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DEVELOPMENT OF ANALYTICAL METHOD FOR DETERMINATION OF DITHIOCARBAMATE RESIDUES, EXPRESSED AS CS_2 IN FRUITS AND VEGETABLES BY GC-ECD

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Abstract.This report presents the development of analytical method for determination of dithiocarbamat fungicides (DTCs) residues in vegetables through CS_2 using gas chromatography with electron capture detector (GC-ECD). Research results show that the method recovery ranges from 84.9 – 98.7% and meets the requirement for pesticide residues analyzing. This method has also been applied for analyzing 12 vegetable samples collected in Tan Dinh and Thi Nghe markets. The results showed that the majority of DTCs levels of these samples are lower LOD value except sample BC2 (0.08 μg/g) and sample X3 (0.11 μg/g).

Keywords: Dithiocarbamate, residues, CS_2, fruits and vegetables, GC-ECD.

1. INTRODUCTION

Pesticides or agricultural chemicals are substances that are derived from nature or synthetic, used to protect crops and agricultural products, against the destruction to plant resources of harmful creatures. The main pests include insect pests, diseases, weeds, rats and other factors. Dithiocarbamate are organosulfur containing dithiocarbamic acid, which are the most effective and popular pesticides. Dithiocarbamate compounds with metals such as zinc, iron, manganese have greater effect and lower toxicity level than elemental sulfur [1]. They help prevent the growth of pest population in a short time, contribute to increased crop productivity and protect the quality of harvested products to meet the demand for food that is increasing all over the world, including Vietnam, whose agricultural production was primarily.

However, when pesticides are used improperly (not comply to the isolated time after spraying, increase the amount arbitrarily as well as mix several pesticides together against the instructions) can cause the opposite effects when pests cannot be only stopped but also disrupt the balance of ecosystems and create new pest generations that affect negatively on crop productivity. On the other hand, they can leave a toxic residues in agricultural products that affect the health and life of people and livestock that consume those products. Pesticide residues in agricultural products are now a major food safety issue, which are interested by many people and organizations to determine solutions to reduce the maximum levels of toxic residues.

1634 active ingredients of pesticides with 3902 number of trade names are permitted to use in Vietnam in 2014. Pesticides that are in dithiocarbamate group used in agriculture are mainly fungicides. According to reports from the pesticide industry in 2014, Vietnam imported more than 55,000 tons of technical materials and pesticide products every year. Herbicides accounted for 26%, fungicides accounted for 37%, insecticides accounted for 31% and the rest were some other products. The above data shows that the demand to use pesticides in general and fungicide in particular in Vietnam is great [2].

Currently, in the world, dithiocarmate group has been analyzed using different analytical methods on a variety of analytical equipment such as gas chromatography coupled with mass spectrometry (GC-MS), with electron capture detector (GC-ECD), and with flame photometric detector (GC-FPD), or high performance liquid chromatography coupled with electrochemical detector (HPLC-ECD) [3]. Among them, GC-ECD instrument is relatively common in Vietnamese laboratories due to cheap price and easy to use. Meanwhile, the price of analytical equipment such as GC-MS, GC-MS/MS, HPLC-ECD are expensive and they are not easy to use. Therefore, the development of an analytical method to determine dithiocarbamate group in fruits and vegetables with GC-ECD, a rather popular device in Vietnamese laboratories, is very necessary.

2. MATERIALS AND METHODS.

2.1. Material

Toluen, isooctane, acetone, n-hexane (pest grade Merck, Darmsta dt, Germany), hydrochloric acid fuming (37%) (p.a. Merck), conversion tin(II) chloride 2% in aqueous HCl 5M solution (100 g of tin (II) chloride is dissolved in 5000 ml hydrochloric acid 5M), carbon disulfide (CS_2), purity 99%, density 1,260 mg/ml at 20^{o}C were used as supplied. All reagents must have a purity level of p.a. or pestigrade. Fungicides DTCs such as mancozeb, metiram, propineb, thiram, zineb, ziram are used as agents representatives for DTC group in this research.

