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IDENTIFICATION AND QUANTIFICATION OF HERBICIDE CONTAMINANTS FROM INPUTS USED FOR ORGANIC AGRICULTURE

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Abstract

Organic agriculture is increasing in Romania, especially due to a better understanding of the effect of conventional agriculture on biodiversity. Being a relatively new implemented concept, there is a gap in the regulations regarding contaminants in organic inputs. Therefore, there are no available standard methods used to quantify contaminants such as pesticides from inputs (fertilisers) used for organic farming. This study aims to develop and to validate a quantification method to analyse the triazine contaminants (Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn) from organic foliar fertilizer using UPLC-PDA. Commercialy available types of fertilizer were used as complex matrix (Codamix). The quantification method was evaluated in terms of linearity, limit of detection and limit of quantification, BIAS, recovery, precision of repeatability and precision of reproducibility. Based on the acquired data, the standard uncertainty of the method was also evaluated. The developed and validated method can be successfully optimised for other type of organic fertilisers.

Key words: *triazine; organic inputs; UPLC-PDA; method validation.*

INTRODUCTION

In Romania they are over 3000 certified producers in organic agriculture in 2021 (MADR, 2021), which in order to maintain their certification they need to use special fertilizers according to regulation EC 2008/889. Fertilizer obtained from biomass waste, using different types of processing (Aro Fatehi, 2017), may contain pesticide residues. In order to be used in organic agriculture all fertilizers should be tested to make sure that no contamination is present.

Triazines were one of the most frequently used group of herbicides, until they were suspected to be endocrine disrupters, moreover to cause different types of cancers and birth defects (Baranowska et al., 2012). The most extensively applied triazine prior to 2003 were Atrazine (2-chloro-6-ethylamino-4-isopropylamino-1,3,5-triazine) and Simazine (2-chloro-4,6-bis-ethylamino-1,3,5-triazine), members of s-triazine groups. Although atrazine has been banned since 2003 in EU, the parent compound and its metabolites are still detected in waters (Sass & Colangelo, 2006), soil and plants.

At EU level several triazines are still allowed in food products in different maximum residue amounts, for example: Atrazine - 0.05 mg/kg (EC 2016/440), Simazine - 0.02 mg/kg (EC 2011/310), Terbuthylazine - 0.01 mg/kg (EC 2021/618), depending on commodity type. Simetryne, Hexazinone, Ametryn, Prometryn, and Propazine are not accepted in food products, according with EU MRL database vers.2.2 (EU, 2021).

According to Romanian regulation regarding the triazine contaminants in environment, the normal values of total triazines is < 0.1 mg/kg of dry matter in soil (Order 756/1997). In case of alert steps should be less than 1 mg/kg of dry matter of in case of sensible soils and be less than 2 mg/kg of dry matter in case of less sensible soils. Specifications regarding pesticides in the fertilizers used for organic agriculture are missing from organic regulation, mainly because no one will expect to find pesticides in this area.

Currently, herbicides are mainly determined by gas (GC) (Dou et al., 2020) and liquid (LC) chromatographic methods coupled with different detectors such as, UV (Khana et al., 2018), PDA (Baranowska et al., 2012), tandem MS/MS (Patrudu et al., 2020), but also by special methods such as surface-enhanced Raman spectroscopy coupled with an advanced chemometric model (Chen et al., 2015). High pressure liquid chromatography (HPLC) coupled with UV-Vis detection is still widely used technique for the analysis of non-volatile and high molecular weight pesticides which depend on its solubility in the mobile phase (Leong et al., 2020). Furthermore, HPLC is known to be more rapid and efficient than the GC-MS. Shah et al. (2011) determined triazine herbicides (Atrazine, Metribuzin, Ametryn, and Terbutryn), in soil samples with HPLC-UV detection. In another study, the HPLC coupled with MS analysis was used for the evaluation of the sorption and persistence of Atrazine, Propazine, Simazine, Terbuthylazine, Prometon, Prometryn, Simetryne, Terbutryn, Metamitron and Metribuzin in agro-industrial and composted organic wastes (sheep manure, spent coffee grounds, composted pine bark and coir) (Fenoll et al., 2014).

