PE – HPLC/DAD ANALYSIS OF BENTAZONE AND AZINPHOS-METHYL IN WATER SAMPLE

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Abstract

A simple and reliable method was developed for the determination of bentazon and azinphos-methyl in water using high-performance liquid chromatography with diode array detection (HPLC-DAD) at 254 nm. Chromatographic separation was carried out on a BDS Hypersil C8 column (15 0mm x 4mm, 5 µm particles) at 20^0C and 0.8 ml/min flow rate with a mobile phase consisting of acetonitrile - ultrapure water (80 : 20, v/v). Satisfactory separation of two pesticides was obtained in 7 minutes by injecting 10µl standard solution. The linearity ranges of the calibration curves ranged from 0.27 µg/ml to 2.47 µg/ml for bentazon and from 0.34 µg/ml to 3.1 µg/ml for azinphos-methyl. The selectivity of the method was tested by injecting standard solution containing a mixture of azinphos-methyl, bentazon, atrazine, simazine and propazin. These compounds were separated at differents retention times, this showing the selectivity of method. The recovery rate was tested using two SPE cartridges: Strata X (Phenomenex) and LiCrholut EN (Merck). The Strata X cartridges were found to be more suitable for extracting the two organophosphorus pesticides from surface water samples. The average recoveries were 95.8% for azinphos-methyl and 105.6% for bentazon.

Keywords: HPLC, bentazon, azinphos-methyl, water

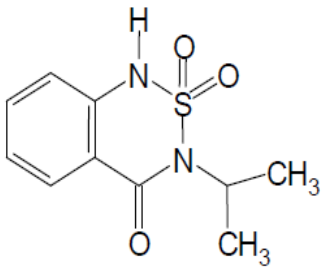
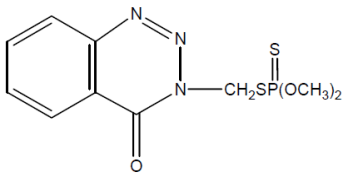
Introduction

Contamination of water resources by pesticide residues is one of the major challenges for the preservation and sustainability of the environment. Their extensive usage in agriculture and the industrial emission during their production has led to substantial occurrence of pesticide residues and their metabolites in food, water and soil.
Azinphos-methyl is a systemic pesticide (contact and stomach action). Exposure of pesticides affects the nervous system by inhibiting the activity of acetyl cholinesterase. At contact of the enzyme from insects with the pesticide results irreversible phosphorylation of cholinesterase, leading to the accumulation of acetylcholine at the neuron/neuron and neuron/muscle (neuromuscular) junctions or synapses [1].
Azinphos-methyl is subject to hydrolysis and decomposes with gas evolution at elevated temperatures [2].

Bentazon is a benzothiadiazinone contact herbicide acting as a photosynthetic electron transfer inhibitor. Its selectivity is based on the ability of the crop plants to quickly metabolize bentazon to 6-OH- and 8-OH-bentazon and conjugate it with sugar. Since most weeds do not possess this metabolic ability, their photosynthesis is disrupted and the weeds die. Bentazon is used in agriculture for selective postemergence control of many broadleaf weeds in soybeans, rice, corn, peanuts, mint, dry beans, dry peas, and succulent lima beans [3]. He has a contact action on the leaves and to a lesser extent an action via the soil. The active ingredient is principally absorbed by the green parts of plants.

In Table 1 are presented the chemical structure and the IUPAC chemical name of the selected pesticides.

Table 1. Chemical structure and IUPAC chemical name of the target pesticides

Compounds/ IUPAC chemical name	Chemical structure	Molecular formula/ Molecular weight (g/mol)	CAS number
Bentazon / 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide		$C_{10}H_{12}N_2O_3$ 240.3	25057-89-0
Azinphos-methyl / *S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl) *O*,*O*-dimethyl phosphorodithioate		$C_{10}H_{12}N_3O_3PS_2$ 317.33	86-50-0

