

Online solid phase extraction LC–MS/MS method for the analysis of succinate dehydrogenase inhibitor fungicides and its applicability to surface water samples

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Abstract A sensitive and selective analytical method, based on online solid phase extraction coupled to LC–MS/MS, was developed and validated to determine traces of several recently introduced fungicides in surface water and wastewater. The list of target analytes included eight succinate dehydrogenase inhibitors (bixafen, boscalid, fluopyram, flutolanil, fluxapyroxad, isopyrazam, penflufen, and penthiopyrad), and two other fungicides with different modes of action, fenpyrazamine and fluopicolide. Detection and quantification limits in various matrices were in the range of 0.1 to 2 and 0.5 to 10 ng/L, respectively. Moderate signal suppression was observed in surface water ($\leq 15\%$) and wastewater ($\leq 25\%$) and was well compensated by the selected internal standard. The intra- and inter-day precisions were generally <10 and $<20\%$, respectively. The applicability of the method was demonstrated in a study on the occurrence of fungicides in the river Glatt, Switzerland, that drains a catchment area of 419 km² with a substantial proportion of agricultural land. Of the studied compounds, only boscalid and fluopicolide were detected in flow-proportional weekly composite samples, generally at low concentrations up to 15 and 5 ng/L, respectively. While fluopicolide was detected in only 30 % of the samples above the LOD of 0.5 ng/L, boscalid was detected in all samples analyzed between March and October 2012.

Keywords Succinate dehydrogenase inhibitors · Fungicides · Carboxamides · LC–MS/MS · Online SPE · Boscalid

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Introduction

The use of synthetic fungicides is an essential component of crop protection in most countries of the world with a history dating back more than a century. The successful control of fungal diseases results in increased crop yields and farmer incomes. Intensive use of fungicides has, in turn, led to other challenges, including development of resistance, risks to farmers, local residents, and bystanders during application, to consumers via residues in food, as well as to the environment by accumulation in soils, contamination of natural waters, and adverse effects on non-target organisms [1].

The group of succinate dehydrogenase inhibitors (SDHIs) represents an important class of modern fungicides. The biochemical mode of action is based on the inhibition of succinate dehydrogenase, an enzyme in the mitochondrial respiration chain, resulting in interruption of the electron flow, and thus disruption of cellular respiration [2].

Early members of SDHIs, such as carboxin, have been used in agriculture since the late 1960s, but had a rather narrow activity spectrum against fungal pathogens. In the past decade, with the discovery and development of a new generation of compounds of the carboxamide class, SDHI fungicides experienced a remarkable revival [3]. These new compounds show a much broader activity spectrum [4], and the market introduction of boscalid in 2002 was a commercial breakthrough in the crop protection area [5].

Fungicides are routinely applied as preventive crop protectants throughout the plant growing season. The number of treatments may add up to several per season, depending on the crop type and crop conditions [6]. SDHI fungicides are slightly lipophilic, neutral compounds and moderately soluble in water under environmental conditions (see Electronic Supplementary Material (ESM), Table S1). Based on their K_{oc} values and the McCall classification scheme [7], these fungicides are expected to have low-to-moderate mobility in soils. However,

some of these compounds are quite persistent in soil and may leach to groundwater to some extent [6]. SDHI fungicides also reach surface waters via spray drift during/after application and via runoff during rain events. Thus, they may be of potential concern due to possible long-term effects on aquatic organisms [6]. Trace levels of flutolanil in the low nanogram-per-liter range were previously found in Asian surface waters [8–12] and boscalid detections were recently reported in Europe [13, 14] and the USA [6, 15].

To achieve detection limits low enough to monitor the occurrence of fungicides in surface waters, pre-concentration is usually required, e.g., by solid phase extraction (SPE), often combined with filtration [16]. Nowadays, the time and resource consuming offline SPE is increasingly replaced by online enrichment (online SPE) prior to liquid chromatography (LC) [17]. The direct coupling of SPE to LC eliminates several working steps, such as evaporation, reconstitution, and transfer. This results in faster and more precise analyses and, when coupled to tandem mass spectrometry (MS/MS), provides high selectivity and sensitivity.