The aim of this study was to develop and to validate an analytical method based on UPLC-PDA for the identification and the quantifycation of eight most common triazine contaminants in organic fertilisers. This method can be successfully optimised for other type of organic fertilisers based on biomass waste material.

MATERIALS AND METHODS

Chemicals and reagents

The standard solution was prepared using reference materials of Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn with a purity of more than 98%, purchased from Dr. Ehrenstorfer. Acetone, ethyl acetate, acetonitrile (ACN), sodium sulphate and sodium chloride were purchased from Merck KGaA. All of the other chemicals used were of analytical grade.

Samples

The development and validation of the analytical method was performed using lignosulfonate fertilizer CODAMIX as test matrix which was purchased from agrochemical market. CODAMIX is a formulation of trace elements chelated with citric acid, lignosulfonic acids, which is completely soluble in water and specially created to complement NPK fertilizers for both hydroponic and fertilized crops. The tested matrices were not contaminated with any of the herbicides investigated in this study and therefore they are suitable to be used as blank samples. The samples were stored at room temperature during the analysis.

Extraction and concentration

The extraction and concentration method were developed based on ISO 11264:2005. In our case, 5 ± 0.05 g of sample was weighed in a 100 mL Erlenmeyer flask, and the necessary water was added in order to keep a ratio between 10: 20: 15 of water: acetone: ethyl acetate. A volume of 20 mL of acetone was added and the samples were homogenised for six hours on a mechanical stirrer (IKA KS 260). After 5 h, 3 g of sodium chloride was added to the samples and 15 mL of ethyl acetate. The samples were stirred again for 5 minutes, and then 14 mL of the extract was transferred through a filter paper containing anhydrous natrium sulphate in order to remove the water excess into a 50 mL pear shaped rotavapor flask. Extract was concentrated to 1 mL volume on a Heidolph Laborota 4000 rotavapor, then 2 mL of acetonitrile was added in the rotavapor flask, and concentrated under 1 mL. The procedure was repeated 2 times. The final extract was adjusted with acetonitrile to 1 mL and additional 1 mL of ultrapure water was added. The final volume was mixed, filtered through a 0.45 µm filter, collected in a 1.8 mL vial, injected and subjected to UPLC-PDA analysis (Figure 1).

Lignosulfonate fertilizer samples were spiked with herbicides standard at a concentration of 2.5 mg/kg. The spiked samples were subjected to extraction according to the method described above in order to assess the 3 important R: repeatability, reproducibility and recovery.

UPLC-PDA instrument and chromatographic conditions

A Waters Acquity I chromatographic system was used for herbicide analysis, equipped with a binary pump, autosampler, PDA detector and a Zorbax Eclipse Plus C18 4.6 x 100 mm, 5 µm column. The following chromatographic conditions were used: the samples were maintained at 10°C and the injection volume was 10 µL. Column was kept at 30°C during the analysis and the PDA detector was set to register the spectrum from 210 to 320 nm, and also at wavelengths of 245 nm for Hexazinone herbicide and 220 nm for the other 7 herbicides. The flow rate of the mobile phase was 1 mL/min and the gradient is presented in the Table 1.

Method validation

For the method validation several parameters were taken into account: linearity, limit of detection, limit of quantification, accuracy, precision of repeatability, precision of reproducibility and recovery (EURACHEM, 2014).

Table 1. Gradient condition

Time (min)	**A% (H_2O)**	**B% (ACN)**	**Curve**
Initial	85	15	Initial
5	85	15	6
25	40	60	6
30	85	15	6
33	85	15	6

Linearity

For the calibration curve, five concentrations in the range 0.1-10 µg/mL of herbicide standard solution in methanol were used. First, a stock solution of individual herbicide Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn in 2-propanol or acetone with a concentration of 1 mg/mL was prepared. The equipment response and the calibration curve validity were checked with a different herbicide solution at five levels of concentration (0.1, 0.5, 1, 5 and 10 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 0.2, 1, 2, 10, 20 µg/mL for Hexazinone). For linearity evaluation, two coefficients were calculated, correlation coefficient (r) and coefficient of variation, in order to obtain the best result.