2.2. Analytical method

A previously reported $SnCl_2$/HCl acid – hydrolysis method was employed for sample preparation [4]. DTCs are not stable and cannot be extracted and analyzed directly. For this reason, DTCs should be converted into more stable form by reacting with tin chloride in aqueous : HCl (1:1) at 80^{o}C. The CS_2 gas released was absorbed into isooctane and measured by GC-ECD [4,5]. The concentration of CS_2 was calculated based on external calibration curve, with metabolite (CS2) residue of dithiocarbamate are generally expressed under µg CS2/g fruit or vegetable. When analyzing DTCs through metabolite (CS_2), they have low boiling temperature and low saturated vapor pressure. This results in clean-up steps in preparation of sample when analyzing DTCs with GC-ECD is not necessary due to the ECD is not affected by the sample matrices [6].

Operating conditions of GC-ECD are as follow: Agilent 6890 gas chromatograph system with ECD detector, capillary column HP-5 (5%-phenyl 95%-methyl polysiloxane) 30m x 0.25mm x 0.25µm, oven temperature: 50^{o}C, injection port temperature: 120^{o}C, detection port temperature: 150^{o}C, flow rate of carrier gas: 1 ml/min, flow rate of make up gas: 60 ml/min, flow ratio of split 1:20.

3. RESULTS AND DISCUSSIONS.

Instrument detection limit (IDL) is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL should always be below the method detection limit, and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments [7]. IDL of CS_2 on GC-ECD instrument is 0.012 µg/ml and shown in Fig. 1 below.

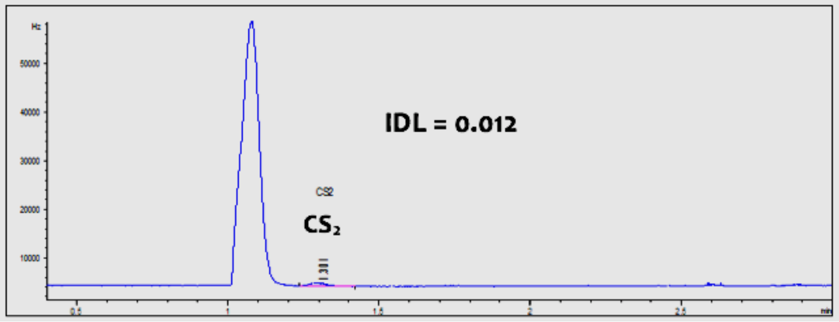

Figure 1. Representative chromatogram for determination of CS_2 IDL

Limit of detection (LOD) is the lowest concentration of a substance that can be distinguished from peak of that substance absence with peak of blank sample noise. The detection limit is calculated from the ratio equal 3 of substance signal to noise. The detection limit is calculated from the ratio equal 3 of substance signal to noise [8].

