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LC/MS/MS METHOD FOR INVESTIGATION OF FIVE USUAL PESTICIDES FROM WATER

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ABSTRACT. Pesticides are chemical compounds used in agriculture to destroy the pests and weeds but with great potential on environment contamination. An analytical method for screening and confirming the presence of 5 pesticides (acetamiprid, cymoxanil, mefenoxam, thiacloprid, thiametoxam) was developed. LC/MS/MS was proved to be a sensitive technique for these classes of compounds (insecticides and fungicides). Five calibration curves at six levels of concentration were obtained with good correlation coefficients (0.9960-0.9990). The method detection limit (MDL) ranges between 0.4–0.75 ng/ml and the method quantification limit (MQL) ranges between 1.2–2.25 ng/ml. Two methods: a liquid-liquid extraction and a solid phase extraction were tested. The selection criteria was the recovery degree which was prove to be more adequate for liquid-liquid extraction (71-102%) than for solid phase extraction (32-87%).

Keywords: *acetamiprid, cymoxanil, mefenoxam, thiacloprid, thiametoxam, LC-MS-MS, solid phase extraction, SPE*

INTRODUCTION

In the modern agricultural practices, different synthetic pesticides such as: insecticides, larvacides, miticides, mollucides, nematocides, fumigants, fungicides, herbicides and defoliants are extensively used to improve the agricultural production by destroying pests and weeds [1]. Besides their positive effects, the use of pesticides may lead to contamination of surface and ground water by drift, runoff, drainage and leaching. This contamination may have ecotoxicological effects for aquatic flora and fauna and for human health if used for public consumption [2]. Neonicotinoids are class of neurotoxic insecticides designed in the ’80s, highly systemic with long-term persistence. The neonicotinoids permanently bind to nicotinic receptors of acetylcholine, blocking the receptors and consequently the passage of nerve impulses [3]. The neonicotinoids are used for pome fruits, stone fruits, citrus, grape, horticultural and industrial crops, flower and ornamental plants against insects such as:

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Aphids, whiteflies, planthoppers, scale insects, Lepidoptera, soil insects, Colorado potato beetle [3]. Acetamiprid, thiacloprid, thiametoxam (Table 1) are among the most used neonicotinoids. Their use is limited in fruits and vegetables by the Regulation (EC) No 396/20053 of European Union, which establishes the rules governing the setting of pesticide MRLs (maximum residues limits) [4-6]. Cymoxanil is a fungicide belonging to aliphatic nitrogen compounds. It acts as a foliar fungicide with protective and curative action. It presents contact and local systemic activity, and also inhibits sporulation [7]. Mefenoxam is the systemic phenylamide fungicide, being widely used in phytophthora disease control which causes late blight, downy mildew, wetting and rot of stems and fruits of many plants, including tomato, cantaloupe, cucumber, eggplant, pepper, squash, grape, pimiento, litchi, watermelon and tobacco. It is the R-enantiomer of metalaxyl that has been on the market since 1996 under various formulations and trade names [8].

Due to their assumed ecotoxicological effects, a method for determining these pesticides is mandatory in order to protect the consumers, for environmental assessment and to establish the quality of agricultural products. In the last years, several techniques such as: ELISA [9], HPLC-UV [10-13], GC–NPD [14], GC-NCI-MS [15], GC-MS-MS [16, 17], LC-MS-MS [18-22], LC/TOF-MS [23] were used to determine some neonicotinoids and fungicides from food, water and soil. The purpose of these studies was to develop an efficient, quick and environmental friendly method at low cost.

This study follows to develop a LC/MS/MS method for determining the residuals of five pesticides (see Table 1) from water samples. These pesticides are widely used in modern agriculture and can occur in waters from the proximity of agriculture's areas.

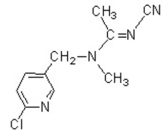
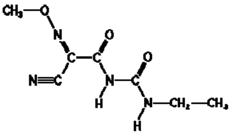
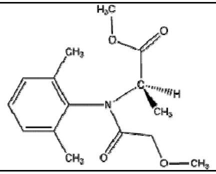
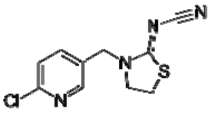
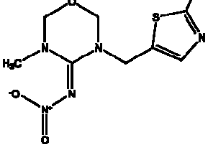
RESULTS AND DISCUSSION

The analytical method developed during this study was intended to investigate several of the most representative pesticides from two classes: fungicides and insecticides. Table 1 contains basic information about the selected pesticides.