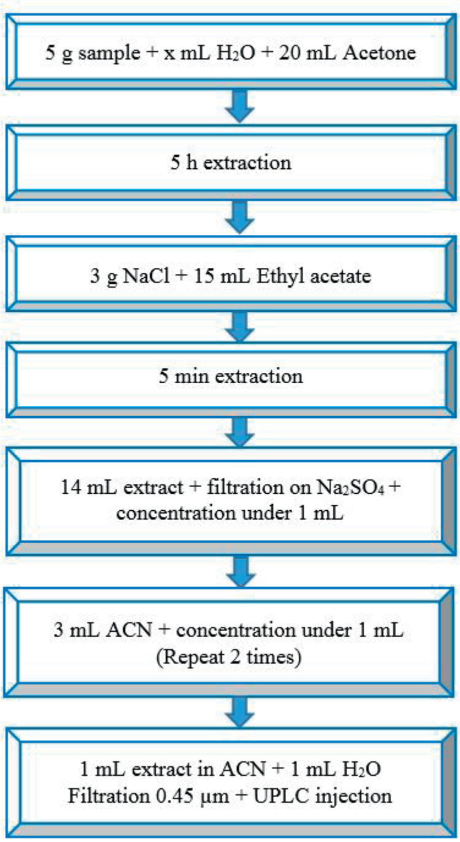

Figure 1. Schematic representation of the extraction and concentration of fertilizer samples

Precision and recovery

The precision of the method was evaluated based on the relative standard deviation (RSD) of the intra-day precision (RSDr %) and the inter-day precision (RSDR %), both for standard solutions and spiked samples. The RSDR values are calculated using Horwitz equation, a generalised precision equation which has been found to be independent of analyte and matrix but dependent on concentration.

One concentration of herbicide standard mix solution was prepared, namely 2.5 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 5 µg/mL for Hexazinone). The accuracy and precision of repeatability was determined based on the analysis of 10 freshly prepared standards on the same day by one analyst, and the precision of reproducibility was determined by analysing the mix solutions in different days using 2 operators. Also, recovery and precision tests were conducted by analysing spiked blank samples at the concentration of 2.5 mg/kg, since there are no imposed MRL, and the only specification in European regulation regarding the use of mixed inputs is "*the use of the EU fertilising product as specified in the use instructions must not lead to the exceedance of those limit values in food or feed*" (EC 2019/1009). For the precision of repeatability six samples have been analysed and for the precision of reproducibility the samples were analysed in eleven different days with three replicates.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limit is the lowest amount of herbicides in a sample which can be detected, under well-specified conditions, but not necessarily quantified as an exact value, and the quantification limit represents the lowest amount of herbicides in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD value is the point at which a true signal is detectable from the noise of the detector, the ratio has to be larger than a three to one signal to noise, and for the LOQ the determined value of signal to noise ratio is larger than 10. For this analytical method, samples were spiked at low concentrations until a detectable signal was obtained, and the results were repeatable.

Measurement Uncertainty

Quantification of measurement uncertainty was determined taking into consideration the following sources: standard uncertainty from control solution, uncertainty derived from linearity (coefficient of variation), uncertainty derived from precision of repeatability of control solution, uncertainty derived from BIAS and uncertainty derived from precision of reproducibility of spiked samples.

RESULTS AND DISCUSSIONS

Optimization of extraction and analysis method

Water, acetone, 2-propanol and ethyl acetate are considered to be chemicals that present the least negative environmental impact. Due to their low environmental impact, they are also popularly denoted as a 'Green Solvent' (Welton, 2015; Byrne et al., 2016). Water has a great importance in extraction of pesticides, low water content can lead to low recoveries in triazine herbicide extraction.

As guided by ISO 11264:2005 different solvents were tested for triazine extraction from lignosulfonate fertilizer, each with different effect on the matrix. Dichloromethane has a polymerization effect on the lignosulfonate fertilizer, making it impossible to separate the liquid phase from the solid phase in order to proceed with pesticide extraction. Petroleum ether showed a good recovery, but is preferred to be avoided due to its high volatility. Ethyl acetate is recognized for its lower recovery (about 5-10% lower) compared to other solvents (petroleum ether, hexane, dichloromethane, and so on) but it was selected due to his smaller impact on environment. Taking into consideration good results for the intra-day precision (RSDr %) and the inter-day precision (RSDR %), the recovery can be introduced in calculation of the final amount of pesticides in the fertilizer matrix.