The aim of this study was to establish an online SPE LC–MS/MS method for the simultaneous analysis of many recently introduced SDHI fungicides in groundwater, surface water, and wastewater. Eight SDHI fungicides were selected: boscalid, bixafen, flutolanil, penflufen, penthiopyrad, fluxapyroxad, fluopyram, and isopyrazam (Table 1). Two additional compounds, fluopicolide and fenpyrazamine, with different modes of action, were included in the method development as they are structurally similar and also new. The analytical method was successfully applied in a field study at the river Glatt, Switzerland, where the seasonality of fungicide inputs to the river was studied during several months.

Materials and methods

Chemicals

The fungicides included in the study are listed in Table 1. Their CAS numbers, IUPAC names, and some properties are listed in the ESM, Table S1. Boscalid (purity 99.9 %), fluopicolide (99.9 %), fluxapyroxad (99.9 %), flutolanil (99.5 %), fluopyram (99.9 %), bixafen (99.8 %), isopyrazam (99.8 %), and penflufen (98.4 %) were purchased from Sigma-Aldrich (Buchs, Switzerland). Penthiopyrad (99.8 %) was obtained from Mitsui Chemicals Inc. (Tokyo, Japan). Fenpyrazamine, which was not commercially available as a pure active substance at the time of method development, was extracted in-house from a formulated product containing 50 % of active substance (water dispersible granules lately introduced to the Swiss market as Prolectus®). Briefly, an aliquot of 10 mL ethyl acetate was added to an aqueous suspension of 2.0 g of the product (1:1, w/v). The mixture was shaken and,

after phase separation, the upper organic layer was removed and transferred to a new vial. The procedure was repeated twice and the organic extracts were combined. During gentle evaporation of ethyl acetate the amount of ≈ 240 mg of fenpyrazamine was precipitating. The purity of the white powder was not determined, since this was not necessary for method development and characterization (see “Performance and validation of the online SPE LC–MS/MS method”). As this fungicide was not detected in environmental samples (see “Application to environmental samples”), no quantification was needed.

Individual stock solutions of all compounds were prepared in methanol with concentrations of ≈ 1 mg/mL and further diluted with water to 1 μ g/mL for MS parameter optimization. Mixed standard solutions at concentrations of 100 and 10 ng/mL were freshly prepared in water and used for optimization of LC parameters, as spiking solutions for sample fortification, and for the preparation of calibration standards.

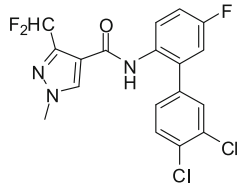
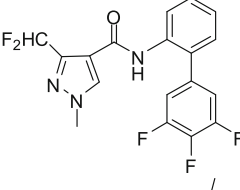
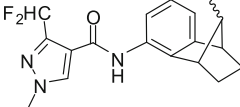
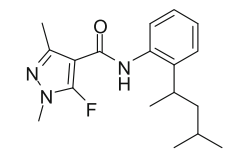
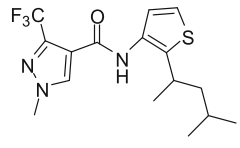
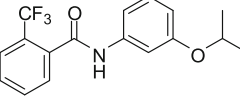
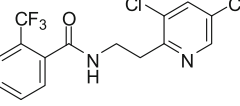
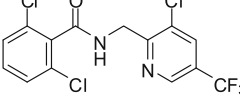
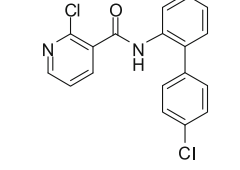
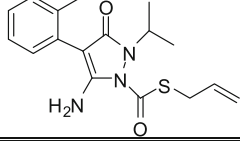
Ammonium acetate (≥ 98 %; Sigma-Aldrich), HPLC grade methanol and acetonitrile (both from Labscan, Gliwice, Poland) were used for preparation of eluents. Acetic acid (100 %; Sigma-Aldrich), ammonia (25 %) and formic acid (> 98 %; both from Merck, Darmstadt, Germany) were used for pH adjustment. Purified water produced by a Millipore water purification system was used throughout the study.

