon residues is high pressure liquid Chromatography Mass Spectrometry (LC-MS/MS). The limit of detection (LOD) Etherphon is 0.92 mg /kg and Limit of quantitation is LOQ = 2.7mg/Kg. The linear range curve is from 0.96ppm to 96ppm with $R^2 = 0.999$ repeatability. The study subjects are green bananas soaked in Ethephon solution with concentrations: 4.8%, 9.6%, and 24%. Soaking time was from 1 day, 2 days, 3 days, and 4 days. Results obtained after 4 days of experiments showed that Ethephon content in banana increased proportionally with the concentration of Ethephon in the solution. The highest increase was on day 1, measured at 90.5 ppm at soaked concentration 24%, and 42.2 ppm at 9.6% and finally is 21.9ppm at soaked concentration of Ethephon 4.8%. Till the second, third, and fourth day, Ethephon content in banana started to decrease. Ethephon content in bananas of the fourth day is 20ppm at soaked concentration 24%, 9.6ppm at soaked concentration 9.6% and 3.3 ppm at soaked concentration 4.8% of Ethephon solution tank. However, after 4 days, bananas soaked in Ethephon solution had blackened and become inedible in comparison with bananas soaked in the Etherphon solution only within 1 or 2 days, the banana still remained beautiful and edible.

Keywords

Ethephon, LC/MS/MS, Banana Analysis, Tanks

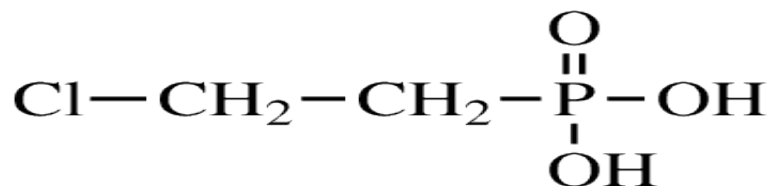
1. Introduction

Ethephon is the chemical used for plant growth regulation, and it belongs to the phosphonate group. Ethephon is used to stimulate the defoliation and releases Ethylene ($\text{CH}_2 - \text{CH}_2$) which helps fruits ripen more evenly and faster, also shortens the ripening time in chambers, and reduces post-harvest losses.

Ethylene is a plant's endogenous hormone produced by itself during the ripening process of fruits. Ethylene generated from Ethephon is called Exogenous Ethylene, so the process of soaking fruits in Ethephon solution or extracting di-

rectly into fruits will include endogenous Ethylene and exogenous Ethylene, which make fruits ripen fast, but also make them become rotten more quickly [1] [2].

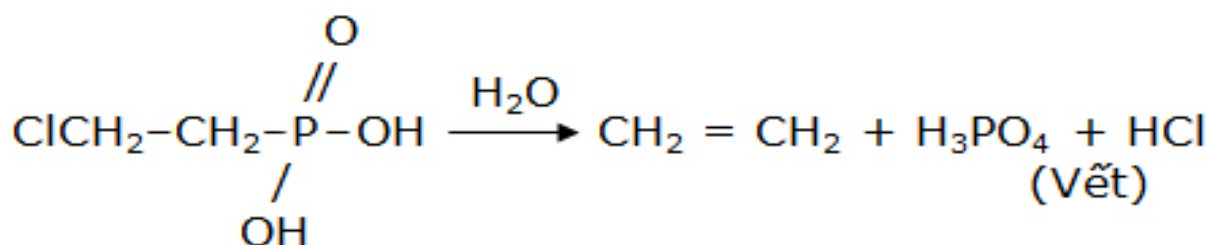
Chemical formula of Ethephon with molecular mass is 144.5



Ethephon (also known as: Bromeflor, Arvest, Ethrel...) is a synthetic substance called 2-Chlorethylene phosphoric acid in the form of liquid with colors ranging from colorless to light amber. It remains stable in acid form and destroyed at pH greater than 3.5.

