

Special Article – Environmental Pollution

The Challenges of Fungicide Analyses Using Gas Chromatography and Liquid Chromatography-Mass Spectrometry Methods

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Abstract

There is growing interest in developing methods for the analysis of fungicides in environment samples and other sample matrices. Strobilurin fungicides and conazole fungicides are two important classes of fungicides with different modes of action that can be found in formulations containing more than one active fungicide ingredient. New conazole fungicides have been introduced into the marketplace and the analysis of conazole fungicides is more challenging due to isobaric interferences such that chromatographic resolution must be obtained for those conazole fungicides with the same selected reaction monitoring transitions. Our liquid chromatography-electrospray positive ion-tandem mass spectrometry method has been expanded to include new conazole fungicides from both agrochemical and pharmaceutical applications along with the addition of more recently available deuterated internal conazole standards. The feasibility of simultaneous liquid chromatography-electrospray positive ion-tandem mass spectrometry analysis of seven strobilurin fungicides including picoxystrobin with conazole fungicides was demonstrated. Further suggestion for other classes of fungicides for inclusion in chemical class specific fungicide methods are provide and require utilization of both gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry approaches.

Keywords: Strobilurin fungicides; Conazole fungicides; Pesticide residue analysis; Fungicides; Liquid chromatography-tandem mass spectrometry

Abbreviations

GC-MS-SIM: Gas Chromatography-Mass Spectrometry-Selective Ion Monitoring; LC-ESI+-MS/MS: Liquid Chromatography-Electrospray Positive Ion-Tandem Mass Spectrometry; SRM: Selective Reaction Monitoring; TR: Retention Time

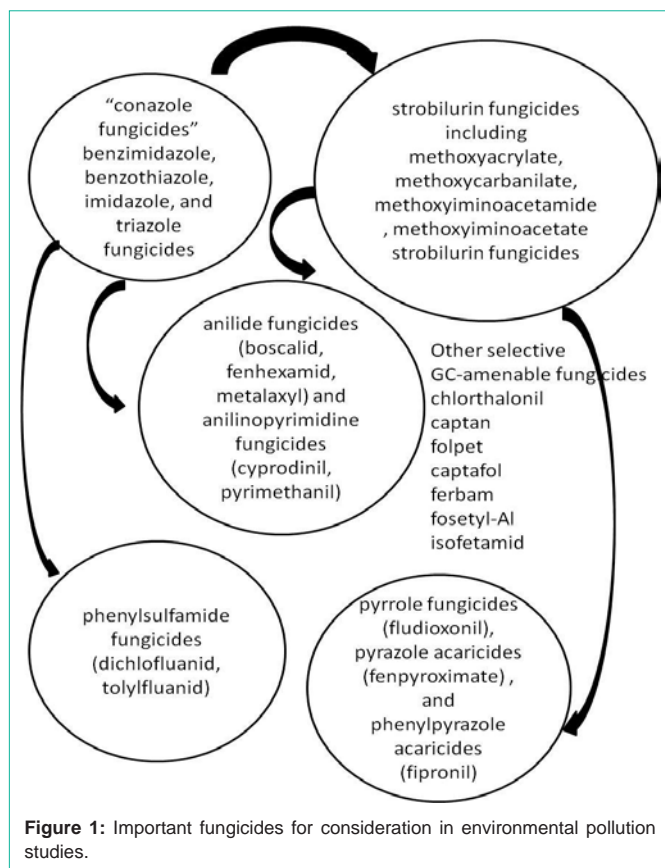
Introduction

Much of the focus of recent studies examining levels of pesticides in environmental matrices or exposure of sensitive species to agricultural formulations has focused on insecticides including neonicotinoids, organophosphorus pesticides, and methyl carbamates, however recent studies have shown occurrence of fungicides in soil, water, and atmospheric samples (gas or particle phase), pollen, wildflowers, beeswax, bees, ants, crops and food products [1-13]. Fungicides are also used as pharmaceuticals in veterinary and human health applications and may be released directly into the environment or may enter water treatment facilities and subsequently be released into surface waters further increasing the complexity of analysis of environmental matrices with potential to have a much wider range of fungicides present in the environment [14-15]. Some fungicide classes including strobilurin and conazole fungicides have been associated with detrimental impacts on aquatic systems, bee populations, farmland ants and are associated with endocrine disruption in humans [14-22]. Herein we tackle the feasibility of chemical analysis of fungicides largely used in applications for disease control in crop production.

Chemical analysis methods for fungicides have received limited attention relative to the extent of method development for classes of insecticides and herbicides with often a few selected fungicides included in multi-residue analysis methods. Sample preparation and clean-up prior to gas or liquid chromatography-mass spectrometry also vary greatly with sample matrix and have been recently reviewed for some classes of fungicides [23]. There are a large range of chemical classes of fungicides to combat diseases in plants aimed at improving yield and appearance of crops to minimize loss of market value. Fungicides can be applied due to their curative properties but more often are applied in a preventative approach to protect from pathogens and some crops have developed resistance to particular fungicides such that management practices often include rotation of different groups of fungicides or formulations with more than one active ingredient from different groups of fungicide classes (different modes of action). Some of the mostly commonly used chemical classes of fungicides that have been analyzed using liquid chromatography-electrospray ionization tandem mass spectrometry or gas chromatography with electron impact or chemical ion ionization-mass spectrometry are shown in (Figure 1).