Collection of water samples

For method development and validation, surface water was collected from several lakes in the Swiss Midland region. Grab samples were taken at the outflow of the lakes at 0–1-m depth in June 2012. A 24-h composite sample from the wastewater treatment plant of Horgen, Switzerland was taken at the effluent after sand filtration. “Fossil” groundwater from a public fountain (Aqui) in Zurich, Switzerland was used as blank samples to study potential contamination from reagents and the experimental procedure as well as cross-contamination between samples. This groundwater, with an age of several thousand years, is expected to not be contaminated with anthropogenic compounds. Purified water was used for recovery experiments and preparation of calibration standards.

A sampling campaign was performed at the river Glatt, Switzerland, during March–October 2012 to monitor the presence of the pesticides and the seasonality of their occurrence in the river. Thirty-three flow-proportional weekly composite samples were taken from an automatic water sampling station installed ≈ 400 m upstream from the mouth of river Glatt, where it flows into river Rhine (coordinates 47° 34' 25" N/08° 28' 33" E). The catchment area of 419 km² is characterized by intensive agricultural production (45 %), mainly arable land and grassland, followed by urban areas (26 %), and forests (24 %) [18]. All water samples were collected into glass

Table 1 Chemical structures of the studied fungicidal active compounds and substance specific MS/MS parameters for the present study (Quantifier: main product ion; qualifier: secondary product ion, and the corresponding collision energy (CE) and collision cell exit potential (CXP))

Common name	Chemical structure	Formula [M+H] ⁺	Quantifier Qualifier	CE [eV]	CXP [V]
Bixafen		C ₁₈ H ₁₂ Cl ₂ F ₃ N ₃ O 414.0	394.1 266.1	20 33	5 7
Fluxapyroxad		C ₁₈ H ₁₂ F ₅ N ₃ O 382.1	362.1 342.1	18 29	9 8
Isopyrazam		C ₂₀ H ₂₃ F ₂ N ₃ O 360.2	340.2 244.1	22 34	9 18
Penflufen		C ₁₈ H ₂₄ FN ₃ O 318.2	234.1 141.1	22 40	18 11
Penthiopyrad		C ₁₆ H ₂₀ ON ₃ SF ₃ 360.1	276.1 256.1	21 29	7 21
Flutolanil		C ₁₇ H ₁₆ F ₃ NO ₂ 324.1	262.0 282.1	25 19	21 17
Fluopyram		C ₁₆ H ₁₁ ClF ₆ N ₂ O 397.1	173.0 208.1	41 30	14 15
Fluopicolide		C ₁₄ H ₈ Cl ₃ F ₃ N ₂ O 383.0	175.1 173.0	34 32	13 13
Boscalid		C ₁₈ H ₁₂ Cl ₂ N ₂ O 343.0	307.1 140.0	28 28	19 10
Fenpyrazamine		C ₁₇ H ₂₁ N ₃ O ₂ S 332.1	230.1 304.1	28 21	16 23

bottles and stored at 4 °C in the dark. Before analysis, water samples were fortified with an aqueous solution of penflufen as surrogate standard (see “Quantification”) to a concentration of 25 ng/L. Analyses were performed within a few days.

Online SPE–HPLC

The instrumental setup was similar to the one reported earlier [19–21] and consisted of an auto sampler (HTC PAL, CTC Analytics, Zwingen, Switzerland) equipped with two six-port valves for column switching, a sample loop, and an online extraction cartridge. The HPLC system included a pump used solely for sample loading (Jasco PU-980, Gross-Umstadt, Germany) and a binary gradient HPLC pump (Agilent 1100 series, Palo Alto, CA) for the mobile phase.

The online SPE procedure involved three main steps: sample loading, pre-concentration, and elution using a column switching technique (see ESM, Fig. S1 and [21]). A 2-mL PEEK loop was over-filled with 2.1 mL of sample via an auto sampler syringe. The sample was transferred from the loop to the SPE cartridge (two stacked Gemini–NX C18 cartridge precolumns, 4×3.0 mm i.d., 5 µm; Phenomenex, Torrance, CA) with purified water at a flow rate of 1 mL/min, providing enrichment of the analytes as well as clean-up of the samples from highly polar components. After valve switching, the enriched analytes were eluted backwards directly on to a Gemini NX C18 column (150×2.0 mm i.d., 5 µm; Phenomenex) equipped with a guard column filled with the same stationary phase (4×3 mm i.d.). The mobile phase consisted of 1 mM ammonium acetate in water (eluent A) and methanol (eluent B). Gradient elution at a flow rate of 0.2 mL/min was done in two steps with a fast initial increase from 5 to 50 % B within 2 min, followed by a slower linear increase to 95 % B within 21 min. Initial conditions were re-established within 0.1 min, and the column was equilibrated for 8.9 min prior to the next analysis, yielding a total run time of 32 min.

