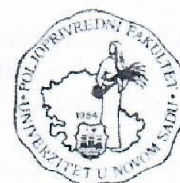




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SOLID PHASE EXTRACTION IN THE ANALYSIS OF BROMOPHOS RESIDUES IN BARLEY MALT

Grahovac, N.¹, Zeremski-Škorić, T., Sekulić, P., Jakšić, S., Radović, B.¹

Summary: Applicability of solid phase extraction (SPE) in determination of pesticide residues in cereals was confirmed by measuring bromophos contents in barley malt. The method entails simple extraction of pesticide residues from sample with acetonitrile followed by solid phase extraction (SPE) clean-up step prior to the final determination by gas chromatography with mass spectrometry. It is evaluated that performance of the method, recoveries bromophos ranged between 80.1 and 129% with associated relative standard deviations between 2 and 13%. Limits of detection of bromophos were less or equal to 0.0013 mg/kg. The applicability of the proposed method to detect and quantify pesticide residues has been demonstrated in the analysis of 13 real samples. The findings for bromophos in barley malt samples were below the EU regulation MRL value for barley as well as in conformance with the currently valid regulation of Serbia.

Key words: SPE, barley malt, bromophos, residues pesticide.

Introduction

A multitude of xenobiotic compounds are released worldwide every year. Among these, pesticides play an important role, as their use cannot be neglected because of the enormous benefits in agricultural outputs. However, owing to their occurrence and possible toxicity in the environment and foods, it is important to monitor their residues. An important aspect of the determination of pesticide residues in complex matrices is the application of efficient extraction and purification of target analytes from the sample matrices. An appropriate method should be capable of separating the target analytes from other substances that might interfere with the analysis as much as possible [1,2]. Therefore, the choice of an adequate determinative technique is of paramount importance to give reliable results in samples with a heavy matrix burden.

Bromophos is a broad spectrum, non-cumulative non-systemic organophosphorus insecticide which is not included as an active substance in Annex I to Directive 91/414/EEC (Commission Regulation (EC) No 2004/129/EC and No 2076/2002). Bromophos as an active substance is banned for trading in the Republic of Serbia [3-6].

The aim of our work was the development of a sensitive and selective method for the quantitative determination of bromophos from barley malt using MS detection. The work focused on SPE/clean-up in the analysis of bromophos in barley malt.

Material and methods

The procedure consists of extracting bromophos from homogenised sample barley malt by mechanical shaking with 20 mL acetonitrile and addition of sodium chloride [7-9]. An advantage of extracting with acetonitrile rather than other solvents is that acetonitrile more easily separates pesticides from sample with the addition of salts, which provides a well-defined phase separation without dilution with hazardous non-polar organic solvents and also achieves high recoveries pesticides [10].

Clean-up is necessary in order to reduce the detection limits of methods and/or to avoid interferences from the matrix barley malt. Solid-phase extraction was carried out using C₁₈ columns packed with 500 mg of highly cross-linked octadecyl sorbent. The extraction columns were conditioned by passing 6ml of acetonitrile through the column. The sorbent was never allowed to dry during the conditioning and sample loading steps. Exactly 4 mL of the extract barley malt (equivalent to 2 g sample) was transferred to the column. Anhydrous sodium sulfate was added to the previously collected eluates in order to remove water from the eluate [11]. Afterwards, a 2 mL (equivalent to 1 g sample) aliquot of the eluates was transferred on connected ENVI Carb (6mL, 500mg) and an amino propyl column (6mL, 500mg). The pesticides were eluted with 20 mL of acetonitrile-toluene 3:1 into a 50 mL tube. The eluate was evaporated to less than 3 mL using evaporator with a water bath at 35°C, and solvent exchange to acetone was performed by adding twice 10 mL portions of acetone and evaporating to low volume after each addition. The extract was quantitatively transferred to a 2 mL GC vial. Fortified samples were prepared by spiking 10 g of barley malt samples, previously homogenised with different known volumes of working standard bromophos solutions.

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A standard stock solution containing bromophos (Dr. Ehrenstorfer, Ausburg, Germany, 98,50% purity) was prepared at concentration of about 100 µg/mL in methanol (J.T.Baker, USA). Calibration standards were prepared by dilution standard stock solution in methanol. Working standard bromophos solutions were used to calibrate the GC/MS system and spike samples barley malt in recovery experiments.

Determinations were performed using Agilent Technology 6890 N gas chromatograph with an HP5975 B mass selective detector employed. The gas chromatograph was equipped with CTC CombiPAL sampler and split/splitless injector with electronic pressure control. The mass spectrometer was operated in the electron impact (70eV) selected ion monitoring (SIM) mode. Identification of the analytes studied was done by comparing mass spectra and retention times of the barley malt samples with the aforementioned standards. The identification was confirmed by comparing the relative abundances of the three ions (one quantifier and two qualifiers) of the experimental standards well-known relative abundances of the US National Institute of Standards and Technology (NIST) library reference spectra. Bromophos was identified using 330.90, 328.90, 125 and 332.90 ions.