Analysis of Fungicides

Formulations of fungicides can include active ingredients from more than one group of fungicides with the major classes for formulas including strobilurin fungicides, conazole fungicides, and anilide fungicides. In addition pyrrole and phenylsulfamide and



other selected GC amenable fungicides (more frequently analyzed in specialized GC-MS methods such as for captan and folpet where cold on-column injection is used to minimize thermal degradation and improve detection limits with the use of large volume injections) may be present in these formulations or applied separately [7-11]. Figure 2 shows some common strobilurin fungicides that are used in combination with azole, anilide, and pyrrole fungicides. In our prior work we developed methods for the simultaneous analysis of neonicotinoid insecticides, degradation products of neonicotinoid insecticides and strobilurin fungicides as well as a separate LC-MS/MS method for the analyses of over 30 conazole fungicides [12-13]. In this paper we will tackle the feasibility of LC-ESI⁺-MS/MS analysis for simultaneous analysis of strobilurin fungicides and conazole fungicides with updates for inclusion of new conazole fungicides to the marketplace. These two chemical classes of fungicides were the focus of the new approach due to the importance of strobilurin fungicides and conazole fungicides in disease management and presence in formulations with more than one active ingredient.

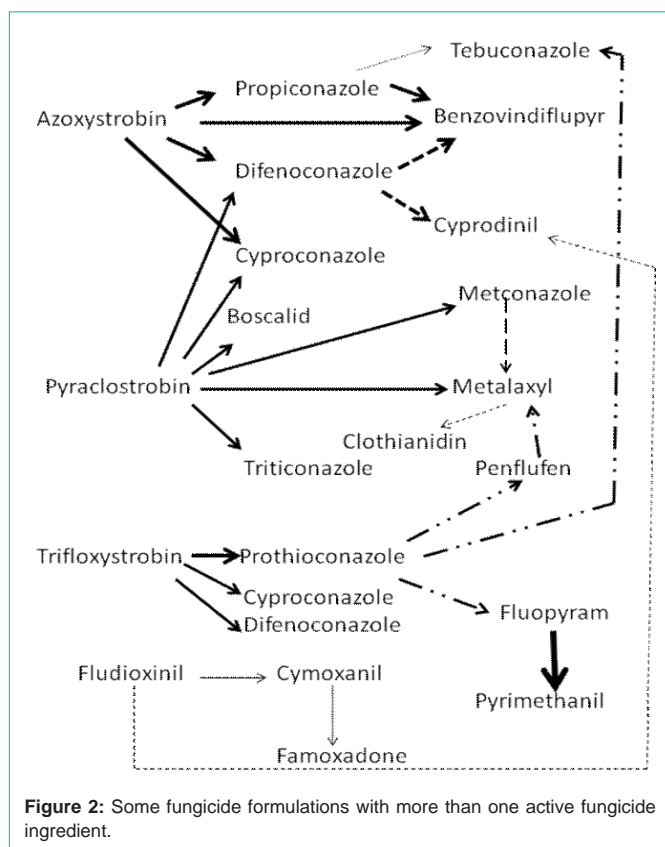
A few selected fungicides have often been included in multi-residue analysis methods, however with the importance of particularly strobilurin fungicides and conazole fungicides to the marketplace there has been increasing demand to develop methods that accommodate a wider range of fungicides within the same chemical class or fungicide group. Some chemical classes such as conazole fungicides are particularly prone to false positives (due to improper identification of target fungicides particularly those with isobaric interferences with low resolution tandem mass

spectrometry). In addition sample preparation methods for target chemical classes allows for improvements in recoveries of these fungicides from extraction and clean-up steps [12-13, 23-24]. Both strobilurin and azole fungicides can be analyzed with gas or liquid chromatography-mass spectrometry methods depending on which particular fungicides from these chemical classes need to be targeted.

Conazole Fungicides

Conazole fungicides can be analyzed by GC-MS methods and LC-ESI⁺-MS/MS methods often with similar sensitivity, however, LC-ESI⁺-MS/MS is often preferred due to the improved confirmation of identity of conazole fungicides as LC-ESI⁺-MS/MS can provide molecular weight information from the protonated molecular ion. In 2012 we developed a LC-ESI⁺-MS/MS method for a wide range of conazoles (over 30 conazole and related fungicides) and with new fungicides commercially available we have further expanded the range of azole fungicides that can be included in the LC-ESI⁺-MS/MS analysis (Table 1) [13]. In addition in prior methods there were no deuterated internal standards commercially available such that conazole fungicides for pharmaceutical applications such as voriconazole and thioconazole were used as internal standards and evaluated for their presence in environmental samples collected in a targeted agricultural region. However there has been increasing potential for conazole fungicides to enter the environment from veterinary or human health pharmaceutical use [14-15] such that this approach is no longer viable. In recent years commercially available deuterated conazole pharmaceutical fungicides have become available including aripiprazole-d₈, fluconazole-d₄, hydroxyitraconazole-d₄, itraconazole-d₄, and imidazole-d₄. These conazoles are deuterated analogs of conazole anti-fungals with retention times ranging from 1.3 to 18 min on reversed-phase columns (Figure 3C) is similar to other conazoles or metabolites of azoles and cover a large mass range in mass spectrometry for [M+H]⁺ → fragment ion to evaluate potential matrix effects (Figure 3 and Table 1). New conazoles added to our method that can be separated from other azoles include azaconazole, itraconazole (pharmaceutical), etaconazole, etoconazole along with the deuterated conazole fungicides (Figure 3C and 3I). Etriazazole can only be analyzed by LC-MS/MS at high concentrations and it is recommended to be analyzed using GC-MS methods [25]. Fluconazole-d₄ and imidazole-d₄ have the best MS sensitivity.