Mass spectrometry

Detection of fungicides was accomplished using an API 4000 triple quadrupole tandem mass spectrometer equipped with a TurboIonSpray source (AB Sciex, Foster City, CA). For MS parameter optimization, standard solutions were infused to the ion source at a flow rate of 20 µL/min via a syringe pump combined with 200 µL/min of 70:30 methanol/water (v/v) via a T piece. After optimization of pre-collision cell voltages (declustering potential and entrance potential) for precursor ions, product ion scans were acquired. Two product ions with the greatest intensity were used to generate multiple reaction monitoring (MRM) transitions. Finally, the collision energy (CE) and collision cell exit potential (CXP) were manually fine-tuned to obtain the highest MRM signals for each

transition (Table 1). The mass spectrometer was operated with the following ionization conditions: positive mode, spray voltage 5 kV, nebulizer gas pressure 15 psi, heater gas pressure 10 psi, and temperature of the heater gas 300 °C.

Quantification

The most specific or most intense product ion of each target analyte was used for quantification, and a secondary product ion was used as a qualifier ion for confirmation. Together with the retention times, they were used to ensure correct peak assignment. The use of an isotopically labeled internal standard for each substance would be favorable for trace analysis to correct for the loss of analyte during sample preparation and for matrix effects. However, no such labeled standards were available for the fungicides tested in this study and, therefore, penflufen was selected as the most suitable surrogate standard. Penflufen has a medium retention time (21.2 min; range of all analytes=17.0–24.3 min) and its chemical structure resembles most other fungicides investigated. Furthermore, penflufen has so far not been authorized in Europe and, thus, it was not expected to appear in natural waters. The concentration of each compound was calculated by comparing the peak area ratios of the analytes and penflufen to the corresponding ratios in the calibration standards. Calibration curves were constructed from two sets of 11 standards spiked in purified water (concentrations, 0.1–1,500 ng/L), acquired at the beginning and at the end of a measurement series, and obtained by a weighted (1/x) linear least squares regression.

Method validation

Three different water matrices were used for validation of the method: purified water, surface water from Swiss Midland lakes, and treated wastewater. The following parameters were determined: limits of detection (LOD) and quantification (LOQ), linearity, precision, and accuracy.

The LODs were determined on the basis of the lowest calibration standard where the peak in the quantifier ion trace reached a signal-to-noise ratio of ≥ 3 . The lowest concentration levels for which the quantifier signal-to-noise ratio was ≥ 10 , was set as LOQ.

The precision of the entire method (reproducibility) was determined in water samples fortified with analytes at two different concentrations (25 and 100 ng/L). It was calculated as the relative standard deviation of repeated analyses ($n=10$). For the intraday precision, ten replicates of each concentration level were measured within one run. For the inter-day precision, fortified samples were divided into ten aliquots. One aliquot of each matrix was analyzed with each sequence. The latter samples served, furthermore, to assess the possible analyte loss during storage (transformation or sorption to the storage vessels).

To assess the extent of matrix effects, matrix-matched and purified water-based calibration curves were measured and the corresponding ratio of the slopes in matrix and in purified water, respectively, were calculated for each of the analytes.

The suitability of different filter materials for filtration of water samples was tested by assessing recoveries in filtered samples from water matrices spiked at 100 ng/L: regenerated cellulose (RC), regenerated cellulose with glass fiber pre-filter (GF/RC), polyethersulfone (PES), polyester (PE) (all CHROMAFIL[®], pore size 0.45 μm , Macherey–Nagel, Oensingen, Switzerland), and cellulose acetate (CA) (pore size 0.45 μm , VWR International, Dietikon, Switzerland).

Filtration using GF/RC, PES, or CA material substantially reduced the concentration of all fungicides. Recoveries varied from 2 to 80 %. Regenerated cellulose alone (RC) produced satisfactory results for most analytes (recoveries, 89–112 %), but eliminated more hydrophobic compounds (penthiopyrad (62–85 %), isopyrazam (36–46 %), and bixafen (52–68 %)). In samples filtered through PE, good recoveries were found for all analytes (91–108 %) except for bixafen (58–78 %) and isopyrazam (63–75 %). However, as sorption onto filter material was significant for certain analytes at low environmental concentrations, filtration was not applied in this study.