Ethephon is easily soluble in water, less toxic to humans and animals. Toxicity test on rats from the gastrointestinal tract showed that: LD50 = 7.0 mg/kg. Ethephon is not harmful to bees, less toxic to fish.

Ethylene released from Ethephon by the following mechanism:



In which ethylene is a gas, plants themselves produce ethylene during their development process ($\text{CH}_2 = \text{CH}_2$) which is called endogenous ethylene. Ethylene is the main agent that promotes the ripening of fruits. Ethylene produced during the ripening process will be called exogenous. The residues of Ethephon in fruits are unhealthy when consumers eat these produces with Ehephon.

2. Material and Methods of Analysis

Equipment used for Ethephon analysis: Ultra high-performance liquid.

chromatography-tandem mass spectrometry: LC/MS/MS name ENDURA 2018. For validation method. We have to do as the following: Determined LOD, LOQ, recovery,

$$R\% = \frac{C_{m+c} - C_m}{C_c} \times 100$$

R%: Recovery, %

C_{m+c} : Concentration sample + Concentration of standard added

C_m : Concentration sample which measured on LC/MS/MS

C_c : Concentration of standard added

- **Conditions of running LC/MS/MS machine:** [3] [12]

- The column used to run Ethephon analysis is a HILIC silica polarized column with a particle size of 5 μm , diameter and length of 2.1mm x 50 mm.

- The mobile phase and solvent gradient mode follow the reverse phase program, polar solvent Acetonitrile and H_2O .

- Define linear graph: From 1,000ppm of Ethephon standards will be prepared with 10 different concentrations from 0.96ppm to 96ppm. Each concentration point of Ethephon will be measured on the LC/MS/MS for 5 times

- Analytical procedures: Reference Quechers method with solvent extraction is MeOH: H_2O : 1:1.

- **Set up an experiment model**

Samples: Green bananas

Tank: 4 tanks

Ethephon solution with the different concentrations as following: 4.8%, 9.6%, 24% and one tank is controlled.

Experiment 1: Bananas were soaked in Ethephon solution 4.8% -A

Experiment 2: Bananas were soaked in Ethephon solution 9.6% -B

Experiment 3: Bananas were soaked in Ethephon solution at 24% -C

Experiment 4: Control banana soaked in without Ethephon 0% -D

After 1 day, 2 days, 3 days, and 4 days bananas were removed from the soaking Tanks for the analysis of ethephon.

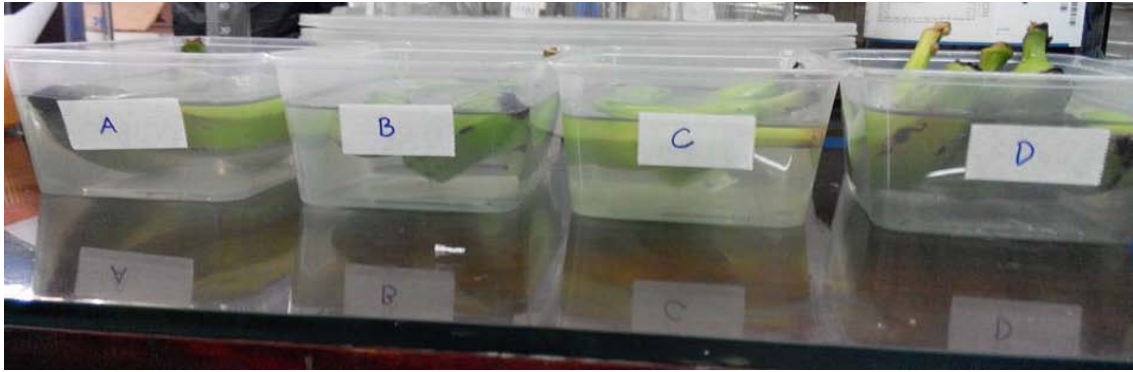


Figure 1. Experiments of Bananas soaked in Etherphon.