We have found that the use of a small percentage of 2-propanol (3%-5%, selected herein as 3% 2-propanol and 2% methanol to optimize peak shapes and selectivity particularly for conazoles that are not retained strongly on reversed-phase stationary phases). The presence of 2-propanol in the aqueous phase in LC-ESI⁺-MS/MS also minimizes carry-over issues and improves the performance of the column over the long-term with no impact on MS sensitivity. In pure aqueous phase there is large potential for conazole fungicides to adsorb to column or tubing components leading to peak distortion and instability of retention times over time. The gradient elution for this large range of conazoles required the gradient program to begin at with high volume percentage of aqueous phase. The mobile phase also has formic acid (0.05-0.10 v%) to improve the ionization efficiency of conazole fungicides for positive electrospray ionization. Good chromatographic resolution is required for those conazole azoles



with isobaric interferences ($[M+H]^+ \rightarrow 70$) such as for triadimenol, paclobutrazol, cyproconazole, uniconazole-P, myclobutanil which are all chlorinated and have the potential to co-elute in reversed-phase separations [13]. High resolution MS can also provide better MS resolution although it is seldom selected in the analysis of conazole fungicides except to aid in identification of new metabolites.

GC amenable conazoles include difenconazole, diniconazole, etriadazole, fenbuconazole, fluquinconazole, hexaconazole, paclobutrazole, penconazole, probenazole, propiconazole, tebuconazole, tebufenozide, tetraconazole, thiabendazole, and triflumizole. Their inclusion in multi-residue analysis methods is often for selected conazoles rather than as a group as recoveries in sample preparation methods may also vary depending upon the entire target list of pesticides [25-29]. Conazole fungicides exhibit a high degree of fragmentation of the molecular ion in the electron impact ion source such that the molecular ion is not high enough in abundance to use for quantitation or confirmation and fragment ions are frequently $m/z < 250$ for GC-MS-SIM (see Table 1). Consequently GC-MS methods for conazole fungicides (pending sample clean-up procedures) are more prone to interferences from matrix components in environment samples. Sample clean-up approaches depend upon sample matrix [23].

There is potential for inclusion of degradation products or metabolites of conazole fungicides into LC-MS/MS methods including 5-hydroxythiabendazole (metabolite of thiabendazole), new metabolite of prochloraz, M1-M3 and HWG 2061 metabolites of tebuconazole, 1,2,4-triazole and its two conjugates triazolylalanine

and triazolylacetic acid, RH-9129 and RH-9130 metabolites of fenbuconazole, triadimenol (metabolite of triadimefon), RPA-4-4886 and RPA-406341 metabolites of cyproconazole, difenoconazole, tebuconazole, epiconazole, tetraconazole, and triticonazole or enantioselective separation of epoxiconazole, fenbuconazole, imazalil, or triadimenfon with MS detection [30-37]. Conazole degradation products such as 1,2,4-triazole are weakly retained on reversed phase LC columns (imidazole4 best internal standard) and needs conditions of high aqueous content in the mobile phase such that care should be taken to avoid carry-over issues of other parent conazoles [26].

Strobilurin Fungicides

Strobilurin fungicides are a class of fungicides in the Quinone outside Inhibitors (QoI) group. For environment analysis LC-ESI-MS/MS was almost exclusively used for the analysis of strobilurin fungicides (Table 1) and in most cases high-resolution MS is not necessary to resolve co-eluting compounds as the protonated molecular ion generally differs by more than one unit mass resolution such that their a few isobaric interferences of nearby eluting compounds (Figure 3A). Picoxystrobin and fluoxastrobin co-elute but can be identified by their unique SRMs. As can be seen with Table 1 the most common strobilurin fungicides produce the protonated molecular ion ($[M+H]^+$) with electrospray ionization which has the advance over GC-EI-MS methods where the most abundant ions produced in the ion source are fragment ions and the molecular ion is too low in abundance to be used as a quantitative or confirmation ion for the analysis (no molecular weight confirmation in GC-MS SIM) [38-41]. Strobilurin fungicides such as kresoxim-methyl and trifloxystrobin share common fragment ions ($m/z=116, 131$) when GC-EI-MS-SIM is used for analysis such that chromatographic resolution is essential. Picoxystrobin has somewhat lower sensitivity in LC-ESI-MS/MS methods (Figure 3) such that GC-MS methods may be preferred [38]. Strobilurin fungicides can also be analyzed by LC-diode array detector for additional confirmation such as for azoxystrobin, dimoxystrobin, kresoxim-methyl, metominostrobin, picoxystrobin, pyraclostrobin [40]. The most common strobilurin fungicides include azoxystrobin, fluoxastrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin and they have been analyzed in environmental and food matrices using liquid chromatography-electrospray positive ion-tandem mass spectrometry [13]. A variety of reversed phase stationary phases can be used include octyldecylsilane and Polar RP can provide additional selectivity. The mobile phase is generally selected to have acetonitrile as the organic modifier and formic acid as an additive to improve ionization of strobilurin fungicides for improved MS sensitivity. Ammonium format in the aqueous phase has also been used particularly when neonicotinoid insecticides or other chemical classes of pesticides are present [13]. Fluoxastrobin and kresoxim-methyl co-elute with many reversed-phase separation conditions but can be distinguished easily by their unique SRMs (Table 1). Some strobilurin fungicides have only been analyzed in a few studies including metominostrobin, oryastrobin, and pyraoxystrobin as they are not registered yet for agricultural usage such as in some countries such as Canada.