Quality assurance

Fortified quality control samples were measured after every ten injections to check for instrumental drift. The analysis was stopped and a new calibration curve was constructed if the quality control standard was not within ± 10 % of its theoretical value. Laboratory blank samples of fossil groundwater were injected after highly concentrated standards to prevent potential carry-over. Fossil groundwater spiked with internal standard solution was analyzed to quantify blank concentrations.

Results and discussion

Method development

Optimization of MS parameters and HPLC conditions

Compound-specific MS/MS parameters (MRM transitions and corresponding instrumental settings) are listed in Table 1. The majority of target compounds showed better sensitivity in positive mode. Fluopyram, fluopicolide, fenpyrazamine, and penflufen showed only slightly better sensitivity in negative mode and were, therefore, also included in the method applying positive ionization to avoid polarity switching within the same chromatographic run.

The effect of different organic solvents and ionic additives that control pH and ionization state was investigated. Methanol showed better peak resolution and gave a more favorable elution pattern than acetonitrile. Further experiments demonstrated that (i) formic acid suppressed ionization, resulting in a lower sensitivity than ammonium acetate, (ii) from different ionic strengths tested (1, 5, and 10 mM), 1 mM ammonium acetate added to the aqueous mobile phase provided highest signal intensities for all fungicides and was thus the preferred mobile phase additive.

Representative chromatograms of the fungicides analyzed in spiked (10 ng/L) purified water, surface water, and treated wastewater are shown in Fig. 1. The retention times in the reversed phase column fell within quite a narrow range and the elution of the earliest eluting compounds occurred at a methanol content of 70 %. To increase the resolution of these analytes, a shallow elution gradient was used in the range where the analytes elute. The strong retention of the target compounds on the HPLC column and, consequently, elution at high proportion of organic solvent in the mobile phase, likely contributed to the observed excellent sensitivity of mass spectral detection.

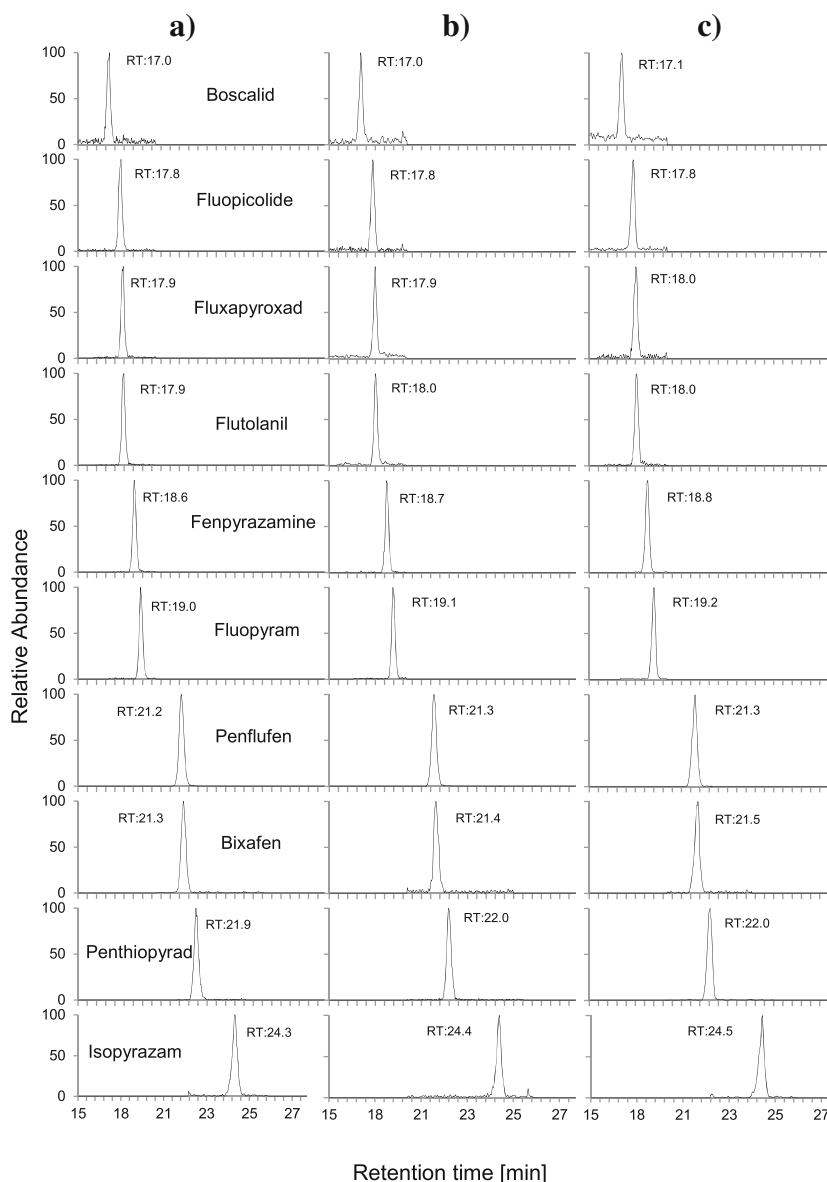
Several of the target compounds are chiral. Penflufen and penthiopyrad both consist of one pair of enantiomers, present in the technical active substance as a racemic mixture. Because enantiomers are not separated on a conventional achiral HPLC column, only one peak was observed for each compound. Isopyrazam consists of two diastereomeric pairs of enantiomers, the *syn* and *anti* diastereomers. The technical active substance contains the enantiomers as racemic mixtures, whereas the *syn/anti* ratio may vary around 85:15. The diastereomers were not separated on the column used in this study (Fig. 1). Because they have the same mass spectral properties, they are reported as the sum of two diastereomers.