Another important parameter in the triazine extraction from fertilizer matrix is the mixing time between sample, water and acetone. Lowering the contact time between samples and extraction solvent under 5 h could lead to lower recoveries (ISO 11264:2005), hence when developing a new method, careful consideration should be applied to adjusting the extraction time to obtain an acceptable recovery (in general higher of 70%).

Good separation between the triazine herbicide was obtained as showed by Figure 2, with the following elution order: Hexazinone (13.107 min), Simazine (13.531 min), Simetryne (16.323 min), Atrazine (16.899 min), Ametryn (19.381 min), Propazine (19.954 min), Terbuthylazine (20.817 min), and Prometryn (22.222 min). Even though Hexazinone was quantified at 245 mn, the herbicide showed lower intensity in absorbance compared to the other herbicides, therefore the concentration of the analytical standard and spiked sample had to be doubled.

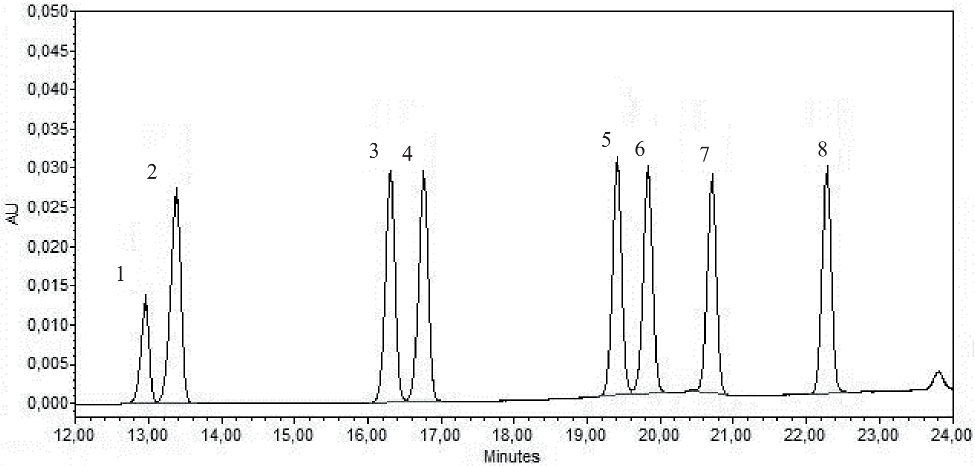

Figure 2. Analytical standard chromatographic profile at 220 nm at a concentration of 2.5 µg/mL (1-Hexazinone, 2-Simazine, 3-Simetryne, 4-Atrazine, 5-Ametryn, 6-Propazine, 7-Terbuthylazine, and 8-Prometryn)

Linearity

Under the described optimum conditions, the plotting of the calibration curves (Figure 3) was achieved using five concentration levels of the reference material of herbicide solutions, with two replications each. An excellent linearity over the relevant working range of 0.1-10 µg/mL for Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, Prometryn and 0.2-20 µg/mL for Hexazinone, as shown by correlation coefficient r, which was higher than 0.9999 (Table 2) was obtained. The values of the parameters that characterize the calibration curve for triazine herbicides are also listed in the Table 2. Another coefficient taken into consideration for the linearity of an analyte, is the coefficient of variation, calculated based on ANOVA, which shows a good linearity for all herbicides, with values from 0.441% for Atrazine to 1.866% for Simetryne (Table 2).

Accuracy and BIAS

The lowest accuracy was observed for Simazine, 89.47% mainly due to a poor dissolution of the powdered standard in the organic solvent. Even though the other 7 triazine herbicides were dissolved in 2-propanol, Simazine shows a low solubility to this solvent, hence acetone was used instead. Particular care should take place when preparing the intermediary solution, due to Simazine tendency to precipitate when the stock solution is stored at 4 ± 2°C.

