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Determination of important azoles in soil solution

Ninni Takala • Heli Sirén • Michal Jakl • Jana Jaklová Dytrtová

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Abstract The azoles (represented by penconazole, cyproconazole and tebuconazole in this study) are frequently used agrochemicals to protect various crops against mildew and fungi. They are considered as endocrine disruptors, because they block the biosynthesis (on the level of enzymes inhibition) of biochemicals with steroid structure. Besides targeted efforts, they can partly get into the soil with the rainfall or litter fall and influence/block the biosynthesis of sterols of non-target organisms. In this sense, the risk of disruption of rhizosphere plant-microbial symbiosis and dynamic processes in the soil solution by azoles is of high importance to be evaluated. We have developed an analytical methodology for determination of penconazole, cyproconazole and tebuconazole in soil solution using capillary electrophoresis with UV detection. ~~The separation efficiency is approx. 85%.~~ The results were also compared with mass spectrometric measurements using μ -TOF mass spectrometry. There approx. 90% of present azoles were bound in the soil solution matrix. The detection limit for these azoles is about 10^{-7} mol dm⁻³. Because of very low pK_a of azoles we

have to consider deprotonation of azoles and consequently the high affinity to create complexes with cations. The majority of present azoles in soil solution might form neutral adducts with mono-cations, making them invisible in electrospray mass spectra.

Keywords Triazoles • CE-UV • Separation • Fungicides • ESI- μ -Q-TOF-MS

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1 Introduction

2 Azoles are agrochemicals frequently used for crop treatments against fungi
3 and mildew. Mostly, they are biologically active chemicals qualified as
4 endocrine disruptors. They block the biosynthesis of steroids on the level of
5 inhibition of C-14 demethylase and aromatase [1,2] of the living organisms.
6 Consequently, they mostly interrupt: (i) integrity of biological membranes
7 (what is significant for small to one-cell organisms) and (ii) biosynthesis of
8 steroid hormones important e.g. for proper gender development and other
9 hormonal regulations ordered by steroid hormones. Azoles are relatively
10 high chemically reactive. Therefore, they create complexes/adducts with
11 nutrients, and essential as well as hazardous elements [3,4]. In this way, they
12 may influence (iii) the plant uptake of nutrients. Additionally, they may (iv)
13 influence the redox properties of natural antioxidants such as resveratrol [5].

14 The desired place of their action is commonly on the leaves or stems
15 of fruit trees – aboveground parts. However, within the rainfall, the
16 chemicals can be washed away and potentially they can get into the near root
17 zone (rhizosphere) < Fig. 1 >. The rhizosphere is very vivid part of soil. It is
18 rich in microorganisms, root exudates, and nutrients in potentially available
19 forms [6]. Therefore, it is a sensitive soil part to disruptions. It is not easy to
20 study the rhizosphere directly [7], because it includes processes on the
21 interface of liquid and solid phases. However, the liquid part of the soil – soil

solution is useful for the assessment of the current processes in soil [8]. It provides many options to be studied and it can be sampled relatively easily [9]. It represents very interesting and informative soil part which is influenced by plant root exudations and microbial activities as well as the bulk soil composition [10]. Soil solution contains soluble portion of chemicals present in soil (mostly nutrients, root exudates, fulvic acids, and also xenobiotics [11-14]. The presence of some xenobiotics in soil solution represents high risk for the ecosystem, because they can be bioavailable (e.g. for crops) or leached into water catchments.

< Fig. 1 > **Fig. 1** Schematic illustration of the possible fate of azoles after their application

Conventional analytical techniques have, of course, some limitations in sensitivity and selectivity, in spite of that usually some kind of extraction to isolate and to concentrate the studied compounds from the matrix is needed [15].

The main aim of this study is to develop a CE-UV method for easy detection of penconazole ($C_{13}H_{15}Cl_2N_3$), cyproconazole ($C_{15}H_{18}ClN_3O$) and tebuconazole ($C_{16}H_{22}ClN_3O$) < Fig. 2 > in a soil solution without previous treatments of the sample, applicable to the assessment of the azoles risk in the soil environment.

< Fig. 2 > **Fig. 2** Chemical structures of the investigated azoles

Results and Discussion

The determination of all three azoles (Pen, Cyp, Teb) in a mixture is possible with relatively good separation efficiency and reproducibility in relatively broad concentration ranges < Table 1 >, < Table 2 >.