Online SPE setup

Important online SPE parameters such as injection volume, enrichment time, and flow rate were selected to optimize the retention of the analytes on the SPE column. A sample loading rate of 1 ml/min was chosen. Due to the setup of the system, it was only possible to rinse the sample loop with the same solvent that was used for the transfer of sample from the loop to the online SPE cartridge. Consequently, only an aqueous wash was performed once the sample was loaded on to the online SPE column. Minimal carryover between samples (<0.2 %) indicated that this rinsing procedure was adequate in this case.

To prevent peak broadening, the stationary phase used for online SPE was the same as in the analytical column. Experiments with different injection volumes up to 2 mL showed that no analytes were lost due to breakthrough. Since this

Fig. 1 Online SPE LC–MS/MS chromatograms of fungicides spiked at 10 ng/L in **a** purified water, **b** surface water, and **c** treated wastewater. The chromatograms were generated from MRM transitions with the respective quantifier ion



volume gave satisfactory results and the LOD was in the range of 0.1–2.0 ng/L, larger volumes were not tested.

Performance and validation of the online SPE LC–MS/MS method

Limit of detection, limit of quantification, and linearity

Validation data, determined for purified water, surface water, and treated wastewater, are presented in Table 2. The LODs were in the low nanogram-per-liter range, which allows the detection of trace amounts of selected contaminants in natural water samples. An LOQ value of ≤ 5 ng/L was obtained for all analytes in all matrices, except for bixafen in wastewater (10 ng/L). Standards in purified water yielded the lowest

LOQs, followed by surface water, and then wastewater. The differences between purified and surface water were minimal.

The calibration curves were linear over the calibration range from 1 to 1,500 ng/L with correlation coefficients ≥ 0.998 (r , $1/x$ weighted calibration curves) for all target compounds (Table 2). The lowest calibration points below or at LOQ were occasionally excluded if the response of an analyte deviated by more than $\pm 10\%$ from the average response in the calibration curve. Four analytes showed linearities down to the low level of 0.5 ng/L and the other four showed linearities at least down to the targeted LOQ of 5 ng/L.