Develoment of ESI-MS profile

The development of ESI-MS profile started with the analysis of all the investigated pesticides both in positive and negative ionization modes. The protonated molecule $[M+H]^+$, representative for positive ionization, was proved to be more abundant in all five cases and therefore was selected for further investigation. Each compound was injected directly in MS, scanned in the first quadrupole (Q1 mode) to select the parent ion, defragmented in the collision cell (Q2) and then scanned in the third quadrupole (Q3 in product ion mode (PI)) to select the product ion. This process of selecting the parent ion and the product ion was followed by an automatic optimization of the compound dependent parameters (Infusion) for each pesticide. These parameters are: DP (declustering potential), EP (entrance potential), CE (collision energy) and CXP (collision cell exit potential) and the obtained values are presented in Table 2. The automatic optimization of the compound dependent parameters for each precursor ion → product ion transition has optimized the MS/MS conditions in a MRM method (multiple reaction monitoring) characteristic for each compound. An automatic function (Merge MRM) was used to merge all MRM methods in one MRM characteristic to all compounds.

Table 1. Basic information of selected pesticides

Chemical name	**Chemical class**	**Chemical formula**	**Molecular weight (g·mol^{-1})**	**Structural formula**
Acetamiprid	Insecticides (Neonicotinoides)	$C_{10}H_{11}ClN_4$	228.68	
Cymoxanil	Acetimide (Fungicides)	$C_7H_{10}N_4O$	198.2	
Mefenoxam	Phenylamide (Fungicides)	$C_{15}H_{21}NO_4$	279.33	
Thiacloprid	Insecticides (Neonicotinoides)	$C_{10}H_{9}ClN_4S$	252.72	
Thiametoxam	Insecticides (Neonicotinoides)	$C_8H_{10}ClN_5O_3S$	291.71	

Development of LC/MS/MS profile

The next step in developing the LC/MS/MS method consists is performing FIA (Flow Injection Analysis), an automatic optimization of the source dependent parameters. FIA is accomplished by injecting 100ng/mL standard solution in MeOH:H2O (60/40, v/v) through the LC connected to the MS/MS by a restriction capillary, using the MRM method developed earlier. The source dependent parameters are: CUR (curtain gas), CAD (collision gas), IS (ionspray voltage), TEM (temperature), GS1 (gas 1) and GS2 (gas 2) and their role is to optimize the compounds signal in LC conditions. The obtained values are presented in Table 2.

Development of chromatographic method

In order to establish the optimum mobile phase, different composition of H_2O (A) and MeOH (B) mixture were tested in isocratic conditions. The tested mobile phase compositions were: A:B (80/20,v/v); A:B (60/40,v/v); A:B (40/60,v/v) and A:B (20/80,v/v). In order to obtain a good separation and well defined shape of the chromatographic peaks the following LC parameters were chosen:

Injection volume: 50µL;

- Flow rate: 500µL/min;
- Column temperature: 28°C;
- Mobile phase: A:H_2O+0.1% ammonium acetate; B:MeOH+0.1% ammonium acetate;
- Gradient program:

Time (min)	Flow rate (µL/min)	A (%)	B (%)
0.00	500	20.0	80.0
7.00	500	50.0	50.0

Table 2. The compound and source dependent parameters obtained by automatic optimizations (Infusion and FIA) for the investigated pesticides

Compound	Q1 Mass (u)	Q3 Mass (u)	DP (V)	EP (V)	CE (V)	CXP (V)
Acetamiprid	223.10	126.00	41.00	7.50	27.00	4.00
Mefenoxam	280.20	220.30	26.00	6.50	17.00	4.00
Cymoxanil	199.10	128.00	11.00	12.00	11.00	4.00
Thiacloprid	253.10	126.10	36.00	4.50	27.00	4.00
Thiametoxam	292.10	211.20	26.00	9.00	17.00	4.00
FIA parameters CUR:30.00 psi; CAD:Medium; IS:5500.00V; TEM:400°C; GS1:35 psi; GS2:35 psi.						

The 100ng/mL standard solution was injected using the LC/MS/MS method developed earlier with the chosen analytical column. The chromatogram obtained for the investigated pesticides in a single run are presented in Figure 1 and their MRM transitions are presented in Figure 2.

