THE STUDY FOR DETERMINATION CHLORPYRIFOS RESIDUAL FROM FRUIT SAMPLES

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Abstract

The aim of this study is the presentation a method for the determine chlorpyrifos from fruits samples by HPLC/UV techniques. Pesticides residues are extracted from the test portion following the acetone. The mixture is centrifuged, filtered and directly analyzed by the method proposed. The tested samples showed contamination with low, but measurable amounts of residues. The limit of detection for the method was calculated from regression data, and recovery results were in the range of 95- 106%. Calibration curves that were constructed for the analytes followed linear relationships with good correlation coefficients (R^2> 0.9). The method developed can be used to determine the chlorpyrifos residues in concentration lower than the maximum residues limits, 0.04mg/kg.

Key words: chlorpyrifos residue, apples samples, HPLC, UV.

INTRODUCTION

Many fruit and vegetables sold in supermarkets and greengrocers contain pesticide residues that are above the maximum legal level. The different classes of pesticides have different types of effects on living organisms. According to the Environmental Protection Agency (EPA), 60 percent of herbicides, 90 percent of fungicides and 30 percent of insecticides are known to be carcinogenic, or cancer causing. Laboratory studies show that pesticides can cause health problems such as: birth defects, nerve damage, cancer, blocking the absorption of important food nutrients necessary for normal healthy growth in children, other long-term effects [1–6]. Various attempts have been made to describe and quantify the negative impacts that pesticides have on the environment and human health [7 – 11]. Symptoms of acute OP poisoning are: nausea, vomiting, abdominal cramps, diarrhea; excessive salivation, rhinorrhea; headache, vertigo; fixed pinpoint pupils, blurred vision ocular pain; muscle twitches, especially of face, tongue and neck; difficulty in breathing, primarily due to excessive secretion and bronchoconstriction; random jerky movements, convulsions; respiratory paralysis, death.

For most foodstuffs. Chlorpyrifos analysis is usually carried out using gas chromatography (GC) with flame photometric detection or nitrogen phosphorus detection [12-14]. However. High sample lipid content

virtually requires extensive sample cleanup to remove fats and oils [12] when GC is used. Black oil sunflower seeds contain an content of approximately 40% by weight, depending on growing conditions and the sunflower cultivar usedm [15]. Due to the exploratory nature of the current study and the comparatively high chlorpyrifos concentrations expected. Fast sample throughput ease of extraction, and minimal sample cleanup were of greater importance than detection sensitivity. High performance liquid chromatography (HPLC) has been used previously for chlorpyrifos determinations in oranges [15], as well as leaves ans soil [15]. We have adapted the method for determination chlorpyrifos residual from apples sample.

Chlorpyrifos, fig.1 (name: *O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate) is a crystalline organophosphate insecticide, acaricide and miticide used to control foliage and soil-borne insect pests on a variety of food and feed crops that inhibits acetylcholinesterase. It is known by many trade names ex. Tricel, Brodan, Eradex etc. Chlorpyrifos is moderately toxic and chronic exposure has been linked to neurological effects, developmental disorders, and autimmune disorders.

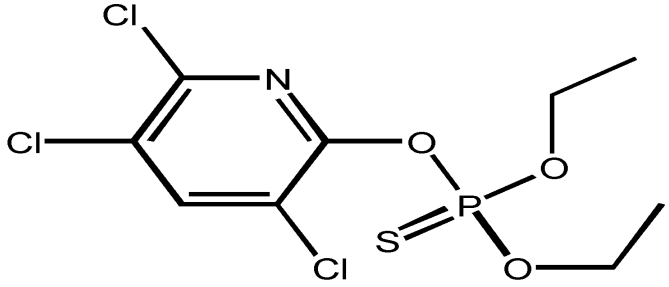

Fig. 1 O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate

Chlorpyrifos is soluble in the solvents like benzene, acetone, chloroform, methanol and ethylacetate. It is slightly soluble in water [7].

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. Five samples of vegetables comprising apples (5) were collected from different farmers around Oradea. Samples were collected from fields when the samples were ready for sale and were refrigerated and analysed with in a week of collection. All the samples were extracted fresh.

Materials:

- acetone, HPLC quality;
- acetonitrile, HPLC quality;

- citric acid monohydrate;
- dimethylamine;
- ammonium formate;
- water deionizate;
- chlorpyrifos standard;
- apple

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 2000g;
- syringes, 2 or 5 mL disposable syringes;
- syringe filters, 0,45 µm pore size;
- rotavapor;

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, acetone;
- eluent B, 1 mM citric acid in water adjusted to pH 11 with dimethylamine;
- column C18 ;

Of 50 g of apples was macerated with 5 -10 g anhydrous sodium sulfate in blender to make a fine paste. The macerated sample was extracted with 100mL acetone , then the ultrasound extraction is applied for 30 minutes at a temperature of 55C. The extract is filtered, washed for 2-3 times with acetone and then introduced in a 25 ml balloon.

