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# ASSESSEMENT OF TRIAZINE HERBICIDES CONTENT IN HONEY SAMPLES BY SOLID-PHASE EXTRACTION AND HPLC ANALYSIS

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**ABSTRACT.** Triazines are a group of compounds used as selective pre-emergence herbicides in different types of crops, such as corn, soybean, as well as orchards and vineyards. Due to their large use in the agricultural sector triazines have been detected in different types of food matrices. In the present study, the triazines content in different honey samples collected directly from private producers in various countryside areas from Romania is evaluated. The clean-up and extraction procedure was carried out by solid phase extraction and further analysis and quantification undertaken with high performance liquid chromatography. In the analyzed samples, the presence of triazine herbicides was detected. Their concentrations ranged between 4.97 and 997.5 µg/kg honey, exceeding in almost all analyzed samples the EU MRLs requirements for triazines in honey.

***Keywords****: triazine herbicides, honey, solid phase extraction, high-performance liquid chromatography*

## INTRODUCTION

The agricultural sector is the most significant user of pesticides. In the long term, these chemicals can cause deleterious effects upon the environment [1]. Contamination of different matrices allows pesticides to spread through the food chain, thus impacting human health [2, 3]. Therefore, the European Union has established Maximum Residue Levels (MRLs) in order to meet food safety requirements, the maximum residual levels of pesticides being established in Regulation (EC) No. 396/2005, the lowest limit being 0.01 mg/kg and the highest 1 mg/kg.

Triazines represent a class of compounds used as selective pre-emergence herbicides in different types of crops, such as corn, soybean, as well as orchards and vineyards Their persistence in soil has been shown to

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have a $DT_{50}$ (half-life) of 40 days and can reach up to 166 weeks, depending on the soil type [4, 5].

Different studies showed the presence of triazine herbicides in various matrices such as water [6-10], soil [4, 5] agricultural products [11], seaweeds [12] food samples [13-15] etc. which suggest that these chemicals are mobile in the environment.

Taking into account that triazine compounds can act as endocrine disruptors, mimicking hormonal activity [16], in the last period different methods for the analysis of these compounds in various matrices have been developed.

The most employed techniques involved in the quantification of triazine herbicides are gas and liquid chromatography [6-18].

For the extraction, different methods, such as liquid phase extraction (LPE) [8, 9, 11, 13, 14], solid phase extraction (SPE) [10, 12, 17] or supercritical fluid extraction (SFE) [18, 19] have been used.

Since LPE requires the use of significant volumes of toxic organic solvent and SFE involves an expensive instrumentation, SPE remains the most suitable method for the analysis of triazine herbicides in liquid samples.

Honey is a staple product consumed by a large percentage of society due to its beneficial properties on human health. One of the most important checked parameter from honey samples is the pesticides residue.

Pesticides residue can get into honey via spraying the crops during the collection of pollen and nectar [20]. Due to this reason the assessment of pesticides pertaining to this matrix presents importance for quantifying their risk to human health.

In this paper, the assessment of triazine herbicides content in honey samples collected directly from private producers in various countryside areas from Romania located in Transylvania, Moldova and Dobrogea is reported. The analysis of triazines was performed by solid-phase extraction followed by high performance liquid chromatography analysis. The results of the analyzed samples showed the presence of triazine herbicides in concentrations ranging from tens to hundreds μg/kg honey.

The novelty of this work consists in the assessment of triazine content in matrices that is not subjected to routine monitoring. Thus, the risk associated with their consumption is difficult to predict.

## RESULTS AND DISCUSSION

### 1. Analytical performance of the analysis method

The performance of the HPLC method used for the analysis of triazine herbicides in honey samples was expressed by precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) (Table 1).

Precision was expressed as intra-day precision (repeatability) by means of five replicates (n = 5) of a triazine standard mixture in concentration of 1.25 µg/mL. The obtained results were situated under 3% which prove a good repeatability of the method.

