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Sensitive method for the simultaneous detection of ten azole contaminants in water samples by SPE-LC-MS/MS

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Abstract. A new analytical method was developed for the determination of azole contaminants from environmental samples (surface water, drinking water, wastewater) by solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantification. The separation of the compounds was achieved using a Zorbax Eclipse XDB C18 chromatographic column (2.1 x 100 mm, 3.5 µm). The quantification limits varied in the range of 3.6 ng/L (ipconazole) to 8.4 ng/L (climbazole), for wastewater samples, 1.44 ng/L ÷ 3.36 ng/L for surface water samples, and 0.72 ng/L and 1.68 ng /L in drinking water, respectively. The values of the recovery percentage and the precision data obtained after applying the extraction procedure for all compounds indicated values in accordance with LC methods.

Keywords: azoles, SPE-LC-MS/MS, detection, environmental waters, validation

1. Introduction

Azoles are used as antifungal agents, against fungal infections, for plant protection, in the treatment of human mycosis, and in veterinary medicine, due to their antifungal activity (Shi et al 2012, Porsbring et al 2009). Azolic compounds are also used as antifreeze fluids, anticorrosive, biocides in vegetables and fruits, wood preservatives and adhesives. Due to the versatility and diversity of chemical structures, applications for azoles continue to grow in agriculture. Because of their presence in the environment, there is concern about harmful effects on aquatic organisms. Due to their intensive use, these compounds are ubiquitous in the aquatic environment (sewage effluents, surface waters) and can have toxic effects on the environment. Azole compounds were recently included in the "Watch List" of the European Union for monitoring in the surface waters of the EU countries (EU Implementation Decision no. 2022/1307). Excessive use of azole compounds in agriculture, pharmaceuticals, and personal care products has resulted in contamination of water, soil, and aquatic organisms with these compounds.

After application, these azole fungicides enter wastewater treatment plants (WWTP) and are then discharged, through effluents, into receiving surface waters. Thus, the main source of river pollution with azole fungicides is household sewage (Chen et al 2014), with a partial contribution from hospital wastewater (Lindberg et al 2010) The maximum concentrations of some azole fungicides in the influent were determined up to micro-grams per liter. Clotrimazole, ketoconazole and miconazole were detected both in the liquid phase and adsorbed on suspended matter particles (Peng et al 2012). In the effluent, the levels of azole fungicides are much lower than in the influent, especially in the range of tens to hundreds of nanograms per liter. The maximum reported concentrations of climbazole, clotrimazole, ketoconazole, miconazole and fluconazole were 443, 8650, 34.8, 35.7 and 448 ng/L, respectively (Lacey et al 2012, Van De Steene et al 2010). Huang et al. (2021) discovered that azoles have negative effects on zebrafish (Danio rerio) by disrupting their metabolism, highlighting the potential for mitochondrial dysfunction and lipid dysregulation by triazoles. Residues of azole fungicides can cause toxic effects on aquatic organisms such as algae and fish. Azole fungicides adversely affect the mammalian endocrine system and several azoles have been identified as endocrine disruptors (Draskau et al 2021). The aim of the work was the development of a new analytical method (sensitive and precise) for the identification and nano-detection of azole contaminants from the two chemical classes triazoles and imidazoles (clotrimazole, imazalil, ipconazole metconazole, penconazole, prochloraz, tebuconazole, tetraconazole, climbazole, epoxiconazole) from environmental sample matrices (surface water, potable water, waste water) by solid phase extraction (SPE) followed by liquid chromatography coupled with mass spectrometry (LC-MS/MS).

2. Materials and methods

The LC-MS parameters were optimized so as to allow obtaining the lowest possible quantification limits of the ten analytes. The experiments to establish the optimal

conditions for chromatographic separation and detection were carried out on an Agilent 1260 LC system coupled with an Agilent 6410B triple quadrupole mass spectrometer (MS) equipped with an ESI electrospray ionization source operated in positive mode. The optimized parameters of the chromatograph and the mass spectrometer are summarized in table 1 and table 2.

Table 1. Chromatographic and mass-spectrometric parameters of the method for the analysis of azoles in water samples.

Optimized LC parameters	**Optimized MS parameters:**
Chromatographic column: Zorbax Eclipse XDB C18 (2.1 x 100 mm, 3.5 µm)	Ionization mode: Electrospray negativ ESI(+)
Column temperature: 30°C	Drying gas temperature: 300°C
Injection volume: 5 µl	Drying gas flow: 10 L/min
Mobile phase: 5 mM ammonium acetate/ Acetonitrile	Nebulizer pressure: 40 psi
Flow rate: 0.2 ml/min	Capillary voltage: 4000V
Elution: gradient	MS tranzition: Multiple reaction monitoring/ MRM
Run-time : 17.5 min.	