The sample is placed in a concentration rotary evaporator. The concentrated product is introduced in a balloon of 10ml and brought to sign with acetone 100 µg After concentrating the eluate on rotary evaporator, final volume was made to 2 mL for HPLC analysis.

Sample Preparation

Stock standard solution were prepared by accurately weighing 0,0104 mg of standard chlorpyrifos and dissolved it in methanol and made the volume 100 ml.

Working standard solution of 25 μg/ml, 50 μg/ml and 100 μg/ml were prepared by diluting standard stock solution. Stock standard solution and working standard solutions were stored under refrigeration. Purity of standard was higher than 95%.

In the literature the commonly used column type for the analytical separation of OP pesticides is the reversed phase C-18. For this reason we started with variants of C-18 columns.

Residues are extracted from the test portion following the acetone. The mixture is centrifuged, filtered and directly analyzed by HPLC with detection UV using HPLC system with UV detector- VIS, model Young – Lin AT 7000.

RESULTS AND DISCUSSION

For compound a linear calibration (figure 2) plot was established by using linear regression and 1/concentration weigthing. The vertical axis of the calibration curve was the area ratio (area of analyte peak divided by the internal standard peak area). The horizontal axis was the concentration. Once the calibration curve was defined, for each concentration and each analyte a "calculated concentration" was determined, based on the slope and intercept of the calibration curve and the response factor for the particular measurement. Next, for each concentration of the standard series the standard deviation of the "calculated concentrations" was determined and plotted over the actual concentrations. Limit of detection was determined by establishing a "standard deviation – concentration"relationship trend line and extrapolating it to zero concentration. LOD is defined as three times the expected value of the standard deviations of the calculated concentrations at zero concentration.

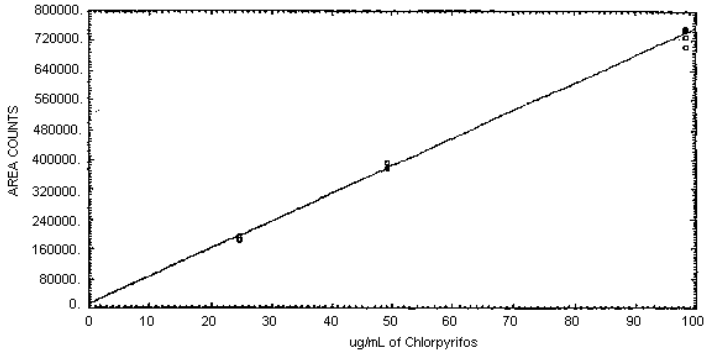

Fig. 2 Calibration curve for Chlorpyrifos, slope = 7400 area counts per microgram per milliliter.

Pesticide was identified from its retention time and confirmed by comparison with authentic standard.

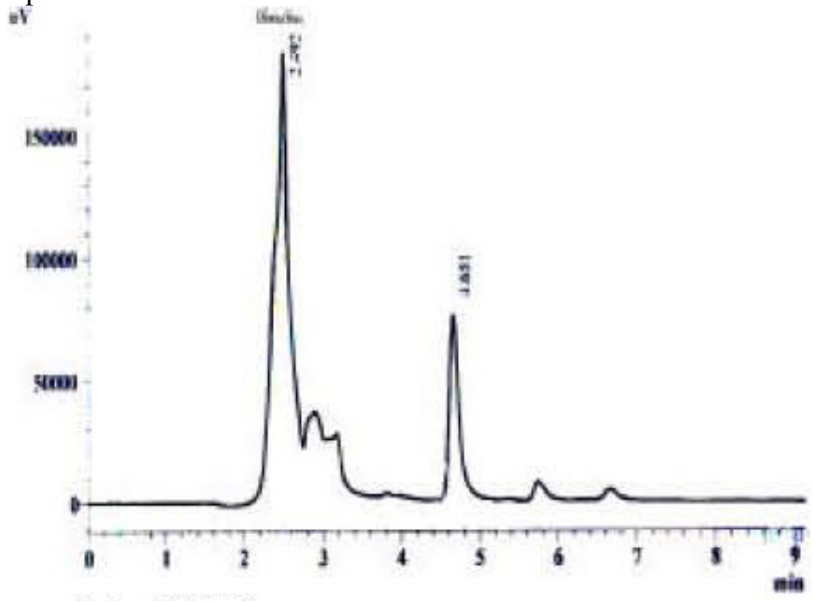

Fig. 3 Chromatogram of plant sample by HPLC

The method developed can be used to determine the chlorpyrifos residues in concentration lower than the maximum residues limits, 0.04mg/kg.

CONCLUSIONS

The limit of detection for the method was calculated from regression data, and recovery results were in the range of 95- 106%. Calibration curves that were constructed for the analytes followed linear relationships with good correlation coefficients (R^2> 0.9).

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 monocrotophos.The impurities and matrix effects from cauliflower were minimal and did not interfere with the quantitation of any target compound.

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