**Table 1.** Analytical performances of HPLC method

| Compound | Linear curve equation (linear range 0.62-10 ng) | $R^2$ | Slope | SD | LOD (µg/L) | LOQ (µg/L) | RSD % |
|---|---|---|---|---|---|---|---|
| Simazine | y = 49916x - 3596.3 | 0.996 | 49916 | 379 | 0.023 | 0.076 | 1.24 |
| Prometon | y = 46067x - 782.58 | 0.992 | 46067 | 542 | 0.035 | 0.118 | 1.75 |
| Atrazine | y = 42868x - 3430.5 | 0.999 | 42868 | 797.9 | 0.056 | 0.186 | 2.98 |
| Ametryn | y = 43269x + 1969.7 | 0.998 | 43269 | 452 | 0.031 | 0.104 | 1.55 |
| Propazine | y = 43269x + 1969.7 | 0.999 | 43269 | 586.1 | 0.041 | 0.135 | 1.98 |
| Prometryn | y = 37949x + 2154.8 | 0.996 | 37949 | 441.9 | 0.035 | 0.116 | 1.67 |
| Terbutryn | y = 37711x + 3049.9 | 0.999 | 37711 | 673.0 | 0.054 | 0.179 | 2.64 |

$R^2$ - coefficient of determination; SD - standard deviation; LOD - limit of detection, LOQ - limit of quantification, RSD - relative standard deviation for (n = 5);

The quantification of the target compounds in real samples was made using the calibration curve method. The data from Table 1 shows a good linearity for all target triazines and the R2 values ranging from 0.992 to 0.999. LOD and LOQ of studied triazines were determined using the standard deviation and the slope of each calibration curve. LODs were situated in the range of 0.076 - 0.056 µg/L and LOQs in the range of 0.076 – 0.18 µg/L, respectively.

## 2. Accuracy of SPE procedure

Because for the extraction of the triazines from honey samples a slightly modified Albero et al. [21] method was used, the accuracy of the extraction procedure was tested.

The accuracy was expressed by extraction recovery (ER) and was calculated using the follow equation:

$$ER\,(\%) = \frac{(amount\ found - initial\ amount)}{spiked\ amount}\ x\,100$$

The results presented in Table 2 show good recoveries of the studied compounds, the values being situated over 80%. Thus the SPE protocol has been used for the extraction of the triazine herbicides from collected honey samples.

**Table 2.** Recoveries of the triazine herbicides in honey samples

| Compound | Initial Amount (ng) | Spiked amount (ng) | Amount found(*) (ng) | ER ± SD (%) |
|---|---|---|---|---|
| Simazine | - | 500 | 446 | 89.2 ± 0.70 |
| Prometon | - | 500 | 446 | 89.2 ± 1.50 |
| Atrazine | - | 500 | 483 | 96.6 ± 1.53 |
| Ametryn | - | 500 | 480 | 96 ± 2.08 |
| Propazine | - | 500 | 437 | 87.4 ± 2.52 |
| Prometryn | - | 500 | 439 | 87.8 ± 3.51 |
| Terbutryn | - | 500 | 460 | 92 ± 3.56 |

(*) - mean value of three replicates

## 3. Analysis of honey samples

The analyses employed in the present study demonstrate the presence of triazines in the honey samples. In Figure 1 it is shown a juxtaposed chromatogram of a honey sample and a standard mixture where the presence of the triazines can be observed.