< Table 1 > **Table 1** Concentration ranges and calibration equations

Azole	Concentration range (mol dm ⁻³)	Linear fit equation	Correlation R ²
Penconazole	1·10 ⁻⁷ - 1·10 ⁻⁵	3·10 ⁶ x+1.388	0.992
Cyproconazole	1·10 ⁻⁷ - 1·10 ⁻⁴	1·10 ⁶ x-0.936	0.988
Tebuconazole	5·10 ⁻⁷ - 1·10 ⁻⁴	2·10 ⁶ x-1.951	0.989

< Table 2 > **Table 2** Chromatographic recovery, matrix effect, LOD and LOQ of azoles determined in the soil solution spiked with equimolar (1.0·10⁻⁵ mol dm⁻³) concentration of each azole

Azole	CE recovery (%)	Matrix effect (%)	LOD (mol dm ⁻³)	LOQ (mol dm ⁻³)
Penconazole	88.6	12.4	4·10 ⁻⁷	8.72·10 ⁻⁷
Cyproconazole	85.2	14.8	5·10 ⁻⁷	1.06·10 ⁻⁶
Tebuconazole	91.3	8.7	8·10 ⁻⁷	1.81·10 ⁻⁶

The separation method was developed using model solutions in methanol, methanol/water (1:1, v/v) and soil solution samples. < Fig. 3 > shows the electropherograms with equimolar mixture of the azoles (1.0·10⁻⁵

1 mol dm^{-3}) in soil solution (a), methanol/water (b) and methanol (c),
2 respectively.

3

4 < Fig. 3 > **Fig. 3** Representative electropherograms of the separation of
5 cyproconazole (Cyp), penconazole (Pen), and tebuconazole (Teb), all $1.0 \cdot 10^{-5}$
6 mol dm^{-3} in (a) methanol, (b) methanol/water and (c) soil solution

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9 The signals of Cyp, Pen, and Teb have slightly higher intensity in the
10 spiked soil solution < Fig. 3c > than in methanol/water mixture < Fig. 3b >
11 and in pure methanol < Fig. 3a > due to the matrix. For the evaluation of the

actual areas and separation efficiency the azoles signal data were corrected with the corresponding background signal of the soil solution matrix < Fig. 4 >. The peaks of azoles in the soil solution electropherograms are slightly wider and better resolved in contrast to the corresponding data of the solvents (methanol and methanol/water). We consider that there can be (i) contribution of weak adducts of the azoles and (ii) internal structure formation between nitrogen (2N carrying the positive charge [4]) and the OH group. The molar masses of azoles increase in order Pen<Cyp<Teb. However, the pK_a of Cyp is higher (1.76) comparing to Pen and Teb (1.57 each) [16], therefore Cyp is protonated easier than Teb and Pen and due to that, it comes to the detector as the first. The second peak belongs to Pen and the last to Teb considering its highest molar mass (bigger surface charge). The identification of the azoles by their absolute migration times in pure solvents by individual analyses does not provide reliable quality estimation in the simultaneous azole group separations, because the migration times are influenced by the sample matrix [17]. However, the order is the same, but their electrophoretic mobility is different. That is why the identification and order of the compounds were determined by spiking and by modification at few concentrations.

1 < Fig. 4 > **Fig. 4** Chromatogram of the background soil solution matrix

2
3
4 Azoles have very low pK_a (below 2) [16], considered as weak bases,
5 therefore, they are deprotonated (Cyp, Teb) at pH 6.9 of our soil solution.
6 Usual pH of the soil solution from undisturbed agricultural soils ranges from
7 4 to 8 [8,18,19]. Therefore, in the azoles analyses the behaviour or impact of
8 soil composition and present biota has to be considered.

9 To identify the azoles as well as to measure the matrix effect of the
10 soil solution samples reasonable ESI- μ -TOF-MS < Fig. 5 > was involved.
11 The concentrations of all azoles in both solvents (in methanol/water as well
12 as in soil solution) were identical ($1.0 \cdot 10^{-6}$ mol dm $^{-3}$). The studied azoles
13 were clearly visible and the main signals originated in the protonated
14 penconazole (H^+ Pen), cyproconazole (H^+ Cyp) and tebuconazole (H^+ Teb),
15 since the protonation is the usual result of the electrospray process in the
16 positive mode. In addition, the minor signals identified in the spectra were
17 created by sodium adducts (Na^+ Cyp and Na^+ Teb), which are also usual

