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SUPERCRITICAL FLUID EXTRACTION OF TEBUPIRIMPHOS RESIDUES IN SUGAR BEET

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ABSTRACT

Tebupirimphos or phostebupirim is an organophosphorous type insecticide (OP). A method for the determination of tebupirimphos residues in sugarbeet was developed. The tebupirimphos was extracted from the samples using supercritical fluid extraction (SFE) and analyzed by capillary gas chromatography coupled with nitrogen-phosphorus detection. The LOD of tebupirimphos was 0.0001 mg/kg, with the LOQ of 0.0003 mg/kg. In addition, the intra- and inter-day precision was satisfactory with RSD less than 10.0 %. Good detector response linearity (R^2= 0.9914) was observed in the range of 0.00488-0.975 µg/ml. The recovery of supercritical extraction was 95.08 %, (RSD 8.2 %). It can be concluded that the tebupirimphos can be efficiently extracted using SFE and residues in the sugar beet root are present for a short time after the soil treatment with this insecticide.The resiudes of tebumpiriphos were no more present at harvest time.

Key words: Supercritical fluid extraction, Residues, Tebupirimphos, Sugarbeet.

INTRODUCTION

To evaluate the potential risk associated with the use of pesticides, it is important to know the fate of a pesticide in the plant. Tebupirimphos or phostebupirim is an organophosphorous type insecticide (OP), belongs to class of chemicals that are less persistent in the environment in comparison with the organochlorines. However, they are generally much more acutely toxic to humans than chlorinated pesticides and are generally regarded as safe for crops due their relatively fast degradation rate (Gilani *et al.*,2010). In the available literature only two scientific papers about the fate of this insecticide in the plants were found regarding the degradation of tebupirimphos in the aquatic anaerobic conditions (Halarnkar *et al*., 1997), and the concentration of its residues in dried ground ginseng root by capillary GC-MS and GC-FPD (Wong *et al*., 2007).The current methodologies to determine pesticide residues usually use liquid extraction, often incorporating a partitioning stage, a concentration of extract, as well as a cleanup stage prior to the determination by chromatography. These cleanup procedures are time-consuming with a lot of steps, which can result in lowering the extraction efficiency and the loss of analite. Besides this extraction methods for the extraction of OPs pesticides, solid phase microextraction (SPME) (Asri and Anderson, 2000; Đurović *et al.*, 2010) or matrix solid-phase dispersion (MSPD) using octadecylsilica as dispersant material can be used (Acosta-Tajada *et al*., 2011). Recently, the microwave-assisted extraction (MAE) has been applied in the pesticide residue analysis, but with some stability problems with thermally labile OPs (Niell *et al*., 2011). The supercritical fluid extraction (SFE) is an environment-friendly technique and has become increasingly popular in the recent years (Abd El-Aty *et al.*, 2009). Supercritical fluids have densities similar to liquids, but lower viscosities and higher diffusion efficiency. This combination of properties results in a fluid that is more penetrative has a higher solvating power, and will extract solutes faster than liquids. Supercritical fluid strength depends on the temperature and pressure relation. These two parameters define the solvent strength of fluid. The possibility of varying the solvent strength is an attractive characteristic that makes SFE an excellent choice for extracting organic chemicals from solid matrices. Supercritical carbondioxide (SC-CO_2) offers considerable potential as a replacement solvent in the laboratory due to its moderate critical constants (T_c = 31°C, P_c = 73 atm), non-toxic, non-flammable, sufficient solvating power, and availability in pure form (Yu, 2002). There are numerous reports on the applications of SFE for the extraction of different analysts in diverse matrices as pesticide residues are frequently extracted with the SFE (Pucarević *et al.*, 2003; Norman and Panton, 2006, Mirosavljević *et al*.,2012). The aim of this paperis to set up the SFE extraction conditions and apply it for investigating the dissipation rate of tebupirimphos in sugar beet root. As a

supercritical fluid CO_2 was used. The extracts were analyzed by capillary gas chromatography coupled with nitrogen-phosphorus detection (GC-NPD).