3. Results and Discussion

3.1. Evaluation results of analytical methods of Etherphon on the LC/MS/MS

3.1.1. Determine LOD, LOQ when measure Etherphon on the LC/MS/MS

By the method of standard substances addition measured at 5 ppm. Each point of standard will be analyzed 5 times and then will calculate average and standard deviation—SD. Results are shown on Table 1.

Table 1. Results of LOD, LOQ

Sample-SP	Sample: Sp	Etherphon was measured by LC/MS/MS ppm (mg/kg)
spike 5ppm Ethepon in Banana	Sp1	4.42
	Sp2	4.12
	Sp3	4.37
	Sp4	4.67
	Sp5	4.29
Calculating	Mean	4.37
	Sd	0.2
	LOD	Sd*t = 0.2*4.604 = 0.92
	LOQ	3MDL = 3*0.92 = 2.76

Comment: Standard for Ethepon 5ppm was injected in LC/MS/MS with 5 times replicates. Results obtained are: mean is 4.37ppm, Standards deviation is 0.2. According to AOAC **LOD = Sd*t = 0.2*4.604 = 0.92 and LOQ = 3*LOD = 2.76ppm**

3.1.2. Determine Recovery of method –R% [12]

By the method of standard substances addition measured at 5 ppm and 100ppm to the samples. At each point, it will be analyzed 5 times replicates and then will be calculated Recovery. Results on the table 2

Table 2. Recovery of method on the LC/MS/MS

STT	Spike 5ppm of ethepon	Actual concentrations measured ethepon by LC/MS/MS	Recovery (R%)	Repeatability (RSD%)
1	5	4.42	91.3	4.5
2	5	4.37		
3	5	4.12		
4	5	4.29		
5	5	4.67		
6	100	79.2	78.26	4.2
7	100	79.6		
8	100	79.2		
9	100	77.8		
10	100	75.5		

Comment: By method of adding standard to the sample at 2 concentrations: 5ppm and 100ppm. Results obtained are shown in Table 2. From results obtained show that recovery obtained 78.26%-91.3%. These results are well, because according to AOAC permissible and acceptable for trace analysis from 75% to 110%.

3.1.3. Calibration Standard Solutions of Ethephon

Preparation of standards solution for Ethephon from 0.96ppm to 96ppm from 1,000ppm Standard Ethephon and then they were measured on the LC/MS/MS.