adduct of the electrospray process in positive mode. Nevertheless, the signals of H^+Pen , H^+Cyp and H^+Teb in soil solution have approx. 10 times lower intensity in contrast to that in methanol/water solution < Table 3 >. In the spectra of soil solutions < Fig. 5b > not any significant signal of charged azoles adducts were observed. In pH around 7 (in the soil solution as well as in methanol/water solution we studied) the azoles have tendency to deprotonate because of their low pK_a ($pK_a(Pen) = 1.57$, $pK_a(Cyp) = 1.76$ and $pK_a(Teb) = 1.57$, [16]). This evidence was also supported by the results received in the mass spectra of negative ionization modes < Fig. 6 >. Cyp and Teb are present as deprotonated anions and Pen as a radical. The tested azoles have high affinity to metal cations [3,4,13,16,20]. We expect the majority of the azoles due to the hydroxyl group are combined with metal cations present in soil solution matrix (neutral pH) to non-ionic complexes.

1 < Fig. 5 > **Fig. 5** Positive ESI-MS spectra of equimolar ($1.0 \cdot 10^{-6}$ mol dm⁻³)
2 mixture of penconazole (Pen), cyproconazole (Cyp) and tebuconazole (Teb)
3 in (a) MeOH/water (1:1, v/v) and (b) soil solution

4
5

6 < Table 3 > **Table 3** Characteristics of the MS signals coming from the
7 equimolar mixture of penconazole (Pen), cyproconazole (Cyp) and
8 tebuconazole (Teb) in methanol/water (1:1, v/v) and soil solution (SS)

9

Exact mass		Absolute intensity		Intensity ratio
Adduct	(<i>m/z</i>) [Da]	MeOH/W	SS	SS : MeOH/W (%)

H ⁺ Pen	284.0643	11817	1172	10
H ⁺ Cyp	292.1217	11859	1318	11
H ⁺ Teb	308.1530	12487	1152	9
Na ⁺ Cyp	314.1036	670	168	25
Na ⁺ Teb	330.1350	798	207	26

< Fig. 6 > **Fig. 6** Negative ESI-MS spectrum of equimolar ($1.0 \cdot 10^{-6}$ mol dm⁻³) mixture of penconazole (Pen), cyproconazole (Cyp) and tebuconazole (Teb) in (a) MeOH/water (1:1, v/v) and (b) soil solution (SS)

The intensity of charged azole adducts in the negative mode spectra are about one order lower in magnitude compared to them in the positive mode spectra. It was expected that the anions (deprotonated forms of azoles) would be preferred owing to neutral pH of the solvents and the low pK_a of the azoles. On the other hand, the mass spectrometric system (even if we sprayed the sample in pure solvents) is always rich on Na^+ , K^+ as well as H^+ ions. Therefore, it can be expected that these monocations form neutral adducts with mono-anionic azoles. The reason is that these neutral adducts cannot be distributed according to m/z ratio and detected using ESI- μ -TOF mass spectrometry. The effect of cations is even greater in the soil solution, which is rich on these nutrient cations (which are mostly weakly combined to complexes with low-weight-molecular organic matter).

Conclusion

Azoles are very common agrochemicals with frequent use. They block the biosynthesis of steroids and sterols of practically all living organisms. Therefore, their effect on non-target organisms is also expected and considered into account. The first step of the risk assessment is their determination in the environment. Soil solution might be considered as representative environmental matrix, which analysis elucidate the dynamic process in soil. Moreover, it is easy accessible for sampling. Thus, the

method is expected to be universal. Thus azole results can be comparable between different projects, which is important in presence of such biologically active pollutants. Penconazole, cyproconazole and tebuconazole may significantly disturb the delicate equilibria between the plant (root activities, nutrition uptake, and exudation), soil microorganisms, and availability of nutrients. Therefore, a fast and robust method without prior sample treatment for the determination using CE-UV in the analyses of soil solution was developed. The method is applicable without prior sample pre-treatment, with relatively satisfactory separation efficiency ~85%. The detection limit of this method is sufficient even for trace detection of these azoles ($\sim 10^{-7}$ mol dm⁻³).