MATERIALS AND METHODS

Chemicals and solutions: The analytical standard of tebupirimphos of 99.50% purity was used (Bayer). The stock standard solutions in the concentration of 5.0 µg/ml were prepared in methanol. The working standard solutions were obtained by dilution with methanol and stored under low temperature (-20^{o}C).

Field trials and sampling: Tebupirimphos (Arriba 2.1% GR) was applied to sugar beet at the time of sowing (March, 2012) in the concentration of 62-75 g per 100 m^2. The granules were deposited with the applicator in strips width of 10-15 cm and 1 cm away from the seeds followed by soil covering. The plant samples were collected on the 11thMay, 1st June, 15th July, 15thAugust and 8thSeptember.The plant samples were taken from the control and experimental plots according to the SANCO Document 10232/2006. The 8-10 plants were collected from each replication, placed in polyethylene bags and immediately transported to the laboratory. After washing, the samples were stored at -20 ^{o}C until they were analyzed.. For the extraction, 1 g of homogenized sample was used.

Apparatus and conditions of supercritical extraction and chromatographic determination: The HP 7680A Supercritical Fluid Extraction Unit (Hewlett Packard) was used. The plant samples for SFE were prepared by homogenization of the 1 g root portions with 1 g anhydrous Na_2SO_4 in a mortar with pestle. The mixture was placed in 7 ml extraction cell and following SFE extraction conditions were taken: fluid density 0.85 g/ml, pressure 240 bar, flow rate 1 ml/min, extraction chamber temperature 45ºC, equilibration time 5 min, extraction time 20 min, nozzle temperature 45ºC, trap temperature 15 ºC, trap packing octadecyl silica (ODS) and trap rinsing solvent was methanol at rate 0.5 ml/min.

The extracts of tebumpirimphos were analyzed on GC-NPD (Hewlett Packard Gas Chromatograph 5890 Series II with split injection and capillary column Supelco SPB 608 (30m x 0.53 mm x 0.50µm)). Hellium (column head pressure 55 kPa) was used as a carrier gas with 20 ml/min column flow in the mode of constant flow. The gases flow for the detector was: hydrogen 3.5 ml/min, air 110 ml/min, make up nitrogen 20 ml/min. The temperature conditions applied during the gas chromatographic separation were: the column temperature of 200ºC maintained for 5 minutes, the rise of 30^{o}C /min to 250ºC. The temperature of the injector and the detector were 250 ^{o}C respectively. The total run time was 8 minutes. The retention time of tebumpiriphos was 3.62 min.

Validation: The detection limit (LOD) was defined as the amount of tebupirimphos which produces the signal three times the noise signal. The quantification limit (LOQ) is the amount of tebupirimphos produces a signal ten times the noise signal. The mass concentration of standard for the LOD and LOQ determination was 0.0001µg/ml. The linearity of the detector response was checked for the mass range from0.0048 to 0.97µg/ml, in three replicants. The linearity was studied in matrix and without matrix. The recovery was investigated by the spiked untreated samples under the conditions applied method of interest. The recovery study was carried out in three replicants at the spiking levels 0.194, 0.169 and 0.175µg/ml (SANCO/10232/2006; Bursić, 2011).

RESULTS AND DISCUSSION

Validation studies: The limit of quantification (LOQ) 0.0003 mg/kg and the limit of detecton (LOD) of 0.0001mg/kg were obtained. The GC-NPD method was tested for linearity with standard mixtures at eight concentration levels in the range from 0.0048 to1.20 µg/ml. The correlation coefficient R^2 = 0.991 was obtained. The relative standard deviation (RSD%) for n=6 consecutive injections of a standard, containing 0.97 µg/ml tebupirimphos was 2.64 %. The precision calculated from ten consecutive extractions and defined as the coefficient of variation of solutions containing 1 µg/ml of tebupirimphos was 8.2 %. The recoveries for sugar beet fortified samples, spiked with tebupirimphos ranged from 86.42 to 99.60 % (n =3), and recoveries without the matrix ranged from 81.30 to 106.69 % (n=10). The averaged recovery of fortified sugarbeet sample was 91.56±7.71 %. The extraction efficiency and reproducibility of the SFE GC-NPD procedure were studied by analyzing the standards and fortified sugar beet samples taken in September.