Enantioselective separation of strobilurin fungicides with chiral LC-high resolution mass spectrometry has also been used for the

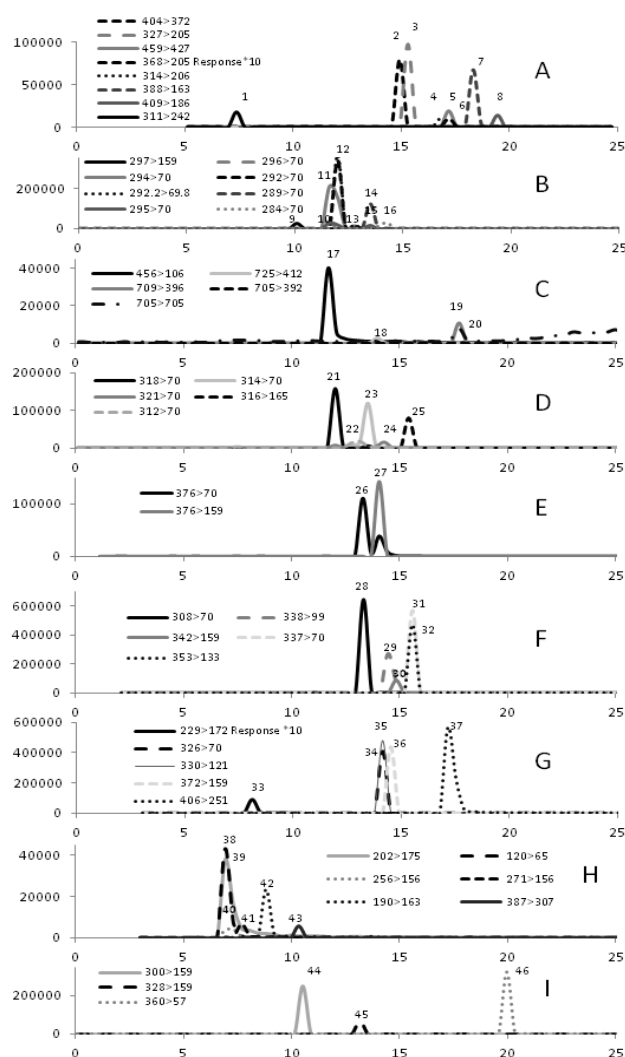


Figure 3: Analysis of strobilurin and conazole fungicides by LC-ESI⁺-MS/MS. Mobile phase gradient from 100% A (3% 2-propanol, 2% methanol, 0.05% formic acid in water) for 1.50 min; 80% A at 2.50 min (20% mobile phase B: 0.1% formic acid in acetonitrile); 60% A at 3.00 min; 50% A at 10.00 min; 40% A at 16 min; 25% A at 20.00 min; and 20% A at 25 min. Stationary phase Synergi Polar RP (550 X 2.00 mm i.d., 2.5 μ m, Phenomenex, Torrance, CA) flow rate 0.15 mL/min. Standard injection for all standards of 50 ng/mL unless noted, 10 μ L injection loop. 100 ng/mL of aripiprazole-d₈ and imidazole-d₄, 50 ng/mL fluconazole-d₄, 250 ng/mL of itraconazole-d₄ and hydroxyitraconazole-d₄. Compounds identified in the separation are as follows: A, 1. fluconazole-d₄ (311 \rightarrow 242); 2. azoxystrobin (404 \rightarrow 372), 3. dimoxystrobin (372 \rightarrow 205), 4. kresoxim-methyl (314 \rightarrow 206), 5. fluoxastrobin (459 \rightarrow 427), 6. picoxystrobin (368 \rightarrow 205) with Y-scale response expanded by 10 and 100 ng/mL injected, 7. pyraclostrobin (388 \rightarrow 163); B, 9. imazilil (297 \rightarrow 159), 10. triadimenol (296 \rightarrow 70), 11. paclobutrazol (294 \rightarrow 70), 12. cyproconazole (292 \rightarrow 70), 13. uniconazole-P (292.2 \rightarrow 69.8), 14. myclobutanil (289 \rightarrow 70), 15. triadimefon (295 \rightarrow 70), 16. penconazole (284 \rightarrow 70); C, 17. aripiprazole-d₈ (456 \rightarrow 106), 18. hydroxyitraconazole-d₄ (725 \rightarrow 412), 19. itraconazole-d₄ (709 \rightarrow 396), 20. itraconazole (705 \rightarrow 392 and 705 \rightarrow 705); D, 21. triticonazole (318 \rightarrow 70), 22. prothioconazole-deshthio (312 \rightarrow 70); 23. hexaconazole (314 \rightarrow 70), 24. metconazole (321 \rightarrow 70), 25. flusilazole (316 \rightarrow 70), E, 26. prochloraz (376 \rightarrow 70), 27. bromuconazole (376 \rightarrow 159), F, 28. tebuconazole (308 \rightarrow 70), 29. biteranol (338 \rightarrow 99), 30. propiconazole (342 \rightarrow 159), 31. fenbuconazole (337 \rightarrow 70), 32. tebufenozide (353 \rightarrow 133); G, 33. tebuthiuron (229 \rightarrow 172 with Y-scale response expanded by factor of 10), 34. diniconazole (326 \rightarrow 70), 35. epoxiconazole (330 \rightarrow 121), 36. tetraconazole (372 \rightarrow 159), 37. difenoconazole (406 \rightarrow 251), H, 38. benzothiazole (120 \rightarrow 65), 39. thiabenazole (202 \rightarrow 175), 40. sulfathiazole (256 \rightarrow 156), 41. sulfamethizole (271 \rightarrow 156), 42. tricyclazole (190 \rightarrow 163), 43. sulfentrazone (387 \rightarrow 07); I, 44. azaconazole (300 \rightarrow 159), 45. etaconazole (328 \rightarrow 159), 46. etoxazole (360 \rightarrow 57). Table 1 provides additional SRM transitions (quantitative SRM shown in Figure). X-scale Time in min and Y-scale Abundance.