These pollutants, present in water resources at low concentrations, could affect ecosystems and impact drinking water supplies. Owing to the toxicity of these pesticides, the European Union (EU) has included pesticides in list of priority pollutants (2000/60/EC) [4] and has established by a directive (98/83/EC) [5] that a single pesticide cannot be present in water intended for human consumption in concentrations higer than 0.1µg/L. High concentrations of pesticides can appear in surface water during runoff in agricultural areas soon after pesticide application to the farm field, and in water from urban basins as a consequence of urban development and abusive household uses.
The Romanian Government Decision, HG 1038/2010, established maximum allowable values for azinphos-methyl in river and ground water (0.1 µg/L) but for bentazon wich is included in regulation is not yet established a limited value [6].
The most widely applied techniques for quantification of bentazon and azinphos-methyl from water samples are liquid-chromatography coupled with

either diode array or mass spectrometer detector (LC-DAD or –MS) [7-16] as well as gas chromatography coupled with mass spectrometer detector (GC) [17-19] .
The main objective of this work was to develop a HPLC-DAD method for determination of bentazon and azinphos-methyl in surface water samples using soild phase extraction for sample preparation.

Experimental

Chemicals and reagents

Bentazon (98.5% purity) and azynphos-methyl (97.8 % purity) were all purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile, methanol were supplied by Merck (Darmstadt, Germany). Strata-X 33µm Polimeric Reversed Phase cartridges (60 mg/3 mL) used for solid phase extraction were purchased from Phenomenex (Torrance, USA). Glass microfibre filters were purchased from Whatman (United Kingdom). Stock standard solutions of about 500µgmL^{-1} were prepared in methanol. The individual dilutions and mixtures of the analytes were prepared in the same solvent. All stocks and diluted standard solutions were protected against light in amber vials and were stored at 4 °C.
Ultrapure water was obtained from a Milli-Q water purification systems (Millipore, Beldford, MA, USA).

Sample collection

Momentary surface water samples were collected from Ciorogarla river in May 2013 upstream and downstream of waste water treatment plant of Magurele city.
Amber glass bottles were used to collect momentary samples from each site. Each bottle was filled to the top to reduce headspace and transported to the laboratory. Samples were stored at 4 °C until analysed. All samples were analysed within 3 days.

Solid-phase extraction procedure

Two sorbents including Strata –X 30um Polimeric Reversed Phase (60 mg, 3 mL) and LiChrolut-EN (200 mg/3mL) were investigated for sample pretreatment and analyte preconcentration.
Prior to extraction, 500 mL of surface water were filtered through 0.45 µm Whatman glass fibre filters to remove any solid particulates and adjusted to pH 2±0.5 with hydrochloric acid (2M). Conditioning of the SPE cartridges was performed with 5 mL of methanol and 5 mL of acidified ultrapure water (pH 2) at a flow rate of 4 mL/min. After loading the sample and subsequent washing with 5 mL of HPLC water at 10 mL/min, the cartridges were dried under vacuum for 10 min. The elution of analytes was performed with 5 mL methanol at a flow-rate of about 1 mL/min. The extract was concentrated to dryness in *automated evaporation system* using nitrogen stream and water bath at 40^{0}C. Finally, the extract was reconstituted in 1 mL of methanol and injected into the HPLC system.

Liquid chromatographic separation

Analytical determination was performed on an Agilent 1200 (Agilent Technologies, USA) system equipped with a degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detector (MWD). The separations were performed on a BDS Hypersil C8 analytical column (150 mm length, 4 mm i.d; 5 µm particle size) acquired from Thermo Scientific (Waltham, Massachusetts, USA) . System control and data acquisition were achieved by means of a computer equipped with an Agilent ChemStation program. Analytes were separated by isocratic elution using ultrapure water and acetonitrile (50/50%,v/v) as mobile phase at a flow-rate of 0.8 mL/min. The HPLC separations were carried out at 20°C and a 10 µL injection volume was employed for this assay. The detection was performed at 254nm which was determined to be the optimum wavelength. Peak area were used for quantitative analyses. Compounds were identified in the chromatograms comparing the retention time of the peaks with that of the corresponding compounds in the standard solution.

Results and discussion

Before solid phase extraction study, the program elution and the wavelength were established, in order to separate the compounds in the shortest possible period of time. Figure 1 shows the chromatogram obtained in the HPLC-DAD analysis of a standard mixture solution (0.27 µg/mL bentazon and 0.34 µg/mL azinphos-methyl) in optimum conditions.