Survey of tebupirimphos in sugar beet: For assessing the matrix effect on the results, the recovery tests were made for a) tebupirimphos standard dissolved in solvent, and b) tebupirimphos added in sugar beet (matrix matched). It can be concluded that there is no significant matrix effect during GC-NPD. Despite that fact, the matrix-matched standard solutions were still used for the the calibration of tebupirimphos in the sample matrix.The results of the investigation of tebupirimphos dissipation in sugarbeet during the five- month period of sugar beet growing are shown in Table 1.

The tests have shown that the sugar beet plants have high metabolic capacity for fast dissipation of the tebupirimphos, which makes it suitable as a soil insecticide in sugar beet growing. The tebupirimphos residues disappear in the root of sugar beet so that after two months and at harvest time (which is in September) they are no longer present in measurable concentrations.

On the assumption that the concentration decrease of the tebupirimphos has an exponential trend with the equation form $y=ae^{bx}$. Based on the data from the table and the linearization of the observed equation, there is: lny=lna+bx, i.e. there is an equation with a linear trend Y=A+bx (Y=lny, A=lna).

Table 1. Tebupirimphos concentrations in sugar beet in the course of time.

	Control plants	Treated plants	µg/ml	Sample weight	Concentration of tebumpiriphos ±RSD %
Month	**C**	**T**	**T-C**	**G**	**mg/kg**
May	0.0011	0.02711	0.0260	0.2354*	0.1104 ± 4.21
June	0.0004	0.00236	0.0020	0.5	0.0040 ± 4.73
July	0.0001	0.00015	0.0001	0.5	0.0001
August	0.0001	0.00008	-0.0001	0.5	< LOD
September	0.0001	0.00021	0.0001	0.5	< LOD

* In May the plant was developing so the amount taken was smaller

Table 2. Data for obtaining a trend equation.

	x^*	y^{**}	Y	x^2	xY
	-1	0.1104	-2.203645	1	2.203645
	0	0.0040	-5.521461	0	0
	1	0.0010	-6.907755	1	-6.907755
∑	0	0.1254	-14.63286	2	-4.704110

*Time (-1→May, 0→June, 1→July), **detected concentrations

A trend equation is obtained by using the data from the table and formulae:

$A=\sum Y_i/n= -4.87762$ (n→number of data)

$b=\sum x_i n_i/\sum x_i^2= -2.35206$

$a=e^A=0.007615$

$y=0.007615e^{-2.35206x}$

The exponential trend is used for the prediction of an occurrence dependent on time in the same manner in which the tebupirimphos concentration is dependent on the month of sampling. On that basis it is possible to predict the tebupirimphos concentrations in August (x=2) and September (x=3). The obtained values would be y=0.000069, i.e. $y=6.564*10^{-6}$.

Conclusions: The sample preparation method based on the two-step SFE with GC-NPD determination has proved to be an efficient and quantitative method for the detection and analysis of the traces of tebumpiriphos in plant samples. The analytical methods provide the possibility to investigate the time of dissipation of tebumpiriphos residues in sugar beet, in particular to identify the hazards and assess the risks to sugar industry. The proposed method is simple, rapid and requires only a small amount of samples (1 g) and a low elution solvent volume (2 ml methanol). Consequently, this paper contributes to covering the increased demand for new extraction techniques with reduced cleanup step (which would drastically decrease the manpower need for a single analysis, and subsequently lower labour cost), extraction time and solvent consumption. In addition, the SFE procedure proposed in this paper should be automated and coupled on-line with the final analytical measurement.

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