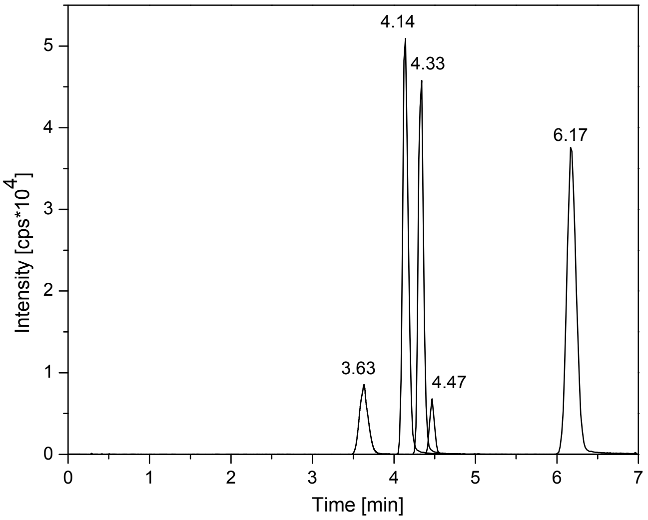


Figure 1. Total ion chromatogram of 100 ng/mL standard solution

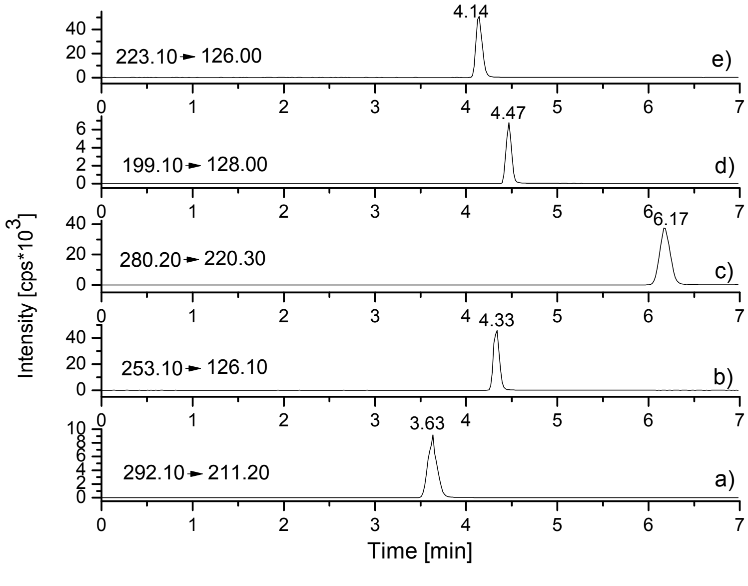


Figure 2. MRM transition of a) Thiametoxam; b) Thiacloprid; c) Mefenoxam; d) Cymoxanil and e) Acetamiprid

Quantitative analysis

Ten concentrations of standard solutions between 0.4 to 100ng/mL were prepared for all investigated pesticides. Five calibration curves at six levels were obtained. The correlation coefficients (r^2) were automatically determined using a regression function (1/x*x). Their values were higher than 0.9960 which proves the linearity of the method. The results obtained are presented in Table 3.

For determining the method detection limit (MDL) and the method quantification limit (MQL) the following theory was applied. The method detection limit (MDL) is defined as the smallest amount of an analyte that can be reliably detected or differentiated from the background of a particular matrix (using the specified method). All the matrix interferences must be taken into consideration while determining the MDL. Similarly, the method quantification limit (MQL) is defined as the smallest amount of an analyte that can be reliably quantified with good reliability from a particular matrix (using the specified method) [24]. The real MQL was defined as the lowest validated spike level meeting the requirements of a recovery within the range 70–120% and a RSD≤20% [19]. The instrument detection limit (IDL) and the instrument quantification limit (IQL) were obtained by injecting six time the standard solutions at three lowest detectable concentration levels (0.4ng/mL; 0.75ng/mL; 1ng/mL) [19].

Table 3. Reteantion time, linear range, linear equation, r^2, MDL, MQL, IDL, IQL and recovery for both extraction methods of the investigated pesticides

Analyte	**Acetamiprid**	**Mefenoxam**	**Cymoxanil**	**Thiacloprid**	**Thiametoxam**
Retention time	4.14	6.17	4.47	4.33	3.63
Linear range (ng/mL)	2.5-75	2.5-75	2.5-75	2.5-75	2.5-75
Linear equation	$2.95*10^4x+ 8.29*10^3$	$2.85*10^4x+ 7.96*10^3$	$4.72*10^3x-163$	$4.14*10^4x+ 7.25*10^3$	$6.37*10^3x+807$
r^2	0.9960	0.9990	0.9980	0.9970	0.9980
MDL	0.4000	0.4000	0.7500	0.4000	0.7500
MQL	1.2000	1.2000	2.2500	1.2000	2.2500
IDL	0,4335	0,5715	0,6345	0,4245	0,6735
IQL	1,4450	1,9050	2,1150	1,4150	2,2450
RSD (%)	5,7800	7,6200	8,4600	5,6600	8,9800
Recovery (%) -1st method	95	73	86	102	68
Recovery (%) -2th method	39	32	55	87	41