Table 2. Linearity criteria of triazine herbicide

Herbicide	RT (min)	Coefficient of correlation (r)	Coefficient of variation (%)
Hexazinone	13.107	1.0000	0.571
Simazine	13.531	1.0000	0.528
Simetryne	16.323	0.9999	1.866
Atrazine	16.899	1.0000	0.441
Ametryn	19.381	1.0000	1.101
Propazine	19.954	1.0000	1.307
Terbuthylazine	20.817	1.0000	1.405
Prometryn	22.222	1.0000	0.571

All other triazines had a good accuracy, in the interval of 100 -110% (Table 3), with respect to EURACHEM Guide, which recommends that the accuracy should be between 70-110%. For the selected triazines BIAS or trueness showed good values, between 0.90% for Hexazinone and 5.44% for Prometryn, with the exception of Simazine, as explained above (Table 3).

Table 3. Accuracy and BIAS of triazine herbicide obtained at a concentration of 2.5 µg/mL

Herbicide	**Mean concentration (µg/mL)**	***Accuracy (%)***	***BIAS (%)***
Hexazinone	5.04	100.90	0.90
Simazine*	2.04	89.47	-10.53
Simetryne	2.53	101.08	1.08
Atrazine	2.55	102.03	2.03
Ametryn	2.63	105.10	5.10
Propazine	2.57	102.76	2.76
Terbuthylazine	2.57	102.64	2.64
Prometryn	2.64	105.44	5.44

**Special consideration should be paid to Simazine due to difficulty of dissolution in solvent*

Precision and recovery

The intra-day and inter-day precision of triazine mix in samples was evaluated using standard addition in the blank samples. The results obtained for both standard solutions and spiked samples are showed in Table 4.

Table 4. Intra-day and inter-day precision

Herbicide	**Conc. (µg/mL)**	***Standard solution***		***Spiked samples***	
		RSDr (%)	***RSDR (%)***	***RSDr (%)***	***RSDR (%)***
Hexazinone	5	0.89	1.39	10.2	16.3
Simazine	2.5	0.91	1.45	7.4	11.9
Simetryne	2.5	0.94	1.50	11.8	18.9
Atrazine	2.5	0.99	1.59	9.5	15.3
Ametryn	2.5	1.41	2.25	6.8	10.8
Propazine	2.5	2.23	3.56	8.3	13.3
Terbuthylazine	2.5	1.15	1.85	8.1	13.0
Prometryn	2.5	0.96	1.53	6.7	10.7

The obtained RSDr for standard solutions ranged from 0.89 % for Hexazinone to 2.23% for Propazine, which indicated that the equipment method is highly repeatable. Moreover, the RSDR ranged from 1.39% for Hexazinone to 3.56% for Propazine, emphasizing a good precision of selected method, and also showing a low influence on the overall uncertainty. When we analyse the results of RSDr % of the spiked matrix, it can be observed that the highest value (11.8%) was obtained for Simetryne, followed by Hexazinone with 10.2%. The lowest RSDr % were obtained for Prometryn (6.7%), closely followed by Ametryn with 6.8%.

The RSDr % values obtained after spiking lignosulfonate fertilizer the triazine herbicide were less than 20% which indicated a good precision of repeatability, within the acceptable limits imposed by the referential in the pesticide field (SANTE, 2017). The same trend is observed also for RSDR % values, the obtained results, even though higher, are still in compliance with validation criteria imposed by SANTE (20%), therefore the overall method doesn't require optimisation or improvement of the extraction method. The recovery values for all the triazine herbicide in spiked lignosulfonate fertilizer were 70%. Simazine coelutes with a compound found in the lignosulfonate fertilizer, the analyte being selected based on the full spectra of the peak at the known retention time (Figure 4). The matrix effect can be removed using SPE (solid phase extraction) but lower recoveries should be expected due to retention of analyte on the sorbent (such as silica, florisil). The best recovery was obtained for Prometryn with 100.82%, followed by Simetryne (80.35%) and Terbuthylazine (80.34%). The rest of the triazine herbicide had a recovery lower that 80% as follows: Hexazinone - 78.07%, Simazine - 79.24%, Atrazine 71.83%, Ametryn - 71.89%, and Propazine - 75.58%. Even though the recoveries were in the accepted interval for pesticide residues analysis, we recommend that when real unknown samples are received, spike with the concern analyte should be performed, in order to obtain a result closer to the true value.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values of LOD and LOQ parameters were found by reporting the processed integrated peak from the UPLC chromatograms to the noise. Thus, for the LOD value the ratio has been 3: 1 signal to noise ratio, and the LOQ value was determined to be larger than 10 values.