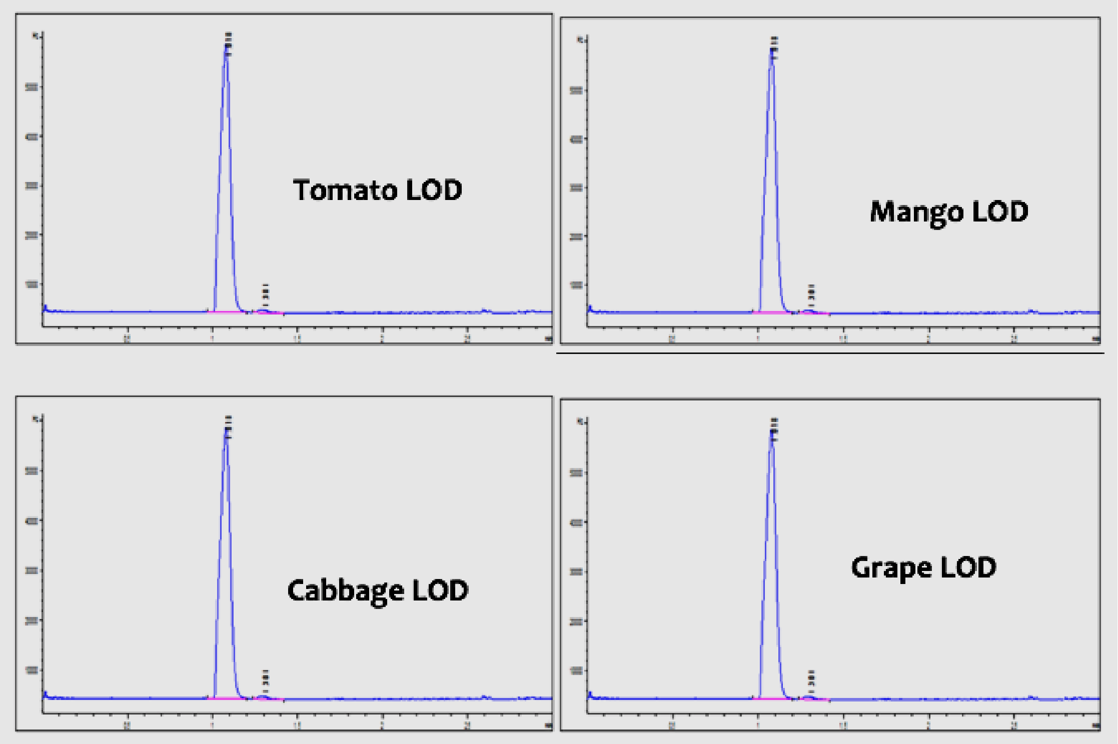

Figure 2. Representative chromatograms for determination of LOD

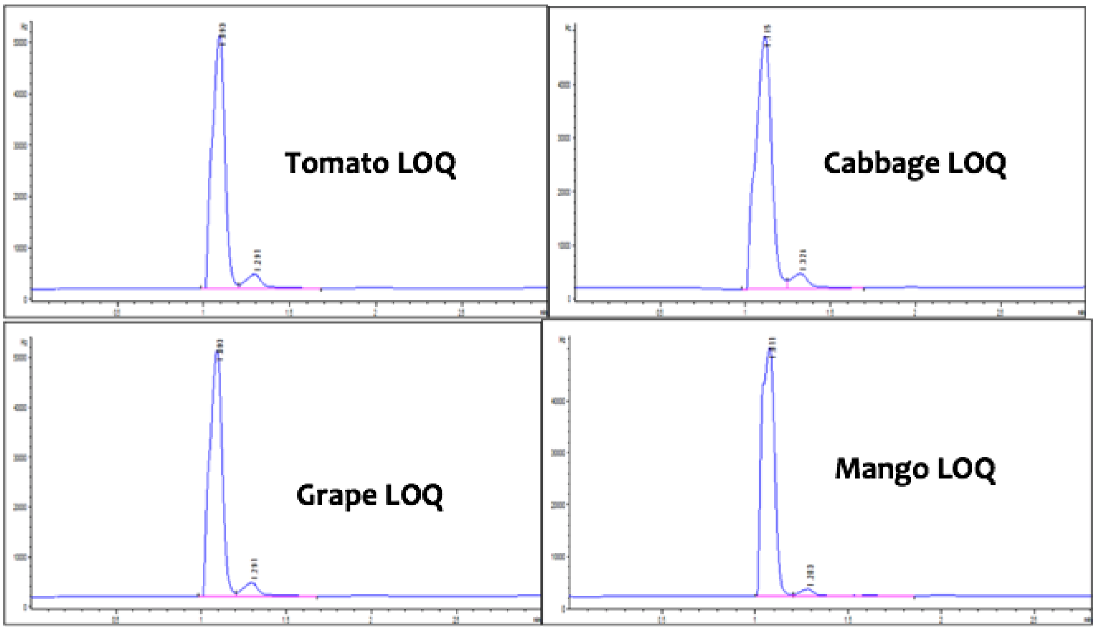

Figure 3. Representative chromatograms for determination of LOQ

LOD and LOQ of the analytical method for tomato, cabbage, grape, and mango matrices are shown in Table 1 below.

Table 1. LOD and LOQ of the analytical method for fruits and vegetables

Sample matrix	**C (µg/ml)**	**Signal/noise**	**LOD (µg/g)**	**LOQ (µg/g)**
Tomato	0.0748	7.908	0.0142	0.0474
Cabbage	0.0748	7.435	0.0151	0.0503
Grape	0.0748	7.205	0.0156	0.0519
Mango	0.0748	7.841	0.0143	0.0480

Calibration standard solutions of carbon disulfide: At least 3 different levels of standard concentration, including lowest point at limit of quantitation (LOQ), medium level and high level are prepared by appropriate dilution of carbon disulfide standard in isooctane solvent. According to Fig. 4, the calibration curve has the value of $R^2 = 0.999$. This result means the range of CS_2 concentrations are very linear each other.