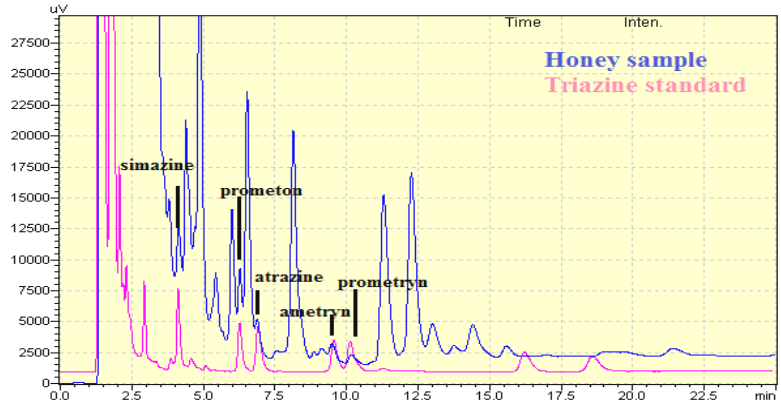


**Figure 1.** Chromatogram of tested triazines in honey sample (honey sample – upper chromatogram; triazine standard mixture (1.25 µg $mL^{-1}$) – lower chromatogram)

The results of the analyzed samples showed that the concentrations of triazine herbicides from the honey samples are dependent on the sampling area (Table 3).

Thus, the highest amounts of triazine herbicides were found in the samples collected from Dobrogea and Moldova areas, where modern agriculture is practiced which involves the use of pesticides for plant treatments. The found concentrations ranged between 50.2 and 102.11 µg/kg in Tulcea County (sample 4T, 5T) and 80.67 and 997.54 µg/kg in Vaslui County (samples 7V, 8V, 9V, 10V, 11V, 12V, 13 V).

**Table 3.** The occurence of triazine herbicides in analyzed honey samples

| Sample code | Concentration (µg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Simazine | Prometon | Atrazine | Ametryn | Propazine | Prometryn | Terbutryn | Total |
| 2 C | nd | nd | 1.19 | nd | 5.65 | nd | nd | 6.84 |
| 3 C | nd | nd | 4.97 | nd | nd | nd | nd | 4.97 |
| 4 T | 50.20 | nd | nd | nd | nd | nd | nd | 50.20 |
| 5 T | 34.59 | 26.08 | 11.18 | 16.34 | 13.92 | nd | nd | 102.11 |
| 7 V | 80.49 | nd | nd | nd | nd | nd | 34.58 | 115.07 |
| 8 V | 58.56 | 54.96 | nd | nd | nd | nd | nd | 113.52 |
| 9 V | 3.80 | 7.80 | 75.97 | 60.29 | 88.74 | nd | 15.00 | 251.6 |
| 10 V | 116.05 | 31.66 | nd | nd | 20.04 | nd | nd | 167.75 |
| 11 V | 82.05 | 17.15 | nd | nd | 4.78 | nd | nd | 103.98 |
| 12 V | 32.73 | nd | nd | nd | nd | nd | 47.94 | 80.67 |
| 13 V | 490.0 | 507.5 | nd | nd | nd | nd | nd | 997.5 |
| 14 B | nd | nd | nd | nd | nd | nd | nd | - |

nd- not detected

The lowest concentrations ranged between 4.97 and 6.84 µg/kg were found in samples collected from Cluj County (sample 2 C and 3 C), while in the sample collected from Bistrita-Năsăud County (sample 14 B) triazines did not occur. In these two regions usually traditional agriculture is practiced and the amount of pesticides use for plant treatments is low or entirely missing.

The high concentrations of triazine herbicides found in acacia honey samples (sample 8V, 9V, 11V,) could be explained by the fact that the flowering of acacia tree corresponds to the period in which these herbicides have been applied on the corn crop.

It is also observed that the most prevalent herbicides are simazine and prometon while prometryn was not found in any samples.

If it is taken into consideration that atrazine, simazine and terbutryn have been recently introduced on the list of priority substances regarding the Water Framework Directive (WFD) (2000/60/EC) their use in agriculture should be limited.

Moreover, analyzing the obtained results and taking into consideration that the MRLs for simazine in honey established by the EU is 0.01 mg/kg it can be observed that in the analyzed samples the total content of triazine exceeds the regulatory framework for those samples collected from Moldova and Dobrogea areas.

## CONCLUSIONS

This study showed the presence of triazine herbicides in honey samples in concentrations which are dependent on the sampling zone.

The highest content of triazines which exceeds the EU regulatory framework was found in the samples collected from Moldova and Dobrogea, areas where modern agriculture is practiced.