The LOD was established as 1 mg/kg for Hexazinone and 0.5 mg/kg for all other triazine herbicides, while the LOQ value was 2 mg/kg for Hexazinone and 1 mg/kg for all other triazine herbicides. Spiked samples were extracted and analysed in triplicates in order to obtain the minimum reliable results. The values of LOD and LOQ can be improved by increasing the injection volume or by decreasing the concentration volume, in case the new regulations will require smaller concentrations to be determined in organic fertilizers.

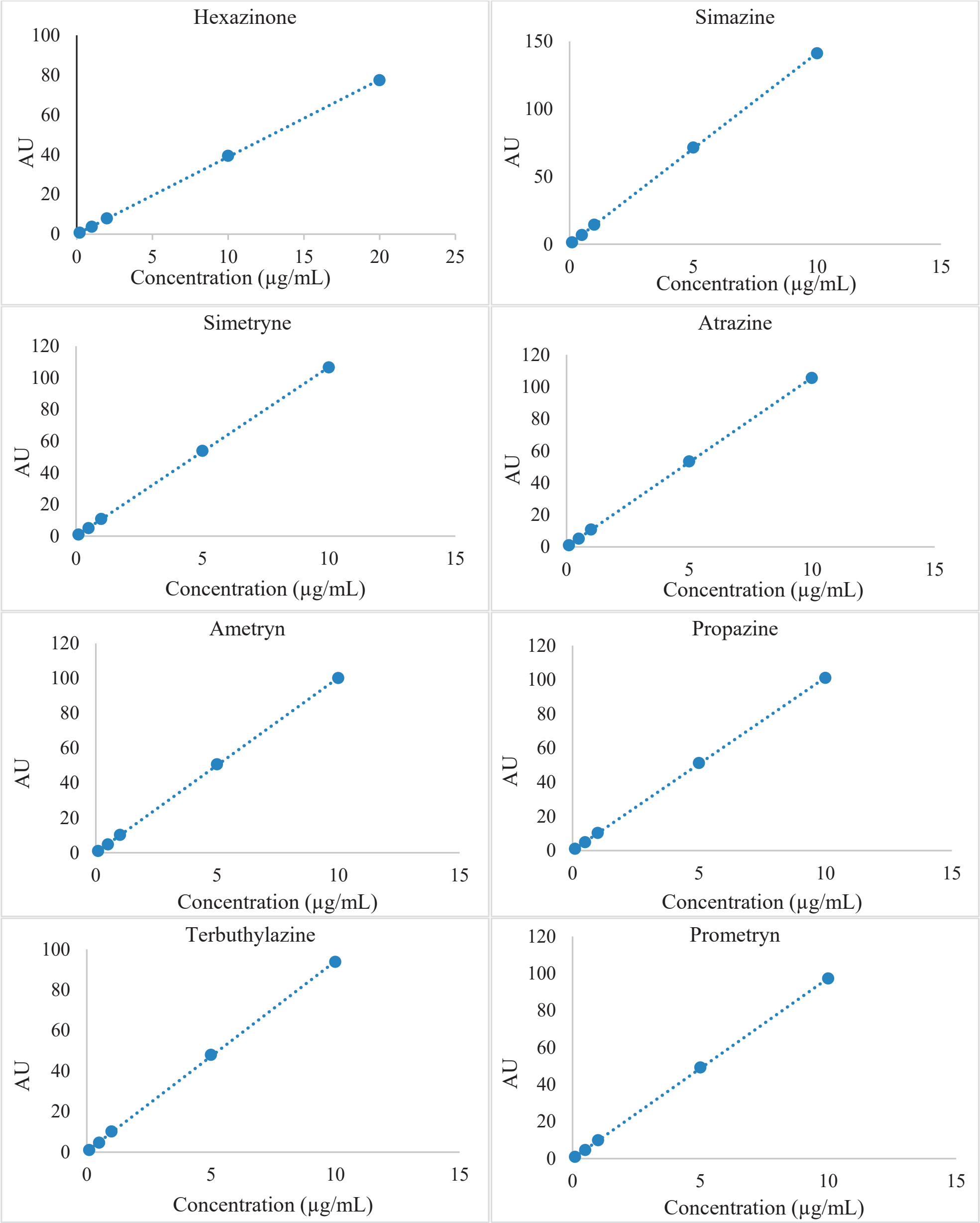

Figure 3. Calibration curves of 8 mixed triazine herbicides

Measurement Uncertainty

The main important factors which influenced standard method uncertainty with more than 30% are BIAS and sample RSDR. From the obtained results it can be observed that higher standard method uncertainty was obtained for Simetryne 19.4%. Similar values of standard uncertainty were obtained for Hexazinone (16.8%), Simazine (16.3%), and Atrazine (15.9%).

The lowest uncertainty was obtained for Prometryn (12.6%), followed by Ametryn (12.7%), Terbuthylazine (13.9%) and Propazine (14.5). For unknown samples the standard uncertainty (U_{sd}) will be reported as expanded uncertainty (U_{ex}) using a coverage factor of 2 (SANTE, 2017):

$$U_{ex}= 2 \text{ x } U_{sd} \quad (1)$$

Based on this estimation, the highest applied expanded uncertainty will be for Simetryne, with a value of 40 %.