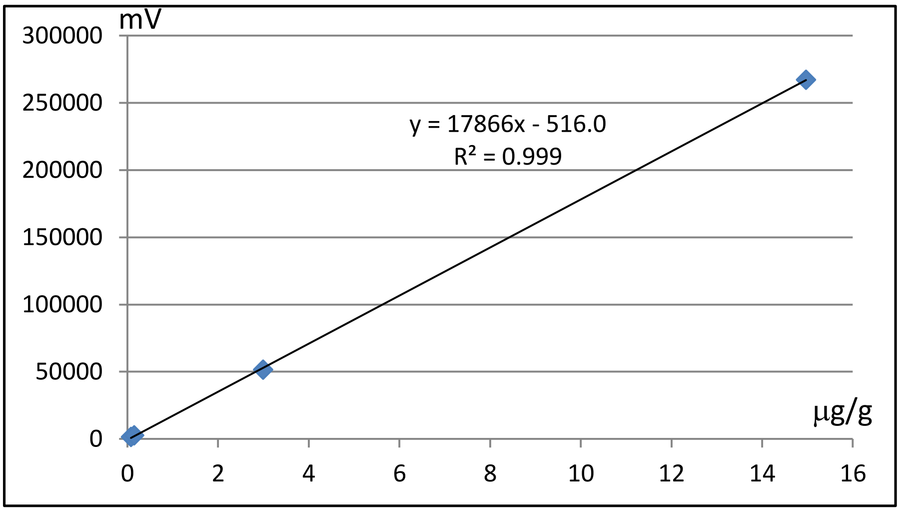

Figure 4. Calibration curve of CS_2

Recovery of the method is the term used to indicate the yield of an analyte in an analytical method. It is calculated by analyzing a sample with known concentrations and comparing the measured value with the true value as spiked into the matrix. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. The expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration [9]. We have analyzed spiked samples (tomato, cabbage, grape, and mango) with the added CS_2 standards of approximately 0.05ppm, 2ppm and 5ppm. Each experimental levels are repeated three times and recoveries for every level are presented in Table 2 below.

Table 2. Repeatability and recovery efficiency of CS_2

Matrix	**$H_{average}$ ± RSD (%)**
Spiking level (mg CS_2/kg) 0.05 ppm	
Tomato	91.3± 2.1
Cabbage	86.6± 4.1
Grape	88.3± 6.5
Mango	84.9± 5.6

Spiking level (mg CS_2/kg) 2 ppm	
Tomato	98.7 ± 4.2
Cabbage	96.8 ± 2.6
Grape	95.6 ± 2.0
Mango	94.5 ± 5.5
Spiking level (mg CS_2/kg) 5 ppm	
Tomato	95.2 ± 6.3
Cabbage	91.2 ± 5.8
Grape	96.9 ± 3.3
Mango	92.7 ± 3.1

Recovery of the method is good, range from 85 – 99% at three levels of 0.05 ppm, 2 ppm, 5ppm on 4 kind of matrices are tomato, cabbage, grape, and mango. The relative standard deviation of the method's repeatability is range from 2 – 6%.

Stability of the method: spiked a standard solution of 2 ppm into cabbage and grapes for 6 weeks to assess the stability of the method over time through a control chart. The stability of the 6-week cabbage method and grape method are shown in Fig. 5 and Fig. 6 below.

