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A METHOD FOR DETERMINATION OF METHOMYL BY HPLC TECHNIQUES WITH DETECTION OF UV IN VEGETABLE SAMPLES

Cozma Alina*, Bota Sanda*, Cărăban Alina*, Sebeşan Mioara*, Borza Ioana, Domuța Cornel****

* University of Oradea, Faculty of Science, Department of Chemistry, Universității st., no. 5, Oradea, Romania, e-mail: acozma@uoradea.ro
** University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania

Abstract

This paper describes a method for the determination of the magnitude of contamination in different vegetables with methomyl using HPLC/UV techniques. Residues are extracted from the test portion following the acidified methanol. The mixture is centrifuged, filtered and directly analyzed by the method proposed. The tested samples showed contamination with low, but measurable amounts of residues.

Key words: methomyl, HPLC/UV, vegetables samples.

INTRODUCTION

It is of primary interest to estimate the fate of pesticides in soils. The increasing number of environmentally significant pesticides requires development of analytical techniques that allow simultaneous detection and quantitative determination of different pesticides with minimum extraction and cleanup steps (Petrovic et al., 2000).

There are nowadays in EU more than 1100 pesticides that can destroy animal bodies. In this respect, there is a maximum level of pesticides residues (MRL) that must be obeyed, to overrun this level meaning a real danger to human health (Tomasevic et al., 2009). The European Commission has established that MRL must be 10 µg/kg for pesticides residues in basic food products and food for children (Tomlin, 2003).

Methomyl was introduced in 1966 as a broad – spectrum insecticide. It is extensively used as an acaricide to control ticks and spider and it is employed for foliar treatment of vegetable, fruit and field crops, cotton, and commercial ornamental plants. The pesticide is effective as both a contact and systemic insecticide (Farre et al., 2002).

Methomyl is a very toxic compound and a major polluting agent for environment because of its high solubility in water (57,9 g/l at 25C) (Tomlin, 2003). Water contamination with methomyl, both surface and deep waters, is caused by its weak absorption in soils. It can be found as an active substance in Lannate 90, Lannate 25WP, Metomex 90SP, etc. It is a carbamate insecticide used to destroy the white fruit-fly in vegetable greenhouses, the solanaceae louse of tomato, and the apple and plum-tree worm. It can also destroy the grape-vine moth and the Californian clover that attacks cucumbers in solariums (Strathmann et al., 2001; Devipriya, Yesodharan, 2005; Poulios et al., 2006).

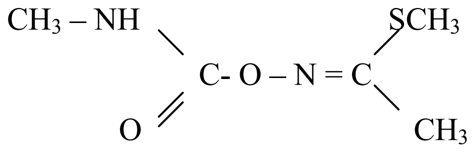

Fig. 1. Structure of methomyl, IUPAC name: S- methyl N- (methylcarbamoyloxy) thioacetimidate

In this paper we describe the method that we elaborate for determination of methomyl in vegetables.matrice.We are using microwave extraction folowing HPLC separation and UV detection.

It has been studied the photocathalytic degradation of methomyl in water, using Tio2 and ZnO, and the UV radiation (366 nm). It has also been studied the influence of the accelerator's concentration, its ph and the NaCl over the photocatalitic degradation process. The result was that the ZnO is a better accelerator than TiO2 for the photodegradation process, and the presence of the Cl ion influences in a major way the process (Tomasevic et al., 2009). The absorption spectrum of the methomyl solution during the photocathalytic degradation at different irradiation times is presented in figure 2.

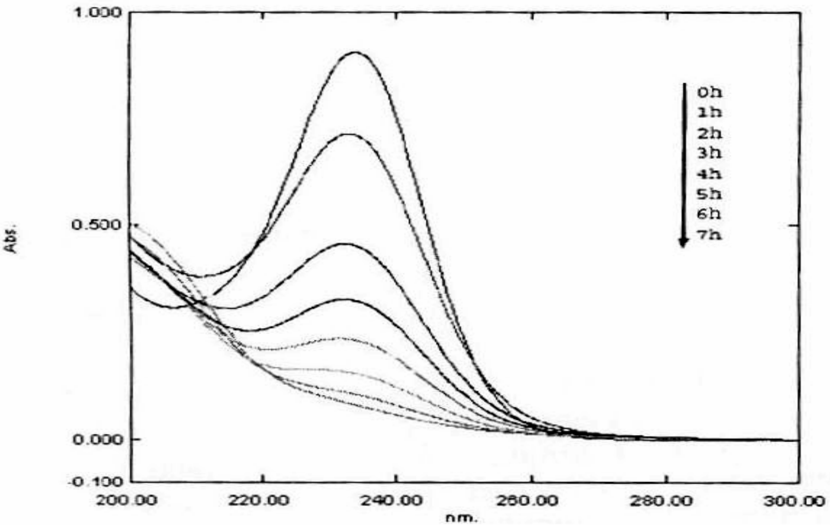

Fig. 2. UV –VIS spectra changes of methomyl
Maximum absorption is noticed at 234nm.

MATERIALS AND METHODS

A method is described for the analysis of polar, non – QuEChERS-amenable pesticides (log K_{ow}< - 2) in vegetables and processed products thereof.

Residues are extracted from the test portion following the acidified methanol. The mixture is centrifuged, filtered and directly analyzed by HPLC with detection UV. Quantification is performed with the help of isotopically labeled internal standards, which are added directly to the test portion at the beginning of the procedure.