Experimental

Chemicals

The analytical standards (Pestanal®) of studied azoles (penconazole, cyproconazole, and penconazole) were purchased from Sigma-Aldrich (Czech Republic). The stock solutions of azoles were prepared in concentration 10^{-2} mol dm⁻³ in methanol (HPLC purity grade, Sigma-Aldrich, Finland). For the working solutions they were diluted to $5.0 \cdot 10^{-5}$ - $1.0 \cdot 10^{-8}$ mol dm⁻³ concentrations. The reference samples and their mixtures to evaluate the migration, electroosmosis, and sensitivity were prepared in

5.0·10⁻⁵ mol dm⁻³ concentrations from the 1.0·10⁻⁴ mol dm⁻³ stock solutions. Concentrations of calibration solutions for penconazole (Pen), cyproconazole (Cyp), and tebuconazole (Teb) were in the ranges 10⁻⁷ - 10⁻⁵ mol dm⁻³, 10⁻⁷ - 10⁻⁴ mol dm⁻³, and 5.0·10⁻⁷ - 10⁻⁴ mol dm⁻³, respectively, with seven different concentrations. They were measured in four replicates. The reliability of the calibrations was checked with selected concentrations during two weeks. All solutions were stored in cold (4 °C) and dark until their use.

Orto-phosphoric acid (85%, grade: ACS, ISO, Reag. Ph Eur) was purchased from Merck Life Science Oy (Finland), 0.1 and 1.0 mol dm⁻³ sodium hydroxide solutions were from Oy FF Chemicals Ab (Finland). The electrolyte solution (BGE) made of 0.16 mol dm⁻³ phosphoric acid in water (pH 1.48) was stored at a stabilized room temperature (20 °C).

Purified water used for the experiments was laboratory-made milli-q water with Direct-Q UV Millipore instrument (Millipore S.A., France), 18.2 MΩ.

Preparation of the soil solution

The soil solution used as a matrix for spiking with azoles was prepared using a procedure published e.g. in [21] and stored in a fridge (4 °C) no longer than 5 days until application. Part of the soil solution was frozen and stored (-20

°C) for further repetitions of the measurements. The soil solution used as representative matrix was prepared from chernozem (strawberries plantation in pots, *Fragaria ananassa* ‘Calypso’) topsoil rich in organic matter (SOM) content (2.73 ± 0.07 %).

CE-UV

A Hewlett-Packard 3D capillary electrophoresis instrument (CE; Agilent, Germany) equipped with a photodiode array detector (wavelength range 190-600 nm) was used for method development and determination of the analytes. The CE was equipped with ChemStation (Agilent) operating software. Bare fused silica capillaries (ID 50 μ m, OD 362 μ m, Polymicro Technologies, USA) were cut to the total lengths of 32.5 cm with the efficient length of 8.5 cm. Before use, they were conditioned by flushing first with milli-q water and then with the electrolyte solution for 20 min each at 13.634 psi (940 mbar).

The standards and the samples were injected at -50 mbar pressure for 10.0 s followed by the introduction of the electrolyte solution at -50 mbar pressure for 17 s. After that, the process was stopped for 30 s to wait for moderation of the pressure. Analyses were realized in an acidic buffer at -10 kV voltage, 25 °C for 15 min, and under -54 μ A current, at the time window of 4.5-6.5 min. The applied electric field was -307.7 V cm⁻¹. The compounds

were detected using the UV-wavelengths 200 nm, 214 nm, 247nm, 260 nm, and 320 nm. The best applicable wavelength for azoles detection was 214 nm, different to recommended 260 nm [22]. Sample volume in vials and injection volume were 250 μm^3 and 26 μm^3 , respectively.

ESI- μ -Q-TOF-MS

The matrix effect of the soil solution on selected azoles (Pen, Cyp, Teb) detection was additionally studied using electrospray mass spectrometry (μ TOF Focus II, Bruker Daltonics, Germany) in the positive as well as in the negative mode. The electrospray ionisation conditions were very soft (spray voltage 4 kV, spray temperature 200°C) and they were optimized to intensify the signals of protonated (positive mode) and deprotonated (negative mode) azoles. The measurements were realized in methanol/water mixture (1:1, v/v) and in the prepared soil solution sample. Concentrations of all azoles were $1.0 \cdot 10^{-6} \text{ mol dm}^{-3}$ in both the solvents.