Matrix effects

Online SPE provides effective analyte enrichment and, at the same time, clean-up by elimination of polar matrix

Table 2 Method validation data (limit of detection, LOD; limit of quantification, LOQ; correlation coefficient, *r* for weighted calibration curve (LOQ–1,500 ng/L); slope in matrix/slope in purified water) determined for purified water (P), surface water (S), and treated wastewater (W)

Analyte	LOD [ng/L]			LOQ [ng/L]			<i>r</i>			Ratio slope in matrix/slope in P	
	P	S	W	P	S	W	P	S	W	S	W
Boscalid	0.5	0.5	0.5	2.0	5.0	5.0	0.9991	0.9998	0.9992	1.04	1.02
Fluopicolide	0.2	0.5	0.5	0.5	2.0	5.0	0.9994	0.9994	0.9992	0.98	0.93
Fluxapyroxad	0.1	0.1	0.2	1.0	2.0	5.0	0.9996	0.9994	0.9991	0.99	0.96
Flutolanil	0.1	0.2	0.2	0.5	0.5	1.0	0.9994	0.9994	0.9992	0.92	0.98
Fenpyrazamine	0.1	0.2	0.5	0.5	0.5	1.0	0.9994	0.9988	0.9984	0.81	1.05
Fluopyram	0.1	0.1	0.5	0.5	0.5	2.0	0.9994	0.9992	0.9987	0.96	0.96
Bixafen	1.0	1.0	2.0	5.0	5.0	10.0	0.9997	0.9996	0.9999	1.04	0.99
Penthiopyrad	0.2	0.2	1.0	1.0	1.0	5.0	0.9997	0.9998	0.9997	1.01	1.02
Isopyrazam	0.2	0.5	1.0	1.0	2.0	5.0	0.9990	0.9992	0.9995	0.89	1.01

components, which are not retained on the SPE material. However, a fraction of medium to non-polar sample matrix is also retained on the SPE column. Co-elution of matrix components may thus affect the analyte response by signal enhancement or signal suppression. The extent of matrix effects was investigated by comparing the analyte response (in this case, the slope of the respective calibration curve) in purified water with that obtained in matrix. For the investigated fungicides, only minor signal suppression was observed ($\leq 15\%$ in surface water and $\leq 25\%$ in wastewater, relative to purified water, data not shown). Signal suppression was similar for all compounds and, therefore, well compensated by the selected internal standard penflufen. This is indicated by the fact that the ratios of the slopes of the calibration curves (response relative to penflufen) in surface water and treated wastewater did not deviate significantly from unity (Table 2). Based on these results, the quantification was performed using calibration curves for all analytes in purified water, thus avoiding the use of matrix-matched calibration.

Precision and accuracy

The intraday precision of the method was estimated by repeated analyses ($n=10$) of purified water (spiked at 25 and 100 ng/L), surface water, and treated wastewater (both spiked at 100 ng/L). The relative standard deviation (RSD, $n=10$) was generally lower than 10%. Boscalid, fluopicolide, and fenpyrazamine showed somewhat higher RSDs (11%) in surface water and penthiopyrad in treated wastewater (Fig. 2).

For the inter-day precision, two spiked samples, one for purified water and one for surface water, were analyzed 10 times over a period of 3 months. The RSD was generally lower than 20% for both matrices (data not shown). This test also confirmed that all fungicides were stable in aqueous solutions stored at 4 °C in the dark over a period of 3 months.

The recoveries relative to penflufen (accuracies) were mostly above 90%, except for bixafen (84%), isopyrazam (84%), and penthiopyrad (86%) in surface water and fluxapyroxad (88%), bixafen (81%), and penthiopyrad (86%) in wastewater (Fig. 2). Overall, the results were very satisfying, despite the lack of isotope-labeled internal standards.