In Transylvania area where usually traditional agriculture is practiced the content of triazines does not exceed EU requirements.

The presence of triazine herbicides in honey sample obtained from tree flowers and poly-flora proves the mobility of these chemicals in environmental compartments.

## EXPERIMENTAL SECTION

### 1. Reagents and solutions

A standard mixture of seven triazine herbicides (simazine, prometon, atrazine, ametryn, propazine, prometryn and terbutryn) with a concentration of 100 µg/mL each herbicide, dissolved in methanol was purchased from Sigma-Aldrich (USA). The working solution with a concentration of 10 µg/mL for each herbicide was obtained by dilution of the standard mixture in methanol. Acetonitrile and methanol (HPLC grade) were obtained from Merck (Germany). Milli-Q water was prepared using a Milli-Q Plus water system from Millipore (USA). Potassium dihydrogen phosphate ($KH_2PO_4$) with a purity of 99% was purchased from Sigma-Aldrich.

### 2. Instrumentation and chromatographic conditions

Chromatographic separation and determination of the triazine herbicides were carried out on a Shimadzu high performance liquid chromatograph, equipped with a 10 LC pump, 10 LSD UV-Vis detector and a manual injection valve with a loop of 5 µL. A NovaPak-C18 column (30 cm × 3.9 mm, 4 µm, Waters, USA) was used for the separation of the compounds.

Separation of the analytes was performed by isocratic elution with a mixture of acetonitrile:phosphate buffer (25 mM) (40:60, *v/v*) at a flow rate of 1.2 mL/min. The detection wavelength was set at 220 nm.

The quantification of the target compounds in real samples was made by means of the calibration curve. For this purpose five standard solutions in concentration of 0.625; 1.25; 2.5; 5 şi 10 µg/mL were prepared by dilution of the standard mixture with methanol. The calibration curves were built using the chromatographic peak area and the concentration of each triazine herbicide.

### 3. SPE procedure

Isolation and preconcentration of the target compounds from the honey samples were carried out on a SPE device (Supelco) using C18 EC (end capping) cartridges purchased from Phenomenex, USA. Before extraction

the SPE cartridges were conditioned in three steps using 5 mL Milli-Q water, followed by 5 mL MeOH and again by 5 mL Milli-Q water.

For the extraction of the triazine herbicides from the honey samples it was employed a slightly modified version of Albero et al. [21] as follows: 10 g of honey were dissolved in 40 mL mixture of Milli-Q water:methanol (70:30 *v/v*) and subjected to sonication for 15 minutes. The obtained solution was passed through the extraction cartridge at a flow rate of 2 mL/min for the retention of the herbicides. After that, the cartridge was washed by passing 5 mL of Milli-Q water through the cartridge in order to remove the interferences. Finally, the target compounds were eluted with 3 mL of methanol and evaporated to dryness under nitrogen. The residue was diluted in 0.5 mL methanol and then injected into the chromatographic system for the analysis of target compounds.

To study the accuracy of the SPE procedure 10 g of honey dissolved in 40 mL mixture of Milli-Q water:methanol (70:30 *v/v*) were spiked with 500 ng of each herbicide and extracted by SPE according to the protocol described above.

### 4. Sampling points

In order to have a better assessment of the triazine herbicides use in agriculture over their content in honey, three areas from Romania were taken into account. One area is situated in Transylvania (Cluj County and Bistriţa-Năsăud County), where traditional agriculture is practiced, and another two situated in Moldova (Vaslui County) and Dobrogea (Tulcea County) respectively, where modern agriculture is practiced (Figure 2).

The honey samples were collected in sterilized polyethylene bottles with a volume of 100 mL and kept at room temperature until analysis. Four types of honey were taken into consideration; acacia flower (sample 3C, 8V, 9V and 11V), sun flower (sample 5T, 7V and 10V), colza (sample 4T and 12V) and poly-flora (sample 2C, 13V and 14B).


**Figure 2.** The map of honey sampling areas

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