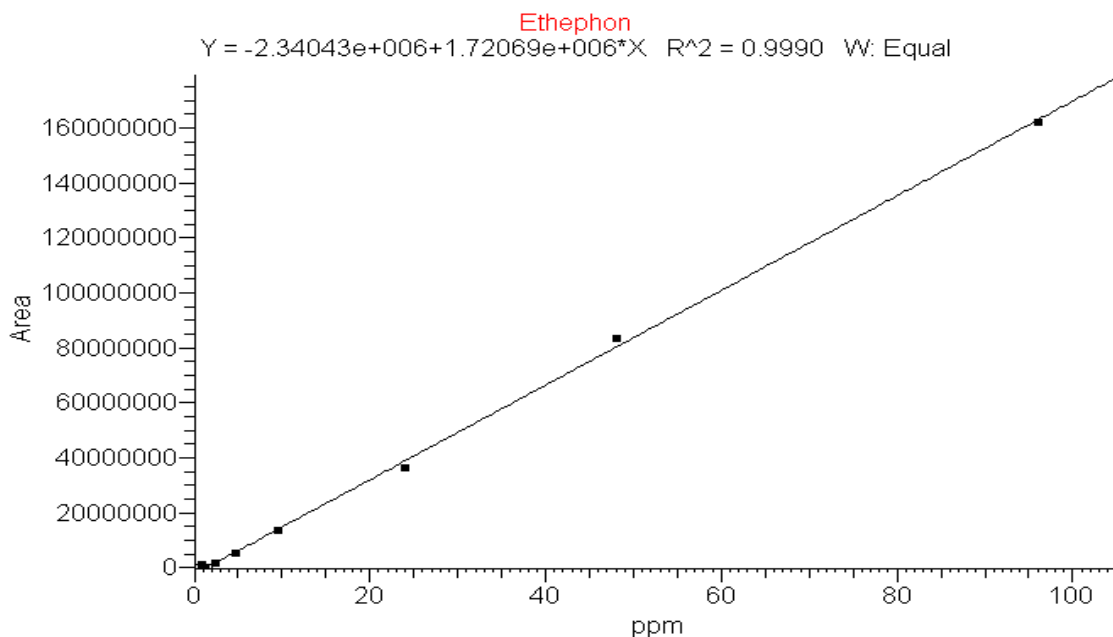


Figure 2. Standard curve of Ethephon.

Comment: From the obtained results on Figure 2, we can see that the concentration ranges from 0.96ppm to 96ppm, the graph has a good linearity with $R^2 = 0.999$ satisfying the requirement of the regression coefficient. Regulation: $0.99 \leq R^2 \leq 1$.

3.2. Experimental Results of the Green Bananas Soaked in Etherphon Solution from First Day, Second Day, the Third and the Fourth Day

Table 3. Residue of Etherphon in Bananas of 0 day, 1 day, 2 day, 3 day and 4 day

Time	Concentration	Etherphon content in bananas ppm (mg/kg)			Control 0% Etherphon
		4.8%	9.6%	24%	
0 ngày		A1	B1	C1	D
	KQ	ND	ND	ND	ND
1 day		A2	B2	C2	D
	Ethephon conten	21.9	42.2	90.5	ND
2 days		A3	B3	C3	D
	Content of Etherphon	8.8	17.6	38.7	ND
3 days		A4	B4	C4	D
	Content of Etherphon	5.8	14	35.4	ND
4 days		A5	B5	C5	D
	Content of Etherphon	3.3	9.6	20	ND

Note: ND—not detected.

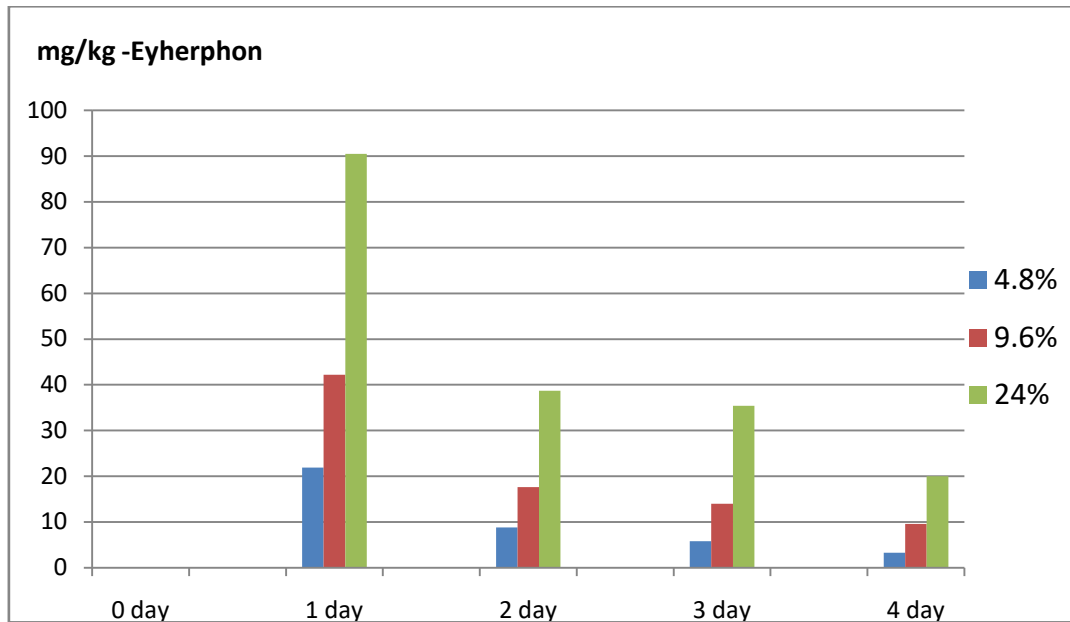


Figure 3. Relationship between Ethephon residue in bannana and time soaked.