analysis of pyrisoxazole [42]. Strobilurin-pyrimidine derivatives have also been identified for their potential for antiproliferative activity against human cancer cell lines [43]. Other candidate thiazole-based strobilurin fungicides are also under development [44]. Trifloxystrobin acid (metabolite of trifloxystrobin) has been detected by GC-EI-MS/MS [45]. Azoxystrobin acid and 2-hydroxybenzoxazole are metabolites of azoxystrobin and have been determined by LC-ESI⁺-MS/MS [46].

Conclusions

Simultaneous analysis of strobilurin and conazole fungicides is feasible using LC-ESI⁺-MS/MS approach as presented. LC-ESI⁺-MS/MS approaches in general provide better confirmation ability than GC-MS-SIM methods for class specific analysis due to the structure similarities of many of these fungicides and in some cases isobaric interferences. Further inclusion of degradation products or metabolites is also feasible with consideration that some of these

Table 1: Ions for GC-MS SIM analysis or Selected Reaction Monitoring Transitions (SRMs) for LC-ESI⁺-MS/MS.

Fungicide	SIM Ions (m/z) for GC Amenable Fungicides Quantitative, confirmation ions	SRM1, SRM2 for LC-ESI ⁺ -MS/MS Amenable Fungicides Quantitative, confirmation SRMs
Conazole fungicides (imidazole)		
Imazalil	41, 215, 173	297→159, 297→201
Prochloraz (and amide fungicide)	70, 43, 180, 308, 310	376→70, 378→70, 376→308
Triflumizole		346→278, 346→73
Conazole fungicides (triazoles)		
Azaconazole		300→159, 300→231
Biteranol	170, 168, 112, 171	338→99, 338→269
Benzotriazole		120→5, 120→92
Bromuconazole	173, 175, 295	376→159, 378→159
Cyproconazole	222, 139, 224	292→70, 294→70
Diclobutrazole		328→70, 328→159
Difenoconazole	265, 323, 267, 325	406→251, 408→253
Diniconazole (diniconazole-M)	268, 270, 70, 232	326→70, 328→70, 326→159
Epoxiconazole		330→121, 332→121, 330→123
Etaconazole	173, 245, 175, 247	328→159, 330→161, 328→187
Etoxazole	141, 204, 300	360→57, 360→141, 360→177.5
Fenbuconazole	129, 198, 125	337→70, 337→125
Fluquinconazole	340, 342	NA
Flusilazole	233, 206	316→165, 316→248
Hexaconazole	83, 214, 216, 231	314→70, 316→70
Ipconazole		334→70, 334→125
Metconazole	125, 83, 70	321→70, 323→70
Myclobutanil	179, 152, 181, 150	289→70, 291→70
Penconazole	248, 159, 161	284→70, 284→159
Propiconazole	259, 173, 261, 69	342→159, 342→69
Prothioconazole (analyzed as prothioconazole-desthio)	186, 188, 125	314→70, 312→70, 312→125
Simeconazole	121, 101	293→70, 293→73
Tebuconazole	125, 250, 127, 252	308.5→70, 310.5→70, 308.5→125
Tetraconazole	336, 338	372→159, 372→70
Triadimefon	57, 208, 181, 210	295→70, 297→70
Triadimenol	112, 168, 128	296→70, 298→70, 296→99
Triticonazole		318→70, 320→70
Uniconazole (uniconazole-P)		292→70, 294→70
Benzimidazole fungicide		
Thiabendazole	201, 174	202→175, 202→131
Benzimidazole precursor fungicide		
Thiophanate-methyl		343→151, 343→311
Others Analyzed with Conazoles		
Paclobutrazol (plant growth regulator with triazole moiety)	236, 238	294→70, 295→70, 296→70
Etriadiazole (thiadiazole fungicide)		Poor sensitivity
Tebufenozide (insecticide)		353→133, 353→297
Tebuthiuron (thiadiazolylurea herbicide)		229→172, 229→116
Tricyclazole (benzothiazole fungicide)	162, 189	190→163, 190→136