The chromatographic separation of the analytes was carried out with a mobile phase gradient composed of acetonitrile and 5 mM ammonium acetate (table 2).

Table 2. Gradient elution program of the mobile phase

Time (min)	5 mM ammonium acetate (A, %)	Acetonitrile (B, %)	Flow rate(mL/min)
0	50	50	0.2
7	50	50	0.2
8	5	95	0.2
13	5	95	0.2
13.01	50	50	0.4
17	50	50	0.4
17.5	50	50	0.2

The parameters of the mass spectrometer for molecular fragmentation and detection of azole compounds are presented in table 3. For example, for climbazole, a fragmentation pattern was obtained from the precursor molecular ion 293.1 to the product ion 69 (m/z). Thus, under the conditions of the developed and optimized method, it was possible to separate the 10 compounds in 13 minutes (figure 1).

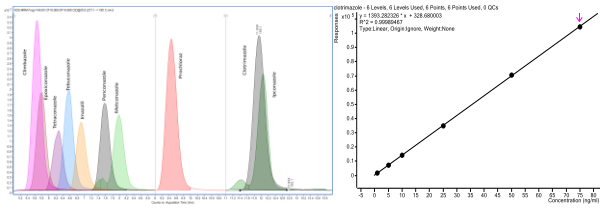

Figure 1. MRM chromatogram for the studied compounds (50 ng/mL), and calibration curve for clotrimazole

In addition, a selective method was developed and optimized using the solid phase extraction (SPE) of the analyte from water samples using Strata X type adsorbents (500 mg /6 mL) styrene divnyl benzene polymer (Figure 2). Water samples (pH 4) were percolated through SPE cartridges using a Thermo Scientific Dionex AutoTrace 280 system. The elution of the analytes from the adsorbent was carried out with 10 ml of methanol, after which the extract was concentrated to dryness and the residue was dissolved in 1 mL of acetonitrile and injected into LC-MS/MS.

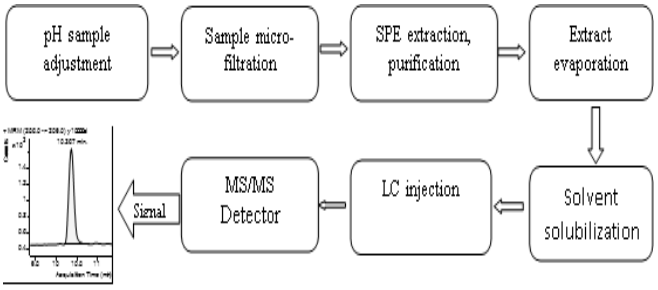

Figure 2. Graphical representation of the main steps followed in the analysis of azole contaminants in water samples

3. Results and discussion

The performance characteristics of the developed method followed during the validation were: linearity, sensitivity (limit of quantification), precision, accuracy and recovery yield. On the calibration range 1 ng/mL to 75 ng/mL, linear regressions were obtained, using the external standard method, and the related coefficients of determination (R^2) were greater than 0.99, according to the data presented in Table 4.

The quantification limits varied in the range of 3.6 ng/L (ipconazole) ÷ 8.4 ng/L (climbazole), for wastewater samples, 1.44 ng/L ÷ 3.36 ng/L for surface water samples, 0.72 and 1.68 ng /L in drinking water. These limits allow the quantification of azoles in surface water and wastewater samples at a trace level. The values of the recovery yield obtained after applying the analyte extraction procedure fell within the range of 72.36 ÷ 98.55% for the wastewater sample (figure 3), in the range of 77.39 ÷ 97.46% for the surface water sample, between 82.66 ÷ 99.45% for drinking water sample The obtained results shows the accuracy corresponding to LC methods. In order to ensure precise quantifications, the analytical variability of the method was evaluated under the same conditions in a short time and in a longer time for a concentration level (50 ng/L). Accuracy was determined from ten sub-samples of wastewater (100 mL), surface water (250 mL), respectively drinking water (500 mL) sample with spiked standard on the same day (repeatability, RSDr) and daily for 5 days (intermediate precision, RSDR). Precision was expressed as a percentage relative standard deviation applying a concentration factor of 100 for wastewater, of 250 for surface water and concentration factor of 500 for drinking water. Table summarizes the calculated validation parameters. The repeatability of the SPE-LC/MC-MS method was characterized by values of the relative standard deviations (RSDr) that varied in the range 2.8 %

to 4.8% in the case of drinking water, 2.9 %÷5.2% for surface water, and 5.4%÷7.6% for wastewater. The reproducibility of the method (RSDR) presented values in the ranges: 4.8%÷8.9% for drinking water, 5.9%÷10.1% for surface water, and 9.7%÷14.3% for wastewater. These values demonstrate that the method developed for the detection of azole compounds is accurate with residual standard deviation below 15%.