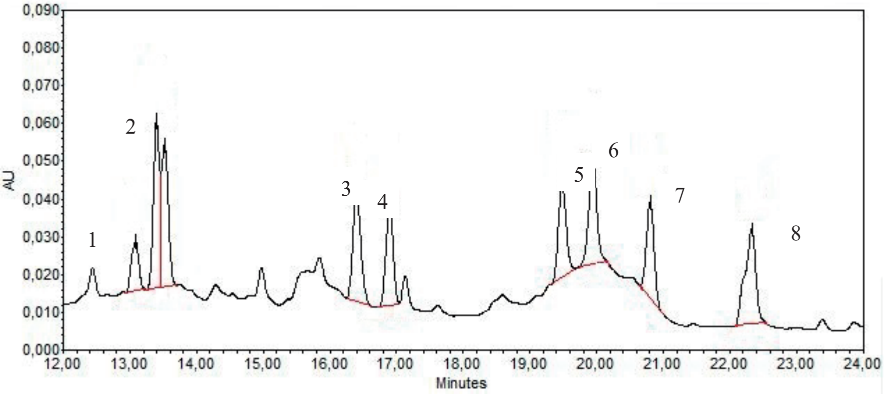

Figure 4. Spiked lignosulfonate fertilizer with triazine herbicide (1-Hexazinone, 2-Simazine, 3-Simetryne, 4-Atrazine, 5-Ametryn, 6-Propazine, 7-Terbuthylazine, and 8-Prometryn)

CONCLUSIONS

In this study an analytical method based on UPLC-PDA for the identification and the quantification of eight most common triazine contaminants (Hexazinone, Simazine, Simetryne, Atrazine, Ametryn, Propazine, Terbuthylazine, and Prometryn) in organic lignosulfonate fertilizer was developed. The method showed good results in terms of linearity, accuracy, precision of repeatability, precision of reproducibility, and recovery. The method can be successfully optimised for other type of organic fertilisers based on biomass waste material. Further investigation should be conducted in order to decrease the limit of detection and quantification, and to improve the sensibility of the overall method.

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REFERENCES

Aro T. & Fatehi P. (2017). Production and Application of Lignosulfonates and Sulfonated Lignin, *ChemSusChem*, 10, 1861–1877, DOI: 10.1002/cssc.201700082.

Baranowska I., Akdogan A., Barchañska H., Divrikli U., & Elçi L. (2012). Determination of Mesotrione, Simazine and Atrazine by RP-HPLC in Thermal Water, Sediment and Vegetable Samples. *Analytical Chemistry Letters,* 2(4), 206–219.