Conazole Pharmaceutical and Deuterated Internal Standards		
Itraconazole (tr=17.73 min)		705→705, 705→392 (note sensitivity lower than other conazoles)
Sulfamethizole		271→156, 271→92
Sulfathiazole		256→156, 256→92
Thioconazole (previously used as IS)		390→131, 388→131
Voriconazole (previously used as IS)		351→127, 351→282
Aripipazole-d8 (tr=11.70 min)		456→106, 456→293, 456→179
Fluconazole-d ₄ (tr=7.39 min)		311→242, 311172, 311→223, 311→70
Hydroxyitraconazole-d ₄ (tr=13.96 min)		725→412, 725452, 725→434
Imidazole-d4 (tr=1.31 min)		70.8→43.8, 70.8→70.8
Itraconazole-d ₄ (tr=17.73 min)		709→396, 709→436
Strobilurin fungicides		
Fluoxastrobin		459.2→427.4, 459.2→188.2
methoxyacrylate strobilurin fungicides		
Azoxystrobin	344, 388, 372, 403	404.1→372.4, 404.1→329.4
Picoxystrobin	145, 335, 303	368→145, 368→205
Pyraoxystrobin		413.4→145.4, 413.4→205.7, 415.4→145.4, 415.4→217.7
methoxycarbamate strobilurin fungicides		
Pyraclostrobin	132, 164	388.1→163.5, 388.1→194.5
methoxyiminoacetamide strobilurin fungicides		
Dimoxystrobin	116, 205, 58	327.6→205.3, 327.6→238.5
Metominostrobin	191, 196, 238	285→194, 285→140
Oryastrobin		392→205, 392→116
methoxyiminoacetate strobilurin fungicides		
Kresoxim-methyl	116, 131, 206	314.1→206.3, 314.1→116.0, 314.1→267.4
Trifloxystrobin	116, 131, 172, 145, 222	409.1→186.4, 409.1→206.4

compounds have weak retention on reverse-phase stationary phases such that gradient elution requires high aqueous content at the start of the separation. Enantiomeric separations for targeted conazole or strobilurin fungicides.

Acknowledgements

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References

- David A, Botias C, Abdul-Sada A, Nicholls E, Rotheray EL, Hill EM, et al. Widespread Contamination of Wildflower and Bee-Collected Pollen with Complex Mixtures of Neonicotinoids and Fungicides Commonly Applied to Crops. *Environ Intern*. 2016; 88: 169-178.
- Hladik ML, Vandever M, Smalling KL. Exposure of Native Bees Foraging in an Agricultural Landscape to Current-Use Pesticides. *Sci Total Environ*. 2016; 542:469-477.
- Perugini M, Tulini SMR, Zezza D, Fenucci S, Conte A, Amorena M. Occurrence of Agrochemical Residues in Beeswax Samples Collected in Italy During 2013-2015. *Sci Total Environ*. 2018; 625: 470-476.
- Kasiotis KM, Anagnostopoulos C, Antastasiadou P, Machera K. Pesticide Residues in Honeybees, Honey and Bee Pollen by LC-MS/MS Screening: Reported Death Incidents in Honeybees. *Sci Total Environ*. 2014; 633-642: 485-486.
- Edder P, Ortellì D, Viret O, Cognard E, De Montmollin A, Zali O. Control Strategies Against Grey Mould (*Botrytis cinerea* Pers.:Fr) and Corresponding Fungicide Residues in Grapes and Wines. *Food Addit Contam*. 2009; 26: 719-725.
- Rose G, Lane S, Jordan R. The Fate of Fungicide and Insecticide Residues in Australian Wine Grape By-Products Following Field Application. *Food Chem*. 2009; 117: 634-640.
- Blandino M, Galeazzi M, Savoia W, Reyneri A. Timing of Azoxystrobin + Propiconazole Application on Maize to Control Northern Corn Leaf Blight and Maximize Grain Yield. *Field Crops Research* 2012;139: 20-29.
- Rickes da Luz S, Pazdiora PC, Dallagnol LJ, Dors GC, Chaves FC. Mycotoxin and Fungicide Residues in Wheat Grains from Fungicide-Treated Plants Measured by a Validated LC-MS Method. *Food Chem*. 2017; 220: 510-516.
- Karaoglanidis GS, Karadimos DA. Efficacy of Strobilurins and Mixtures with DMI Fungicides in Controlling Powdery Mildew in Field-Grown Sugar Beet. *Crop Protection* 2006; 25: 977-983.
- Hvězdova M, Kosubová M, Scherr KE, Šudoma M, Škulcová L, Sážka M, Svobodová M, et al. Currently and Recently Used Pesticides in Central European Arable Soils. *Sci Total Environ*. 2018; 613-614: 361-370.
- Raina R, Belzer W, Jones K. Atmospheric Concentrations of Captan and Folpet in the Lower Fraser Valley Agricultural Region of Canada. *Air, Soil and Water Res*. 2009; 2:41-49.
- Raina-Fulton R, Determination of Neonicotinoid Insecticides and Strobilurin Fungicides in Particle Phase Atmospheric Samples by Liquid Chromatography-Mass Spectrometry. *J Agric Food Chem*. 2015; 63: 5152-5162.
- Raina R, Smith E. Determination of Azole Fungicides in Atmospheric