Results and discussion

The linearity of the calibration curves was evaluated at a concentration range between 0.005 and 0.05 µg/mL using three calibration solutions prepared in methanol (Table 1). For positive identification of pesticides in samples the presence of all three ions and retention time (Table 1) in the correct ratio was necessary [1].

Table 1. Quantitation (target) ions, identification (confirmation) ions and calibration levels of pesticides

Pesticides	Quantitation ion	Identification ion (<i>m/z</i>)	RT (minute)	STD 1 (µg/mL)	STD 2 (µg/mL)	STD 3 (µg/mL)
Bromophos	330.90	328.90, 125	22.251	0.005	0.01	0.05

The response function was found to be linear with a coefficient of determination (R^2) higher than 0.998 in the tested range for bromophos. Calculations were done using the peak areas. Bromophos peak areas, each value calculated as a mean of three experimental runs. Linearity for bromophos is evidently attained in the whole range investigated. The mean recoveries were in the range of 80.1-129% at these two spiking levels with associated relative standard deviations (RSDs) in the range of 2–13%. Some loss in recoveries was noted probably because of the influence matrix. This may be due to matrix effects and suppression of ionization. Using the standard addition method, bromophos concentration in the barley malt was determined at 15.08 mg/kg which were quantified with standards at a concentration range between 5 and 50 µg/mL.

The limit of detection was computed as three times the base line noise of the solvent sample used as a blank and the value of 0.0013 mg/kg was obtained. The concentrated sample extracts may contain a high content of co-extractives, which can damage the capillary GC column as well as result in a matrix enhancement effect [11]. For most cereal samples the final extracts using the proposed SPE method were clean enough for direct GC-MS analysis. The addition of the C18 and amino propyl clean-up did not compromise recoveries for bromophos. Fig. 1 illustrates a comparison of SIM chromatograms obtained for blank extract and corresponding barley malt sample fortified at 10 mg/kg.

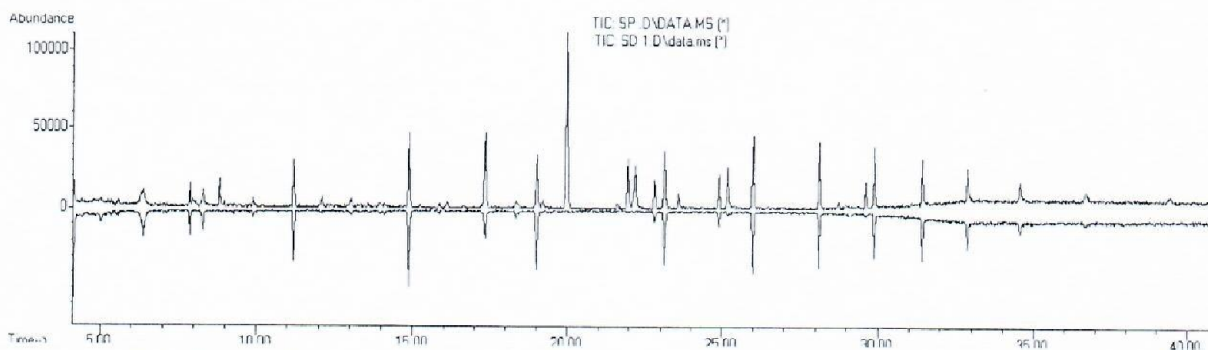


Fig. 1. Comparison of SIM chromatograms of blank barley malt extract (lower) and fortified sample of barley malt (upper)

Besides the regularly shaped and well defined peaks of the pesticides investigated, SPE-GC-MS chromatograms (Fig. 1) contain a group of peaks likely to originate from the barley malt matrix. There were no significant interfering peaks present in the elution region of bromophos. The corresponding range of pesticide concentrations in the samples of barley malt was 0.014 and 0.034 mg/kg for bromophos in the 13 analyzed samples. The positive results could be attributed to the high sensitivity of the developed GC-MS method as well as to the fact that most cereal grain samples, which were stored in granaries, were treated with pesticides to protect against pests.

Conclusion

In this work a SPE-GC-MS method to for determining the bromophos pesticide in difficult matrices such as cereals was developed. The recoveries of the method ranged between 80.1 and 129% for bromophos with associated relative standard deviations (RSDs) between 2 and 13%. Calibration dependencies and the corresponding regression coefficient values show that linearity was achieved in the SPE-GC-MS measuring procedure within the concentration range studied. Measurement repeatability is confirmed by the RSD values calculated for the measuring procedure applied. Comparing the bromophos content measured in the barley malt sample with MRL value of the relevant regulation for barley (0.05 mg/kg), it is obvious that the SPE method is sensitive enough to monitor pesticide residues within the concentration range well below the MRL values for barley [5-6]. Possibly, further improvement in performance characteristics of this method might be achieved by the use of a more extensive clean-up step.

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