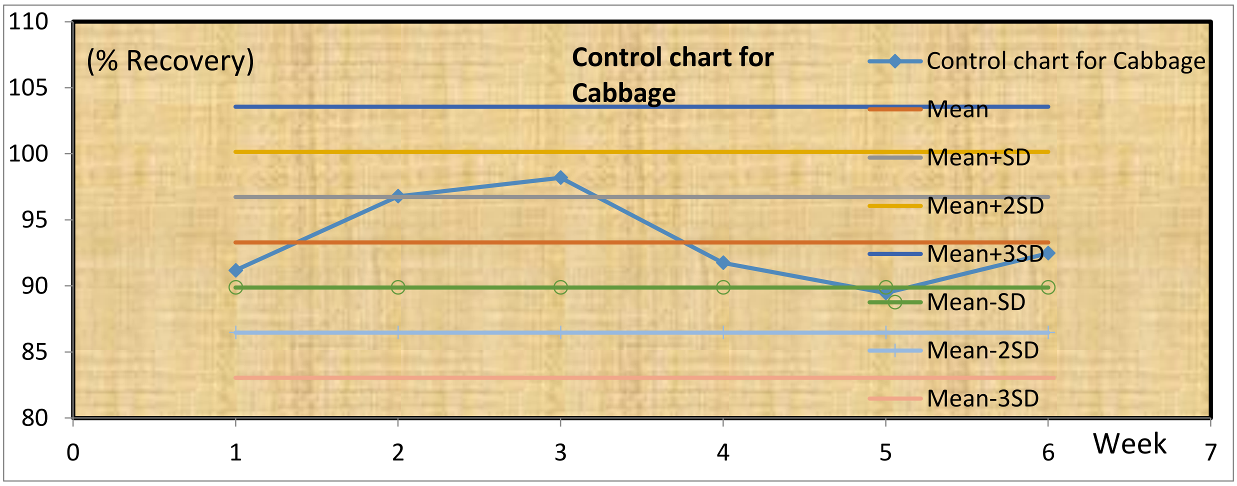

Figure 5. Control chart of the method stability with cabbage matrix in 6 weeks

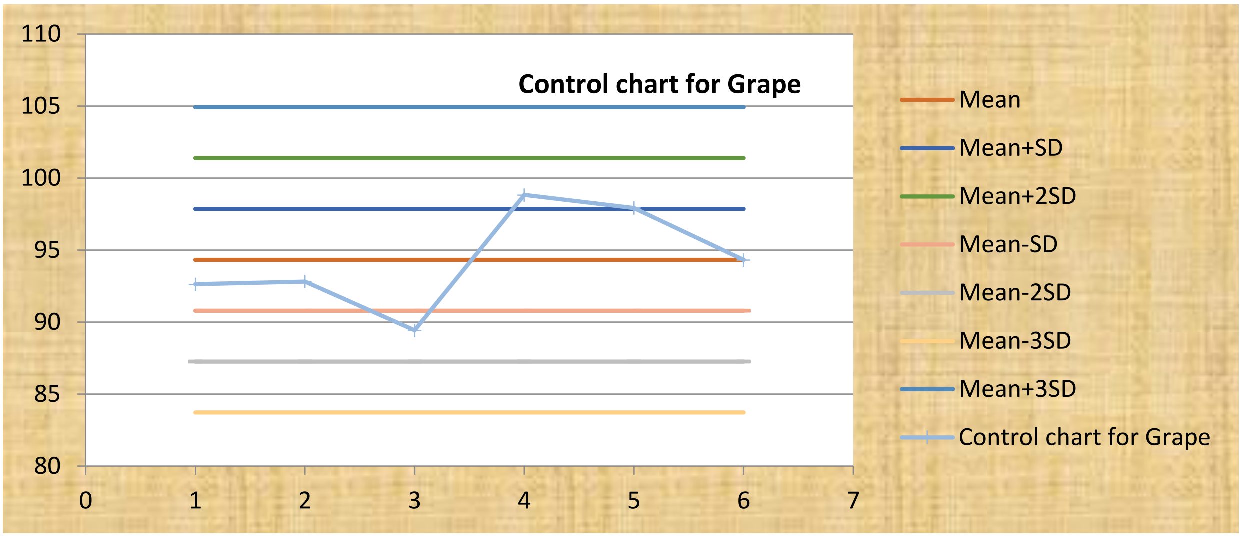

Figure 6. Control chart for the method stability with grape matrix in 6 weeks

Analyzing some real vegetable and fruit samples from the market: The developed analytical method used to analyze some real samples were purchased at Tan Dinh and Thi Nghe markets, including tomatoes, beets, grapes and mangoes. Vegetables and fruits from supermarket are often stored longer than traditional market thanks to better storage facilities. This results in the fact that DTCs content in vegetables and fruits from supermarket is often lower than those in vegetables and fruits from traditional market. Therefore, vegetables and fruits from traditional market were chosen for higher opportunity in finding DTCs. These samples are randomly selected for bias avoidance.

DTCs levels in vegetables and fruits from markets under concentration of CS_2 are presented in Table 3 and Fig. 7 below. These results shown that the DTCs level in the vegetables and fruits from the markets in general, were under maximum residue levels (MRL) of Ministry of Health's regulations, Vietnam (MRL for tomato: 2ppm, cabbage: 5ppm, grape: 5ppm, and mango: 2ppm).