4. Conclusion

In this paper, is presented a new method for the identification and quantification of ten azole contaminants from environmental samples (surface water, wastewater and drinking water). The method was based on the solid phase extraction (SPE) of the analytes (using Strata X polymer cartridges) and on the separation and detection by liquid chromatography coupled with mass spectrometry.

The performance parameters (limit of quantification, repeatability, intermediate precision and recovery) obtained during the validation process demonstrate that it can be successfully applied for detection of azoles in water samples.

5. Acknowledgements

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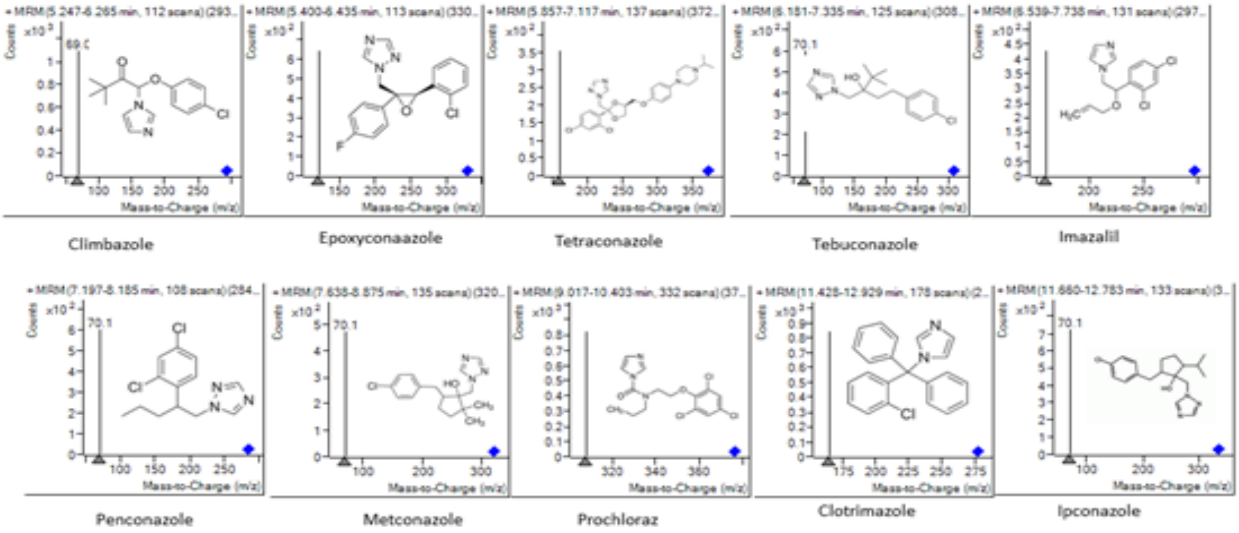

Figure 3. The MS spectra for MRM transitions between precursor molecular ions and product molecular ions obtained for a surface water sample spiked with 25 ng/L

Table 3. Operational parameters of the QQQ mass spectrometer

Compound	Retetion time, minute	MRM tranzition P-Q	Fragmentation voltage (V)	Collision energy (CE, V)	Dwell time (msec)
Climbazole	5.79	293.1→69	115	24	75
Epoxiconazole	5.91	330→121	60	27	75
Tetraconazole	6.41	372→159.1	120	36	75
Tebuconazole	6.67	308.2→70.1	70	20	75
Imazalil	7.03	297.1→159.1	110	24	75
Penconazole	7.71	284.1→70.1	120	20	75
Metconazole	8.107	320.1→70.1	111	36	75
Prochloraz	9.761	376.1→308	80	10	160
Clotrimazole	12.07	277.1→165	140	25	250
Ipconazole	12.14	334.2→70.1	131	24	250

Table 4. Method validation parameters for ten azoles in surface and waste water

Analyte	LOQ, ng/L			Recovery percentage (%)		
	Drinking water	River water	Wastewater	Drinking water	River water	Wastewater
Climbazol	4.3	1.72	0.86	85.36	92.51	97.36
Epoxiconazole	6.2	2.48	1.24	82.64	87.36	89.63

Tetraconazole	3.6	1.44	0.72	72.36	81.93	87.53
Tebuconazole	5.5	2.2	1.1	85.36	88.51	93.42
Imazalil	6.4	2.56	1.28	73.91	80.63	84.65
Penconazole	4.3	1.72	0.86	78.29	87.14	91.23
Metconazole	5.1	2.04	1.02	80.12	79.83	85.46
Prochloraz	7.2	2.88	1.44	98.55	97.46	99.45
Clotrimazole	8.4	3.36	1.68	87.62	84.68	88.63
Ipconazole	4.9	1.96	0.98	73.55	77.39	82.66