- Samples Collected in the Canadian Prairies by LC/MS/MS. *J AOAC Intern.* 2012; 95: 1350-1356.
14. Münze R, Hannemann C, Orlinskiy P, Gunold R, Paschke A, Foit K, et al. Pesticides from Wastewater Treatment Plant Effluents Affect Invertebrate Communities. *Sci Total Environ.* 2017; 599-600: 387-399.
 15. Chen Z-F, Ying G-G. Occurrence, Fate and Ecological Risk of Five Typical Azole Fungicides as Therapeutic and Personnel Care Products in the Environment: A Review. *Environ Intern.* 2015; 84: 142-153.
 16. Calatayud-Vernich P, Calatayud F, Simó E, Suarez-Varela MM, Picó Y. Influence of Pesticide Use in Fruit Orchards During Blooming on Honeybee Mortality in 4 Experimental Apiaries. *Sci Total Environ.* 2016; 541: 33-41.
 17. Lee J, Kim E, Shin Y, Lee J, Lee J, Moon J-K, et al. Whole Body Dosimetry and Risk Assessment of Agricultural Operator Exposure to the Fungicide Kresoxim-Methyl in Apple Orchards, *Ecotoxicology Environ. Safety* 2018; 155: 94-100.
 18. Heneberg P, Svoboda J, Pech P. Benzimidazole Fungicides are Detrimental to Common Farmland Ants. *Biological Conservation* 2018; 12: 114-117.
 19. Rodrigues ET, Lopes I, Pardal MA. Occurrence, Fate and Effects of Azoxystrobin in Aquatic Ecosystems: A Review. *Environ Intern.* 2013; 53: 18-28.
 20. Dimitrov MR, Kosol S, Smidt H, Buijse L, Van den Brink P J, Van Wijngaarden RPA, et al. Assessing Effects of the Fungicide Tebuconazole to Heterotrophic Microbes in Aquatic Microcosms. *Sci Total Environ.* 2014; 490: 1002-1011.
 21. Kjørstad MB, Taxvig C, Nellemann C, Vinggaard AM, Andersen HR. Endocrine Disrupting Effects *in Vitro* of Conazole Antifungals Used as Pesticides and Pharmaceuticals. *Reproductive Toxicol.* 2010; 30: 573-582.
 22. Rieke S, Heise T, Schmidt F, Haider W, Bednarz H, Niehaus K, et al. Mixture Effects of Azole Fungicides on the Adrenal Gland in a Broad Dose Range. *Toxicol.* 2017; 385: 28-37.
 23. Raina-Fulton R, Aborkhees G, Behdarvandan A. Analysis of Herbicide and/or Pesticide Residues in Dietary Botanical Supplements. *Encyclopedia of Analytical Chemistry, Applications, Theory and Instrumentation.* John Wiley and Sons, 2018.
 24. Bailey R, Belzer W. Large Volume Cold On-Column Injection for Gas Chromatography-Negative Chemical Ionization-Mass Spectrometry Analysis of Selected Pesticides in Air Samples. *J Agric Food Chem.* 2007; 55: 1150-1155.
 25. Da Silva Sousa J, Carius de Castro R, de Albuquerque Andrade G, Gomes Lima C, Lima L K, Liberato Milhome M A, et al. Evaluation of an Analytical Methodology Using QuEChERS and GC-SQ/MS for the Investigation of the Level of Pesticide Residues in Brazilian Melons. *Food Chem.* 2013; 141: 2675-2681.
 26. Menezes Filho A, Neves dos Santos F, Pereiro PAP. Development, Validation and Application of a Method Based on DI-SPME and GC-MS for Determination of Different Chemical Groups in Surface and Groundwater Samples. *Microchem J.* 2010; 96: 139-145.
 27. Tankiewicz M, Morrison C, Biziuk M. Multi-Residue Method for the Determination of 16 Recently Used Pesticides from Various Chemical Groups in Aqueous Samples by Using DI-SPME Coupled with GC-MS. *Talanta* 2013; 107: 1-10.
 28. Lian, Y-J, Pang G-F, Shu H-R, Fan C-L, Liu Y-M, Feng J, et al. Simultaneous Determination of 346 Multiresidue Pesticides in Grapes by PSA-MSPD and GC-MS-SIM. *J Agric Food Chem.* 2010; 58: 9428-9453.
 29. Charlton AJA, Jones A. Determination of Imidazole and Triazole Fungicide Residues in Honeybees using Gas Chromatography-Mass Spectrometry. *J. Chromatogr A* 2007; 1141: 117-122.
 30. Cannavan A, Haggan SA, Kennedy DG. Simultaneous Determination of Thiabendazole and its Major Metabolite, 5-Hydroxythiabendazole, in Bovine Tissues using Gradient Liquid Chromatography with Thermospray and Atmospheric Pressure Chemical Ionization Mass Spectrometry. *J Chromatogr B* 1998; 718: 103-113.