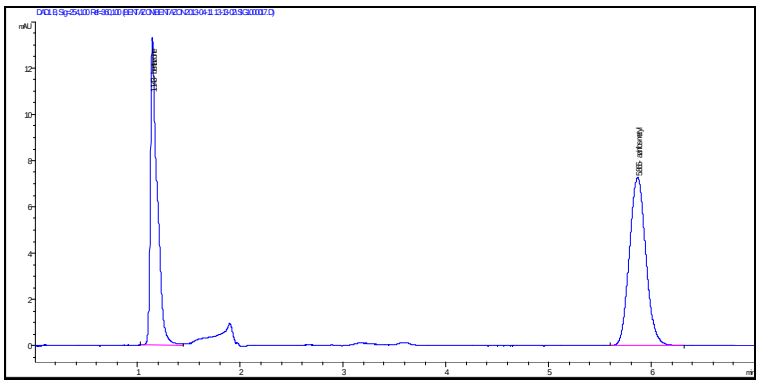


Fig.1. Chromatogram of standard solution of bentazon (0.27µg/mL) and azinphos-methyl (0.34µg/mL) obtained during the HPLC –DAD analysis

Five concentration levels injected in triplicate were used to build the calibration curves, by means of an external standard method based on peak areas. Good linearity was found for all compounds in the range of 0.27-3.1µg/mL with correlation coefficient (R^2) values higher than 0.998. Limits of detection (LOD) were 0.09µg/L for bentazon and 0.11 µg/L for azinphos-methyl and limits of quantification (LOQ) were 0.26µg/L for bentazon and 0.34µg/L for azinphos-metyl, respectively. Table 2 shows quality parameters of the analytical procedure for each compound: concentration range, regression equations, correlation coefficients, limits of detections, limits of quantification, repeatability (intra-day precision) and reproducibility (inter-day precision).

Table 2- Figures of merit of the analytical procedure for bentazon and azinphos-metyl

Compound (concentration range)	Regression equation	R^2	LOD µg/L	LOQ µg/L	Repeatability (RSD) (n=4)	Reproducibility (RSD) (n=4)
Bentazon	***y=25.16x+0.38***	**0.9981**	**0.09**	**0.26**	**3.51**	**7.2**
Azinphos - metyl	***y=25.90·x-0.936***	**0.9992**	**0.11**	**0.34**	**4.2**	**8.4**

Method validation

Linearity (fig. 2), limits of detection, limits of quantification, repeatability and reproducibility were established to determine the accuracy and precision of the overall method. Limits of detection and limits of quantification (LOD and LOQ) were calculated by using a signal-to-noise ratio (S/N) of 3 and 10, respectively.

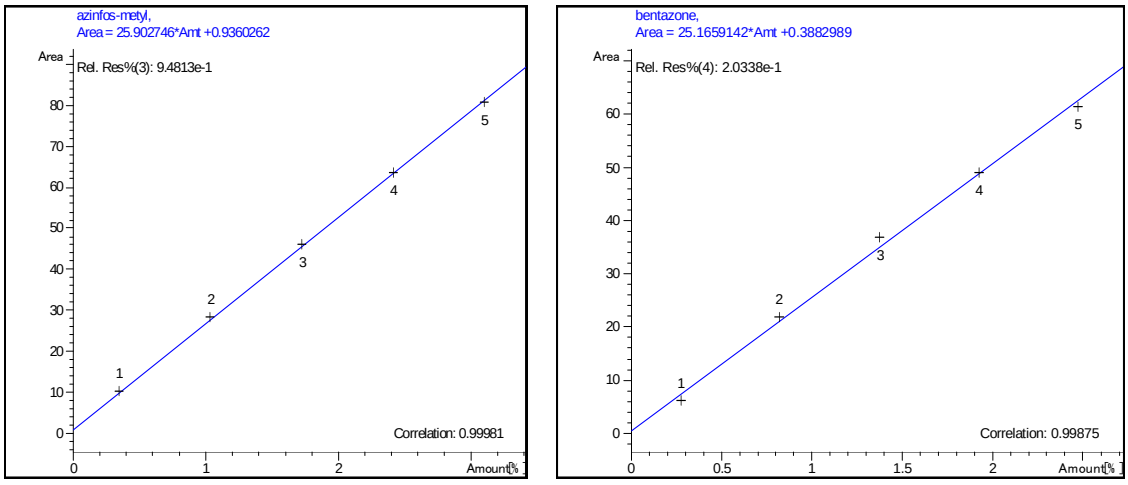


Fig.2. Calibration curves obtained for azinphos-methyl and bentazon

The SPE recovery of analytes was carried out in ultrapure water sample matrices. Samples were spiked with a mixed standard and extracted using two cartridges (Strata-X and LiChrolut-EN) in triplicate. The concentration recovered was compared to the initial spiking concentration.
The results from the evaluation of sorbents used to pack cartridges in SPE, presented as recoveries (%) of bentazon and azinphos-metyl, were calculated on the basis of HPLC-DAD analysis of spiked ultrapure water extracts (0.5 µg/L) and are summarized in figure 3.