Acknowledgements

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References

1. Zarn JA, Bruschweiler BJ, Schlatter JR (2003) Environ Health Perspect 111:255
2. Trösken ER, Fischer K, Völkel W, Lutz WK (2006) Toxicology 219:33
3. Jakl M, Fanfrlík J, Jaklová Dytrtová J (2017) Rapid Commun Mass Spectrom 31:2043
4. Jaklová Dytrtová J, Fanfrlík J, Norková R, Jakl M, Hobza P (2014) Int J Mass Spectrom 359:38
5. Jaklová Dytrtová J, Straka M, Bělonožníková K, Jakl M, Ryšlavá H (2018) Food Chem 262:221
6. Rugova A, Puschenreiter M, Koellensperger G, Hann S (2017) Anal Chim Acta 956:1
7. Neumann G, George TS, Plassard C (2009) Plant Soil 321:431
8. Jaklová Dytrtová J, Šestáková I, Jakl M, Navrátil T (2009) Electroanalysis 21:573
9. Grossmann J, Udluft P (1991) Journal of Soil Science 42:83
10. McGahan DG, Southard RJ, Zasoski RJ (2014) Geoderma 226:340
11. Jaklová Dytrtová J, Jakl M, Schröder D, Navrátil T (2011) Curr Org Chem 15:2970
12. Jones DL (1998) Plant Soil 205:25

13. Norková R, Jaklová Dytrtová J, Jakl M, Schröder D (2012) Water Air
Soil Pollut 223:2633
14. Kim KR, Owens G, Naidu R, Kwon SI (2010) Geoderma 155:86
15. Huang QX, Yu YY, Tang CM, Peng XZ (2010) J Chromatogr A
1217:3481
16. Konášová R, Jaklová Dytrtová J, Kašička V (2015) J Chromatogr A
1408:243
17. Nowak PM, Wozniakiewicz M, Gladysz M, Janus M, Koscielniak P
(2017) Anal Bioanal Chem 409:4383
18. Jakl M, Jaklová Dytrtová J, Miholová D, Kolihová D, Száková J,
Tlustoš P (2009) Chem Speciation Bioavail 21:111
19. Antoniadis V, Levizou E, Shaheen SM, Ok YS, Sebastian A, Baum C,
Prasad MNV, Wenzel WW, Rinklebe J (2017) Earth-Sci Rev 171:621
20. Jaklová Dytrtová J, Jakl M, Schröder D, Čadková E, Komárek M
(2011) Rapid Commun Mass Spectrom 25:1037
21. Jaklová Dytrtová J, Šestáková I, Jakl M, Száková J, Miholová D,
Tlustoš P (2008) Cent Eur J Chem 6:71
22. Gordien JB, Pigneux A, Vigouroux S, Tabrizi R, Accoceberry I,
Bernadou JM, Rouault A, Saux MC, Breilh D (2009) J Pharm Biomed
Anal 50:932

Figure Captions

Fig. 1 Schematic illustration of the possible fate of azoles after their application

Fig. 2 Chemical structures of the investigated azoles

Fig. 3 Representative electropherograms of the separation of cyproconazole (Cyp), penconazole (Pen), and tebuconazole (Teb), all $1.0 \cdot 10^{-5} \text{ mol dm}^{-3}$ in (a) methanol, (b) methanol/water and (c) soil solution

Fig. 4 Chromatogram of the background soil solution matrix

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Table 1 Concentration ranges and calibration equations

	Concentration range	Linear fit	Correlation
Azole	(mol dm ⁻³)	equation	R ²
Penconazole	$1 \cdot 10^{-7} - 1 \cdot 10^{-5}$	$3 \cdot 10^6 x + 1.388$	0.992
Cyproconazole	$1 \cdot 10^{-7} - 1 \cdot 10^{-4}$	$1 \cdot 10^6 x - 0.936$	0.988
Tebuconazole	$5 \cdot 10^{-7} - 1 \cdot 10^{-4}$	$2 \cdot 10^6 x - 1.951$	0.989

- 1 **Table 2** Chromatographic recovery, matrix effect, LOD and LOQ of azoles
2 determined in the soil solution spiked with equimolar ($1.0 \cdot 10^{-5} \text{ mol dm}^{-3}$)
3 concentration of each azole

4

CE recovery		Matrix effect	LOD	LOQ
Azole	(%)	(%)	(mol dm^{-3})	(mol dm^{-3})
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Tebuconazole	91.3	8.7	$8 \cdot 10^{-7}$	$1.81 \cdot 10^{-6}$

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6

Table 3 Characteristics of the MS signals coming from the equimolar mixture of penconazole (Pen), cyproconazole (Cyp) and tebuconazole (Teb) in methanol/water (1:1, v/v) and soil solution (SS)

Adduct	Exact mass (<i>m/z</i>) [Da]	Absolute intensity		Intensity ratio SS : MeOH/W (%)
		MeOH/W	SS	
H ⁺ Pen	284.0643	11817	1172	10
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H ⁺ Teb	308.1530	12487	1152	9
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4

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3

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3

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