 31. Casas ME, Kretschmann AC, Andernach L, Opatz T, Bester K. Separation, Isolation and Stereochemical Assignment of Imazalil Enantiomers and Their Quantitation in an *In Vitro* Toxicity Test. *J Chromatogr A* 2016; 1452: 116-120.
 32. Shen Q, Li S, Xu P, Li W, Zhuang G, Wang Y. Enantioselective Metabolism of Triadimefon and its Chiral Metabolite Triadimenol in Lizards. *Ecotoxicology and Environ Safety* 2017; 143: 159-165.
 33. Li Y, Dong F, Liu X, Xu J, Li J, Kong Z, Chen X, et al. Simultaneous Enantioselective Determination of Fenbuconazole and its Main Metabolites in Soil and Water by Chiral Liquid Chromatography/Tandem Mass Spectrometry. *J Chromatogr A* 2011; 1218: 6667-6674.
 34. Ruiz-Rodríguez L, Aguilar A, Díaz AN, Sánchez. Enantioseparation of the Fungicide Imazalil in Orange Juice by Chiral HPLC. Study of Degradation Rates and Extractive/Enrichment Techniques. *Food Chem.* 2015; 178: 179-185.
 35. Youness M, Sancelme M, Combourieu B, Besse-Hoggan P. Identification of New Metabolic Pathways in the Enantioselective Fungicide Tebuconazole Biodegradation by *Bacillus* sp. 3B6. *J Haz Materials* 2018; 351: 160-168.
 36. Thurman EM, Ferrer I, Zweigenbaum JA, García-Reyes JF, Woodman M, Fernández-Alba AR. Discovering Metabolites of Post-Harvest Fungicides in Citrus with Liquid Chromatography/Time-of-Flight Mass Spectrometry and Ion Trap Tandem Mass Spectrometry. *J Chromatogr A* 2005; 1082: 71-80.
 37. Schermerhorn PG, Golden PE, Krynskiy AJ, Leimkuehler WM. Determination of 22 Triazole Compounds including Parent Fungicides and Metabolites in Apples, Peaches, Flour, and Water by Liquid Chromatography/Tandem Mass Spectrometry. *J AOAC Intern.* 2005; 88: 1491-1502.
 38. Viñas P, Campillo N, Martínez-Castillo N, Hernández-Córdoba M. Method Development and Validation for Strobilurin Fungicides in Baby Foods by Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry. *J Chromatogr A* 2009; 1216: 140-146.
 39. Menezes Filho A, Neves dos Santos F, Pereira PAP. Development, Validation and Application of a Methodology Based on Solid-Phase Micro Extraction Followed by Gas Chromatography Coupled to Mass Spectrometry (SPME/GC-MS) for the Determination of Pesticide Residues in Mangoes. *Talanta* 2010; 81: 346-354.
 40. Campillo N, Viñas P, Aguinaga N, Férez G, Hernández-Córdoba M. Stir Bar Sorptive Extraction Coupled to Liquid Chromatography for the Analysis of Strobilurin Fungicides in Fruit Samples. *J Chromatogr A* 2010; 1217:4529-4534.
 41. Celeiro M, Llupart M, Lamas JP, Lores M, Garcia-Jares C, Dagnac T. Determination of Fungicides in White Grape Bagasse by Pressurized Liquid Extraction and Gas Chromatography Tandem Mass Spectrometry. *J Chromatogr A* 2014; 1343: 18-25.
 42. Qi P, Yuan Y, Wang Z, Wang X, Xu H, Zhang H, et al. Use of Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry for Enantioselective Separation and Determination of Pyrisoxazole in Vegetables, Strawberry and Soil. *J Chromatogr A* 2016; 1449: 62-70.
 43. Chai B, Wang S, Yu W, Li H, Song C, Xu Y, et al. Synthesis of Novel Strobilurin-Pyrimidine Derivatives and their Antiproliferative Activity Against Human Cancer Cell Lines, *Bioorg Med Chem Lett.* 2013; 23: 3505-3510.
 44. Chen L, Zhu Y-J, Fen Z-J, Guo X-F, Zhang X-M, Xu J-H, et al. Synthesis of 1,2,3-Thiadiazole and Thiazole-Based Strobilurins as Potent Fungicide Candidates. *J Agric Food Chem.* 2017, 65, 745-751.
 45. Cao M, Li S, Wang Q, Wei P, Liu Y, Zhu G, et al. Track of Fate and Primary Metabolism of Trifloxystrobin in Rice Paddy Ecosystem. *Sci Total Environ.* 2015; 518-519: 417-423.
 46. Gautam M, Fomsgaard IS. Liquid Chromatography-Tandem Mass Spectrometry Method for Simultaneous Quantification of Azoxystrobin and its Metabolites, Azoxystrobin Free Acid and 2-Hydroxybenzoxazole, in Greenhouse-Grown Lettuce. *Food Additives & Contam. Part A* 2017; 34: 2173-2180.