Table 5. The values obtained for the repeatability and reproducibility at a concentration of 50 ng/L azole compounds added to the wastewater, surface water and drinking water (before SPE)

Analyte	Drinking water		River water		Wastewater	
	RSD_r	RSD_R	RSD_r	RSD_R	RSD_r	RSD_R
Clotrimazole	4.6 ± 0.31	7.5 ± 0.72	4.2 ± 0.36	8.6 ± 0.81	7.30 ± 0.68	14.3 ± 0.39
Imazalil	3.8 ± 0.28	5.9 ± 0.62	3.1 ± 0.29	6.3 ± 0.58	6.9 ± 0.69	12.8 ± 1.2
Ipconazole	2.8 ± 0.21	4.8 ± 0.47	2.9 ± 0.26	5.9 ± 0.55	5.4 ± 0.53	13.9 ± 1.3
Metconazole	4.6 ± 0.34	8.5 ± 0.84	5 ± 0.47	9.4 ± 0.86	6.5 ± 0.61	11.6 ± 1.1
Penconazole	3.9 ± 0.34	6.8 ± 0.67	3.6 ± 0.34	7.2 ± 0.6	6.4 ± 0.63	14 ± 1.3
Prochloraz	4.8 ± 0.42	8.9 ± 0.87	5.2 ± 0.48	10.1 ± 0.97	5.9 ± 0.57	13.5 ± 1.3
Tebuconazole	4.1 ± 0.35	7.5 ± 0.69	4.5 ± 0.41	8.7 ± 0.83	6.7 ± 0.64	12.4 ± 1.2
Tetraconazole	2.9 ± 0.21	6.1 ± 0.58	3.4 ± 0.31	6.8 ± 0.65	7.6 ± 0.74	9.7 ± 0.9
Climbazole	3.8 ± 0.31	7.3 ± 0.71	4.3 ± 0.39	8.2 ± 0.78	7.1 ± 0.68	10.9 ± 1.1
Epoxiconazole	4.4 ± 0.39	8.5 ± 0.82	4.8 ± 0.44	9.3 ± 0.88	6.8 ± 0.63	12.4 ± 1.2

References

Chen Z.F., Ying G.G., Liu Y.S., Zhang Q.Q., Zhao J.L., Liu S.S., Chen J., Peng F.J., Lai H.J., Pan C.G. (2014), Triclosan as a surrogate for household biocides: an investigation into biocides in aquatic environments of a highly urbanized region, *Water Research*, **58**, 269–279.

Commission Implementing Decision (EU) 2022/1307 of 22 July 2022 establishing a watchlist of substances for monitoring throughout the Union under water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council [notified under number C (2022) 5098].

Draskau M.K., Lardenois A., Evrard B., Boberg J., Chalmel F., Svingen T. (2021), Transcriptome analysis of fetal rat testis following intrauterine exposure to the azole fungicides triticonazole and flusilazole reveals subtle changes despite adverse endocrine effects, *Chemosphere*, **264**, https://doi.org/10.1016/j.chemosphere.2020.128468.

Huang T., Zhao Y., He J., Cheng H., Martyniuk C. J. (2022), Endocrine disruption by azole fungicides in fish: A review of the evidence, *Science of The Total Environment*, **822**, https://doi.org/10.1016/j.scitotenv.2022.153412.

Lacey C., Basha S., Morrissey A., Tobin J.M. (2012), Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland, *Environmental Monitoring and Assessment*, **184**, 1049–1062.

Lindberg RH, Fick J, Tysklind M. (2010), Screening of antimycotics in Swedish sewage treatment plants-waters and sludge, *Water Research*, **44**, 2, 649-657.

Peng X.Z., Huang Q.X., Zhang K., Yu Y.Y., Wang Z.F., Wang C.W. (2012), Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China, *Science of the Total Environment*, **426,** 311-317.

Porsbring T., Blanck H., Tjellstrom H., Backhaus T. (2009), Toxicity of the pharmaceutical clotrimazole to marine microalgal communities, *Aquatic Toxicology*, **91**, 203–211.

Shi H.H., Sun Z., Liu Z., Xue Y.G. (2012), Effects of clotrimazole and amiodarone on early development of amphibian (Xenopus tropicalis), *Environmental Toxicology and Chemistry*, **94**, 128–1352.

Van De Steene J.C., Stove C.P., Lambert W.E. (2010), A field study on 8 pharmaceuticals and 1 pesticide in Belgium: removal rates in waste water treatment plants and occurrence in surface water, *Science of the Total Environment*, **408**, 3448–345.