The samples prepared by two extraction methods were analyzed in order to obtain the recovery and the repeatability of each method. Three standard solutions of known concentrations (2.5ng/mL; 5ng/mL; 50ng/mL) were spiked with water samples of known concentration. The samples were analyzed using the LC/MS/MS method developed earlier and the relative standard deviation (RSD) and the recovery obtained for each pesticide were determinate. The values obtained for the method detection limit (MDL), the method quantification limit (MQL), the instrument detection limit (IDL), the instrument quantification limit (IQL), the relative standard deviation (RSD) and the recovery are presented in Table 3.

The LC/MS/MS method was proved to be successful in investigating five pesticides: thiametoxam, thiacloprid; mefenoxam; cymoxanil and acetamiprid from water samples.

CONCLUSIONS

A LC/MS/MS method was developed for screening and determination of five pesticides (herbicides and fungicides). Five calibration curves at six levels were obtained. The correlation coefficients (r^2) range between 0.9960 and 0.9990 depending of the pesticide. MDL (method detection limit), MQL (method quantification limit), IDL (instrument detection limit) and IQL (instrument quantification limit) were determinate. Two methods: liquid-liquid and solid phase extraction were tested. The liquid-liquid extraction was chosen for its superior recovery degree (71-102%) comparing with the one obtained solid phase extraction (32-87%).

The results suggest that the developed method is appropriate for determining the five tested pesticides: acetamiprid, cymoxanil, mefenoxam, thiacloprid, thiametoxam from water samples.

EXPERIMENTAL SECTION

Chemicals and reagents

Acetamiprid, Cymoxanil, Mefenoxam, Thiacloprid, Thiametoxam, anhydrous sodium sulphate, (≥99.0%) and hydrochloric acid (37%) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol LC-MS Optigrade (≥99.8%), Ammonium acetate ULC-MS Optigrade (99-100%), Diethyl ether HPLC Optigrade (≥99%), Dichloromethane HPLC Optigrade (≥99.8%) were acquired from LGC Standards. SPE Strata C18-E 200 mg/ 3 mL cartridges were purchased from Phenomenex, USA. Water was purified using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA).

Standard solution preparation

The stock solutions (1mg mL^{-1}) were prepared by dissolving 1mg of each pesticide in 1mL of MeOH using a vortex mixer. The samples were stored under refrigeration at 4^{o}C in the dark to avoid the alteration. The working standard solutions of 0.4; 0.75; 1; 2.5; 5; 10; 25; 50 and 75 ng mL^{-1} concentrations were prepared by diluting the stock solution with a mixture of MeOH:H2O (60/40, v/v). These standard solutions were used for method optimization and for the calibration curves.

Sample extraction

Two extraction methods, adapted by literature [25, 26], were tested: 1. 0.5L of tap water or ground water, adjusted at pH 2.5 with HCl and spiked with standards, was extracted three times with 50mL of dichloromethane. The residual water was removed from the combined organic extract by addition of anhydrous sodium sulfate. The solvent evaporation was performed in a Laborota 4010 Rotary Evaporator. The solvent was evaporated using a stream of nitrogen gas. 1mL of MeOH:H2O (60/40, v/v) was added to the residue and the solution was then filtered through a 0.45μm membrane before the injection into the chromatograph [25]. 2. The solid phase extraction was performed on a 200mg C18 analytical cartridge. The cartridge was conditioned with 3mL H_2O and 3mL MeOH. 1L of water containing all the investigated pesticides was loaded on the cartridge. After drying the cartridge, the retained analytes were eluted using 3 mL $(C_2H_5)_2O$:MeOH (90/10, v/v) and 3mL MeOH. They were then collected on a conical-bottom centrifuge tube for evaporation to dryness with nitrogen. 1mL of MeOH:H2O (60/40, v/v) was added to the residues [26].