Table 3. CS_2 residue levels (µg/g) in in vegetables and fruits from markets

No.	Sample	CS_2 residue level (µg/g)	Market
1	Tomato (CC1)	not detected	Thi Nghe
2	Tomato (CC2)	not detected	Thi Nghe
3	Tomato (CC3)	not detected	Tan Dinh
4	Cabbage (BC1)	not detected	Tan Dinh
5	Cabbage (BC2)	0.08	Tan Dinh
6	Cabbage (BC3)	not detected	Thi Nghe
7	Grape (NH1)	< LOQ	Thi Nghe
8	Grape (NH2)	not detected	Tan Dinh
9	Grape (NH3)	not detected	Tan Dinh
10	Mango (X1)	not detected	Tan Dinh
11	Mango (X2)	not detected	Thi Nghe
12	Mango (X3)	0.11	Thi Nghe

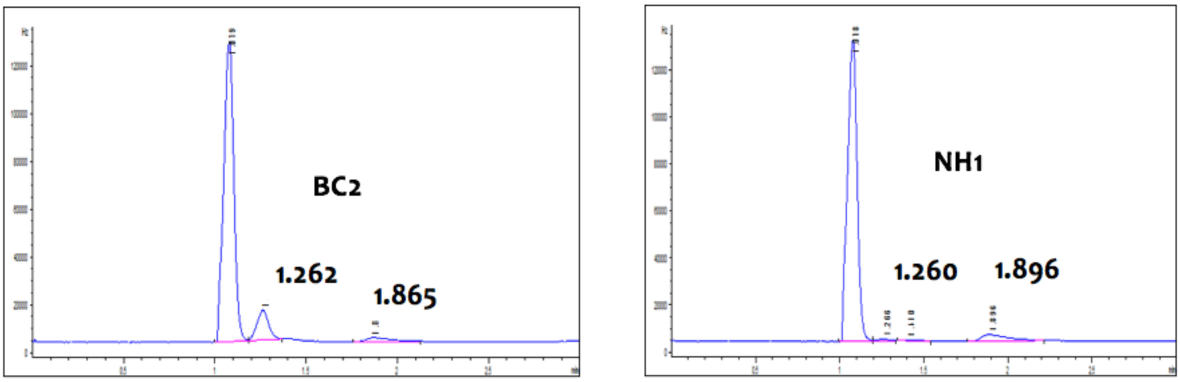

Figure 7. Chromatograms of CS_2 residue (mg/kg) in vegetables and fruits from markets.

Linear range of CS_2 levels in real sample was verified by using the range of 0.5 – 50 ppm. Linearity range were determined by linear regression. Calibration curve equation is $y = 17866x – 516.0$ with R^2 (correlation coefficient) = 0.999. The calculated values for instrument detection limit (IDL), limit of detection (LOD), limit of quantification (LOQ) and calibration curve are appropriate and thus the analytical method is fixed for purpose.

4. CONCLUSIONS

According to the validation result, the method is suitable for determination of DTCs group through CS_2 in trace level in vegetable and fruit matrices. Recovery of analytical method ranges from 85 to 99% and had good repeatability based on standard deviation values, most of them are lower 10%. The analytical method has LOD, linear range, recovery, sensitivity and reliability, which are suitable with real conditions of many labs in Vietnam.

In the future, this method should be applied to other objects such as scallions, potatoes, oranges. In addition, this method should also be implemented on other devices such as GC-FPD, GC- MS, GC-MS/MS, and HPLC-ED to help improve the sensitivity of the method and find the analytical method best suited to the real conditions for each laboratory in Vietnam.

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HÁT TRIỂN PHƯƠNG PHÁP PHÂN TÍCH XÁC ĐỊNH DƯ LƯỢNG DITHIOCARBAMATE DỰA TRÊN CS_2 TRONG RAU QUẢ BẰNG GC-ECD

Tóm tắt: Báo cáo trình bày việc phát triển phương pháp phân tích dư lượng dithiocarbamate trong rau quả thông qua dẫn xuất CS_2 bằng thiết bị sắc ký khí ghép đầu dò ECD. Kết quả nghiên cứu cho thấy độ thu hồi của phương pháp phân tích dao động trong khoảng 84,9 – 98,7% đủ đáp ứng tiêu chuẩn phân tích dư lượng các thuốc bảo vệ thực vật. Phương pháp phân tích dithiocarbamate cũng đã được áp dụng phân tích trên 12 mẫu rau quả thu thập từ các chợ Tân Định và Thị Nghè. Kết quả nghiên cứu cho thấy phần lớn hàm lượng dithiocarbamate trong các mẫu này đều dưới LOD ngoại trừ mẫu số BC2 (0,08 µg/g) và mẫu số X3 (0,11 µg/g).

Keywords: Dithiocarbamate, residues, CS_2, fruits and vegetables, GC-ECD

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