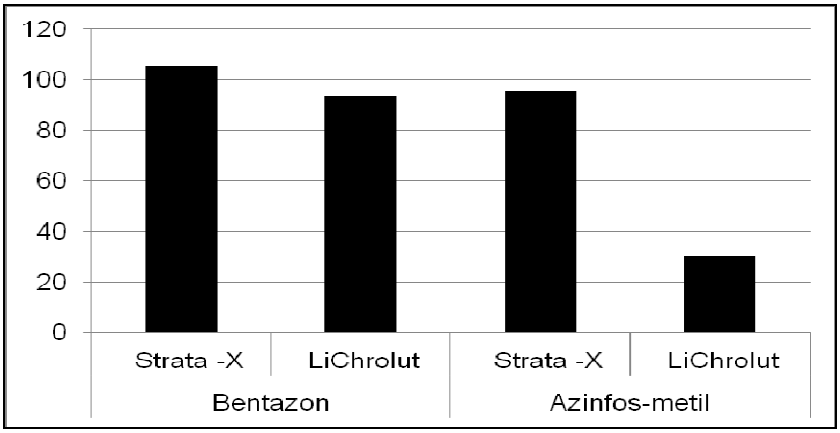


Fig. 3 Analyte recoveries using different SPE cartridges

The Strata-X cartridge gave similar and high recoveries for bentazon (105.7%) and azinphos-methyl (95.8%). Instead, the LiChrolut-En cartridge gave a high recovery only for bentazon (93.9%) but for azinphos-methyl the LiChrolut-EN gave low recovery (30.3%). The compounds investigated being satisfactorily extracted by Strata-X cartridge, this sorbent was chosen for the further investigations concerning the analytical performances of the method.

The precision of the overall method was determined by the repeated (n = 4) intra-day (repeatability) and inter-day (reproducibility) analysis of a spiked surface waters at the following concentration levels: 1 µg/L azinphos-methyl, 0.8 µg/L bentazon. The precision of the method expressed as the relative standard deviation (RSD) of replicate measurements varied by less than 15% in all cases being acceptable data. The results are showed in Table 2 and were for all compounds lower than 4.2 and 8.4 % for intra- and inter-day, respectively.

The selectivity of the method was tested using another class of compounds which can be analyzed by liquid chromatography: simazine, atrazine, propazine. The retention times obtained for these compounds, according to the developed method, are different from those of the analytes of interest (fig.4). The method is selective because it allows distinct separation of other pesticides, the stationary phase of column shows specificity and selectivity in the analysis of these compounds.