Byrne F. P., Jin S., Paggiola G., Petchey T. H. M., Clark J. H., Farmer T. J., Hunt A. J., McElroy C. R. & Sherwood J. (2016). Tools and techniques for solvent selection: green solvent selection guides. Sustainable Chemical Processes, 4(7), 1–24. DOI 10.1186/s40508-016-0051-z.

Chen Y., Chen Z.P., Jin J.W., Yu R.Q. (2015). Quantitative determination of ametryn in river water using surface-enhanced Raman spectroscopy coupled with an advanced chemometric model. *Chemometrics and Intelligent Laboratory Systems,* 142, 166–171.

COMMISSION REGULATION (EC) No 889/2008 of 5 September 2008, laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.

COMMISSION REGULATION (EU) No 310/2011 of 28 March 2011 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for aldicarb, bromopropylate, chlorfenvinphos, endosulfan, EPTC, ethion, fenthion, fomesafen, methabenzthiazuron, methidathion, simazine, tetradifon and triforine in or on certain products

COMMISSION REGULATION (EU) 2019/1009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003

COMMISSION REGULATION (EU) 2016/440 of 23 March 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for atrazine in or on certain products

COMMISSION REGULATION (EU) 2021/618 of 15 April 2021 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for diclofop, fluopyram, ipconazole and terbuthylazine in or on certain products.

Dou R., Sun J., Deng F., Wang P., Zhou H., Wei Z., Chen M., He Z., Lai M., Ye T., Zhu L. (2020). Contamination of pyrethroids and atrazine in greenhouse and open-field agricultural soils in China, *Science of the Total Environment*, 701, 134916.

EU, 2021 *Pesticide database. Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr.*

EURACHEM Guide - The Fitness for Purpose of Analytical Methods (2014).

Fenoll J., Vela N., Navarro G., Pérez-Lucas G. & Navarro S. (2014). Assessment of agro-industrial and composted organic wastes for reducing the potential leaching of triazine herbicide residues through the soil, *Science of the Total Environment* 493, 124–132

ISO 11264:2005 Soil quality - Determination of herbicides - Method using HPLC with UV-detection.

Khan S., Hussain S., Wong A., Foguel M.V., Luís Moreira Gonçalves, Pividori Gurgoe M. I., del Pilar Taboada Sotomayora M. (2018) Synthesis and characterization of magnetic-molecularly imprinted polymers for the HPLC-UV analysis of ametryn. *Reactive and Functional Polymers,* 122, 175–182.

Leong W.-H., The S.-Y., Hossain M. M., Nadarajaw T., Zabidi-Hussin Z., Chin S.-Y., Lai K.-S. & Lim S.-H. E., (2020). Application, monitoring and adverse effects in pesticide use: The importance of reinforcement of Good Agricultural Practices (GAPs), *Journal of Environmental Management*, 260, 109987, https://doi.org/10.1016/j.jenvman.2019.109987.

MADR (2021). *Operators certified in organic agriculture. Retrieved from https://www.madr.ro/agricultura-ecologica/operatorii-certificati-in-agricultura-ecologica-2021.html.*

ORDIN nr. 756 din 3 noiembrie 1997 pentru aprobarea reglementarii privind evaluarea poluarii mediului.

Patrudu T.B., Manohra Naidu T., Parvatamma B., Nageswara Rao T. (2020). Extraction of triazine herbicides in environmental water samples using alumina nanoparticles and determination of residues by LC–MS/MS. *Materials Today: Proceedings*, 27,130–135.

SANTE-11813 (2017). Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.

Sass J. B. & Colangelo A. (2006). European Union bans atrazine, while the United States negotiates continued use. *International Journal of Occupational and Environmental Health*, 2006, 12(3), 260-267, doi: 10.1179/oeh.2006.12.3.26

Shah J., Jan M. R., Ara B. & Shehzad F.-N. (2011). Quantification of triazine herbicides in soil by microwave-assisted extraction and high-performance liquid chromatography, *Environ Monit Assess*, 178:111–119.

Welton T. (2015). Solvents and sustainable chemistry. Proceedings Royal Society A, 471, 20150502. http://dx.doi.org/10.1098/rspa.2015.0502