Apparatus for extraction:

- ultrasound extraction apparatus, Elmasonic S15H;
- 50 mL centrifuge tubes with screw caps;
- automatic pipettes, suitable for handling volumes of 10 to 100µL, 200 to 1000 µL and 1 to 10 mL;
- 10 mL solvent – dispenser methanol;
- centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 2000 g;
- syringes, 2 or 5 mL disposable syringes;
- syringe filters, 0,45 µm pore size;
- rotavapor.

Substances:

- methanol, HPLC quality;
- acetonitrile, HPLC quality;
- concentrated acid formic (> 95%);
- citric acid monohydrate;
- dimethylamine;
- ammonium formate;
- water deionizate;
- methomyl standard;
- N – octadecyl (standard intern).

Method:

Of 100 g of cucumbers treated with methomyl we prepare the sample. It is treated with 20 ml of methanol and it is left to macerate for 20 minutes, then the ultrasound extraction is applied for 30 minutes at a temperature of 55C. The extract is filtered, washed for 2-3 times with methanol and then introduced in a 25 ml balloon.

The sample is placed in a concentration rotavapor. The concentrated product is introduced in a balloon of 10ml and brought to sign with methanol. 100 µg N – octadecyl is added and it is brought to the sign with methanol.

The preparation of calibration solution

There has been a standard stock solution of 1 mg/mL concentration methomyl.

Through several successive dilutions there have been prepared calibration solutions of the following concentrations: 10µg/mL, 40µg/mL, 100µg/mL and 400µg/mL, in 25 mL balloons. In each calibration solutions, there have been introduced 100 µg/mL of internal standard. Each solution was injected for three times and the chromatography was recorded in the conditions described as follows. The average of the obtained areas was formed for the resulted spike of methomyl and the calibration curve was designed as shown in figure 4.

Measurement Conditions for HPLC with detection UV

For the analysis are used:

- HPLC system with UV detector - VIS, model Young – Lin AT 7000;
- gradient pomp930 D, Yuong Lin Acme 9000; flow: 0,3 mL/ min; gradient: 100%A in 8 min to 50 or 10 0% B hold B for about 7 min;
- UV detector –VIS 732 D, Yuong Lin Acme 9000;
- PN Rheodyne injector; injection volume is 10µL;
- eluent A, methanol;
- eluent B, 1 mM citric acid in water adjusted to pH 11 with dimethylamine;
- column Dionex 250mm.

RESULTS AND DISCUSSION

As a result of chromatographic division, the chromatogram obtained is presented in the following figure 3.

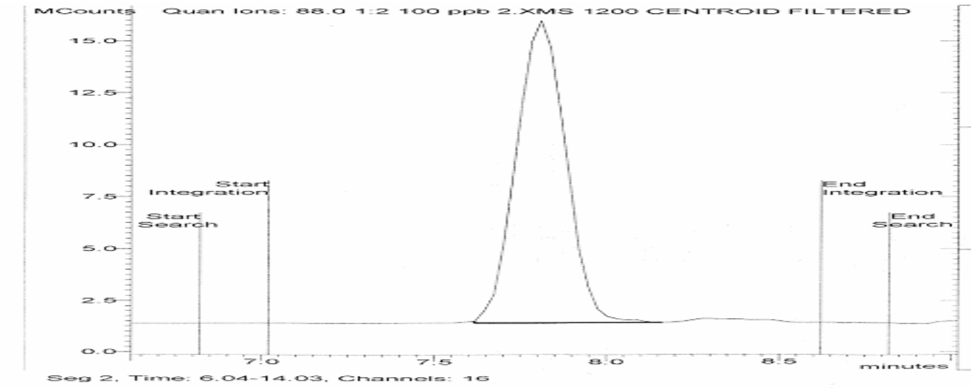

Fig. 3. The chromatogram for methomyl standard

The chromatogram shows that the chromatographic parameters are the best possible, the degree of asymmetry of the spike being 1, 08. The retention time for the active substance was minimum 7,8. Using the calibration solution, the following calibration curve emerged, as shown in figure 4.

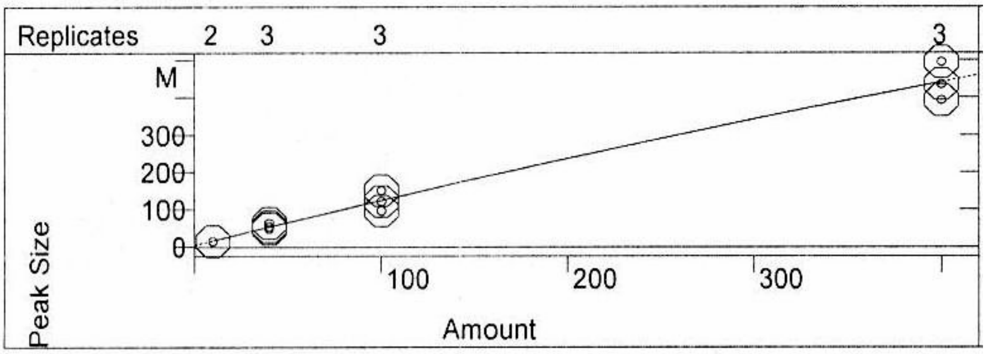

Fig. 4. The calibration curve for the calibration solution

The calibration curve is represented by an equation line: y = - 341, 6232x2 +1,2264e+6x with a correlation value of 0,998727.

Using this method, the cucumber sample was analyzed; through the calibration curve we established a concentration of 13ng/g vegetable product. Based on the retention time, the spike corresponding to the insecticide was determined.

CONCLUSIONS

The proposed method is precise, fast and accurate, a standard relative error being obtained. The recovery and reproductibility, based on matrix spiked standards, were acceptable for methomyl. The impurities and matrix effects from cucumber were minimal and did not interfere with the quantitation of any target compound.

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