LC/MS/MS analysis

The LC/MS/MS analysis were carried out on a HPLC Agilent 1200 Series coupled with an Applied Biosystems API 3200 QTRAP mass spectrometer using a TurboV source. The system was controlled by Analyst 5.1 software. A Zorbax Eclipse XDB-C18 column (4.6 X 150mm, 5μm) purchased from Agilent Technologies was used for the separation. The elution of selected compounds was performed using a gradient mobile phase starting with A:B (80/20,v/v) and finishing with A:B (50/50,v/v), where A is H_2O+0.1% ammonium acetate and B is MeOH+0.1% ammonium acetate, in a 7 min run with a flow rate of 0.5 mL min^{-1}. The column temperature was set at 28°C.

REFERENCES

1. Eun-Mi An, Han-Seung Shin, *Food Science and Biotechnology,* **2011**, *20(2)*, 395.
2. Z. Rahmanikhah, A.E. Sari, N. Bahramifar, Z.S. Bousjien, *Middle-East Journal of Scientific Research,* **2011**, *7(2),* 253.
3. D. Laurino, M. Porporato, A. Patetta, A. Manino, *Bulletin of Insectology,* **2011**, *64(1)*, 107.
4. European Food Safety Authority, *EFSA Journal,* **2011**, *9(5)*, 2166.
5. European Food Safety Authority, *EFSA Journal,* **2010**, *8(7),* 1668.
6. European Food Safety Authority, *EFSA Journal,* **2010**, *8(6),* 1647.
7. European Food Safety Authority, *EFSA Journal,* **2011**, *9(3),* 2093.
8. D. Liu, D. Qin, R. Ji, *Bulletin of Environmental Contamination and Toxicology,* **2010**, *84*, 230.
9. E. Watanabe, S. Miyake, K. Baba, H. Eun, S. Endo, *Analytical and Bioanalytical Chemistry*, **2006**, *386(5),* 1441.
10. M. Gupta, A. Shanker, *Food Chemistry*, **2008**, *111(4)*, 805.
11. P. Fidente, C.D. Giovanni, S. Seccia, P. Morrica, *Biomedical Chromatography*, **2005**, *19,* 766.
12. C. Mohan, Y. Kumar, J. Madan, N. Saxena, *Environmental Monitoring and Assessment*, **2010**, *165(1-4)*, 573.
13. S. Campbell, L. Chen, J. Yu, Q.X. Li, *Journal of Agricultural and Food Chemistry*, **2005**, *53(13)*, 5373.
14. D. Liu, D. Qin, R. Ji, *Bulletin of Environmental Contamination and Toxicology*, **2010**, *84(2),* 230.
15. X. Zhang, N. Mobley, J. Zhang, X. Zheng, L. Lu, O. Ragin, C.J. Smith, *Journal of Agricultural and Food* Chemistry, **2010**, *58(22)*, 11553.
16. X.-B. Yanga, G.-G. Yinga, R.S. Kookanab, *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants and Agricultural Wastes*, **2010**, *45(2)*, 152.
17. P. Paya, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, *Analytical and Bioanalytical Chemistry*, **2007**, *389(6)*, 1697.
18. S.J. Lee, H.J. Park, W. Kim, J.S. Jin, A.M. Abd El-Aty, J.-H. Shim, S.C. Shin, *Biomedical Chromatography,* **2009**, *23(4)*, 434.
19. I.R. Pizzutti, A. Kok, R. Zanella, M.B. Adaime, M. Hiemstra, C. Wickert, O.D. Prestes, *Journal of Chromatography A*, **2007**, *1142(2)*, 123.
20. M.J. Hengel, M. Miller, *Journal of Agricultural and Food Chemistry*, **2008**, *56(16)*, 6851.

21. Y. Fillâtre, D. Rondeau, B. Bonnet, A. Daguin, A. Jadas-Hécart, P.-Y. Communal, Analytical *Chemistry*, **2011**, 83(1), 109.
22. K. Banerjee, D.P. Oulkar, S. Dasgupta, S.B. Patil, S.H. Patil, R. Savant, P.G. Adsule, *Journal of Chromatography A*, **2007**, *1173(1–2),* 98.
23. I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, *Analytical Chemistry*, **2005**, *77(9)*, 2818-2825.
24. M.W. Kujawski, J. Namiesnik, *Food Control*, **2011***, 22*, 914.
25. S.C.N. Queiroz, K. Lazou, P. Sandra, I.C.S.F. Jardim, P*esticidas: R. Ecotoxicol. e Meio Ambiente, Curitiba*, **2004**, *14*, 53.
26. L. Viglino, K. Aboulfald, M. Prevost, S. Sauve, T*hermo Scientific Application Note*, **2009**, *466*, 1.