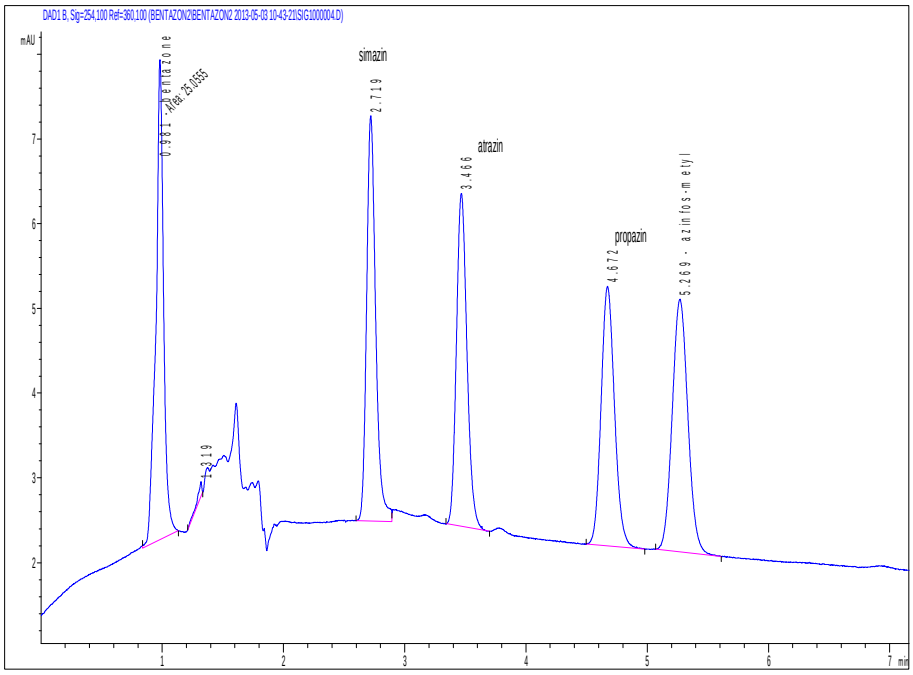


Fig.4. Chromatogram of a mixture standard solution of analytes with triazines pesticides (azinphos-methyl 0.25 µg/mL, bentazon 0.15 µg/mL, simazine 0.2µg/mL, atrazine 0.25µg/mL, popazine 0.18 µg/mL)

Surface water samples

The described method was applied to the quantification of bentazon and azinphos-methyl in surface water samples collected from Ciorogarla river in upstream and downstream of waste water treatment plant (WWTP) of Magurele in May 2013. Both pesticides were detected in concentrations above limit of detection (Table 3).
The higest concentration was recorded for bentazon in downstream WWTP Magurele, these showing probably the possible influence of the WWTP efluent that was poorly removed and then it was evacuated in Ciorogarla river. The concentrations obtained for azinphos-methyl are situated above the admisibile value in accordance with HG 1038/2010 both for upstream and downstream WWTP Magurele.

Table 3: Concentrations of bentazon and azinphos-methyl in Ciorogarla river

Compound	Upstream WWTP Magurele (µg/L)	Downstream WWTP Magurele (µg/L)
Bentazon	0.35	0.82
Azinphos -metyl	0.18	0.26

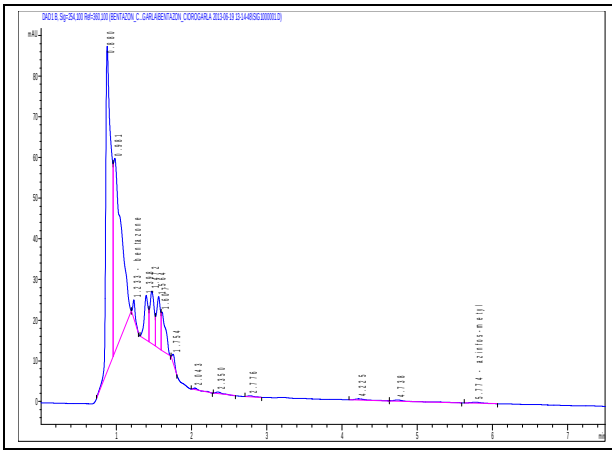


Fig.3a-Chromatogram of Cirogarla water sample Upstream WWTP Magurele

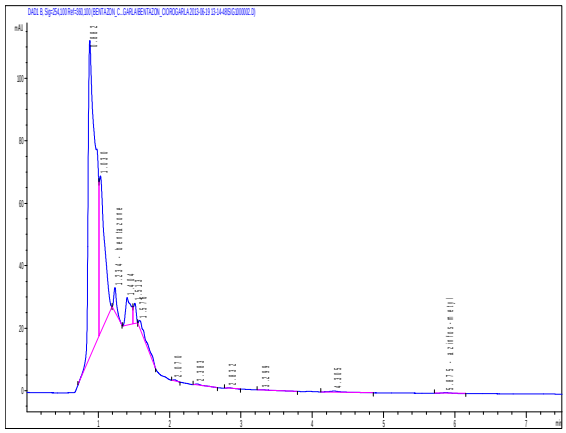


Fig.3b-Chromatogram of Cirogarla water sample Downstream WWTP Magurele

Conclusions

An analytical method for the determination of trace concentrations of bentazon and azinphos-methyl in surface water was devolped. The proposed method includes off-line SPE, followed by HPLC–DAD detection. The chromatographic method is selective and sensitive.
Detection limits (LODs) of bentazon and azinphos-methyl for the overall method were 0.09µg/L and 0.11µg/L, respectively. High recoveries of > 95% were obtained for both analyzed compounds. This illustrates the applicability of this method for monitoring the presence of bentazon and azinphos-methyl in surface waters.
The azinphos-methyl was determined in Ciorogarla river at concentrations (>0.18 µg/L) biger than the admisible value imposed by romanian legislation.

Acknowledgement

This study was financially supported by The Ministry of National Education through The National Research Program (CORE), Project PN 09-13.01.10.

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