Comment:

We have 4 experiments above. Throu 4 days monitoring, we obtained results such as:

Green bababas brought to Labortorie, bananas were removed bark and then measure Etherphon by LC/MS/MS. Obtained results of Etherphon are not detected (Shown on Table 3). Then Bananas were soaked in each tank with different Ethephon concentration: 4.8%, 9.6% and 24%. Time for studying is 4 days. Each day banana took out from each tank for Etherphon analysis.

First day: Obtained results show that Ethephon content in bananas in all tanks were detected. Etherphon content in bananas increased proportionally with the concentration of ethephon in the tank. The highest increase was on day 1 with 90.5 ppm at 24% Ethephon, and 42.2 ppm at 9.6% and finally is 21.9ppm at 4.8% Ethephon. This time bananas green skin turned yellow. Bananas began to ripen (see Table 3).

Second day: Bananas were taken out from each tank for Ethephon analysis. The results obtained show that Ethephon content in bananas decreased proportionally with the concentration of ethephon in the tank (Table 3). During this time bananas had become ripe and edible. Skin of the banana had turned from green to yellow color (see Figure 4).



Figure 4. Bananas after one and two days.

Throughout the 3 days and 4 days, from the obtained results showed that Ethephon content in bananas continued to decrease. To the day 4, residue of Ethephon in bananas was 3.3ppm, 9.6ppm and 20ppm. Ethephon content have decreased approximately 80% compared with Ethephon concentration on the first day and also during this time of experiment, bananas had show some signs of decomposition such as blackened banana peels, crushed bowels and inedible.



Figure 5. Banans after 4 days in Etherphon.

4. Conclusion

Ethephon residue in banana was determined on LC/MS/MS with LOD of 0.92ppm, LOQ of 2.7ppm and Recovery of over 93% [12].

The linear ranges of the method were from the obtained results on Figure 2, we can see that the concentration ranges from 0.96ppm to 96ppm from 1,000ppm, and the corresponding correlation coefficient was 0.9999. Mean recoveries of the analysis at two spike levels (medium, and high) were within the range of 78.26%-91.3% (n = 5). The limit of detection (LOD) is 0.920ppm and limit of quantification LOQ is 2.76ppm

During 4 days, green bananas were soaked in Etherphon solution with concentrations of 4.8%, 9.6% and 24%. For the first day, the Ethephon content in banana increased according to the proportion of lakes with high Ehtephon. Ethephon residues in banana were from 21.9 to 42.2ppm and 90.5ppm. At this point the banana began to ripen and could be eaten (see Figure 3). However, from the 2nd to 4th day, the Ethephon content in banana did not increase, but gradually decreased until the day 4, the Ethephon residue in the remaining banana was 3.3ppm (4.8% Ethephon), 9.6ppm and 20ppm (24% Ethephon). Also at this time, bananas had signs of being rotten. Banana intestines were black, bananas blister... generally could not be eaten. The controlled banana is still green and cannot be eaten. So what we can draw from the 4-day experiment with Ethephon solution is that we can save time by shortening the ripening time of bananas (1-2 days), instead of waiting for 5-6 days for bananas to ripe. However, the Ethephon content in the banana is about 51.53ppm (average of 3 results of Ethephon showed above on first day) with this high Ethephon content like this, people's health will be definitely badly affected. American Fruit Association said that the allowable daily intake of Ethephon is 0.05 mg/kg, if the person weighing 60 kg, content is about 3 mg Ethephon/day [4] [11], but here we exceed 51.53mg/kg, 17.17 times higher than being allowed, of course, this high Ethephon content will be extremely harmful to human health.

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