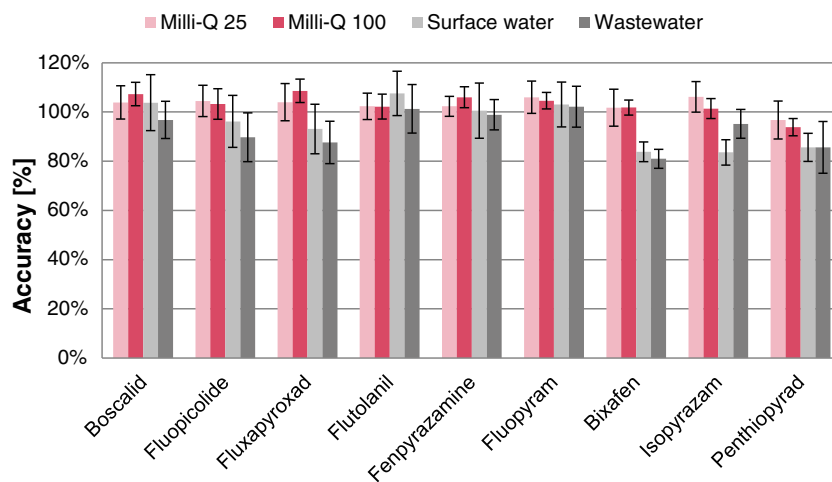
Sample carryover

Instrumental blank samples, analyzed in series with purified water, surface water, and wastewater, did not contain detectable concentrations of fungicides, except for blank samples analyzed directly after the highest calibration standards, indicating some carryover from previous analyses ($<0.2\%$). Consecutive injection of two blank samples after the highest standard was sufficient for removal of any residual fungicides from the system.

Application to environmental samples

The newly developed method was applied in a sampling campaign at the river Glatt, Switzerland from March–October, 2012. At the time when analyses were conducted, only plant protection products containing boscalid, fluopicolide, and bixafen were commercially available on the Swiss market. Boscalid and fluopicolide were found in low, but detectable concentrations in the range of 0.5–15 ng/L, with boscalid being detected in all samples collected (Fig. 3). Fluopicolide was detected in 30% of the collected samples above the LOD of 0.5 ng/L. The third compound, bixafen, was not detected at all. Only a small number of samples contained concentrations above the respective LOQs. The maximum concentrations were 15 and 4.5 ng/L for boscalid and fluopicolide,

Fig. 2 Accuracies (recoveries relative to surrogate standard) for all compounds in purified water (Milli-Q) spiked at levels of 25 and 100 ng/L, surface water and wastewater spiked at 100 ng/L. The error bars denote the precision as relative standard deviation of ten replicates (RSD, $n=10$)



respectively. In a recent study, Reemtsma et al. [22] reported the presence of a fluopicolide metabolite (3-chloro-5-trifluoromethyl-pyridine-2-carboxylic acid) in a single sample of urban runoff. Boscalid has been found in Europe in headwater streams in Denmark (up to 27 ng/L) [14] and a coastal lagoon in Spain (up to 58 ng/L) [13]. A similar frequency in boscalid detections was observed in US surface waters [6]. The authors attributed its regular presence to frequent preventive application of boscalid against many fungal diseases, which in turn, may contribute to the continuous input of this chemical to streams receiving runoff or drainage water [6, 13].

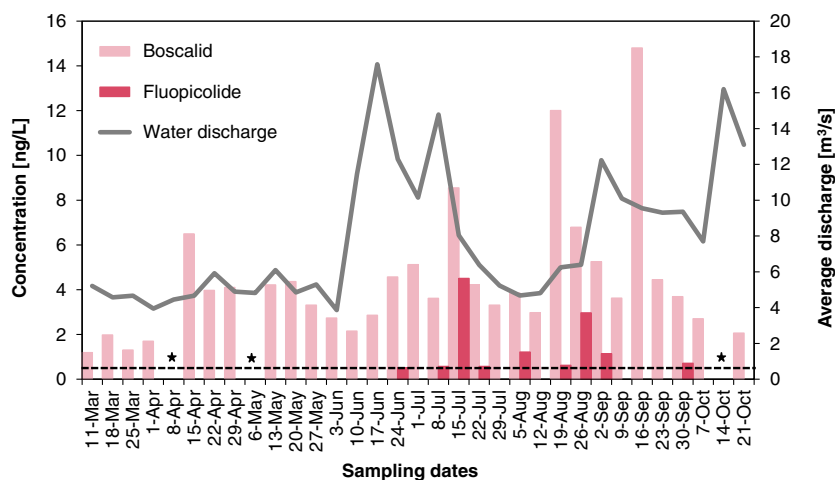
Weekly fungicide loads were calculated from the measured discharge of the river and the fungicide concentrations in the flow-proportional weekly composite samples. The loads ranged between ≈ 4 and 85 g for boscalid and ≈ 2 and 22 g for fluopicolide (ESM, Fig. S2). For boscalid, weekly loads were somewhat correlated to discharge volumes which indicates that inputs of the compounds to the river are related, at least to some extent, to rain events. However, contrary to weekly loads, there was no clear correlation between weekly concentrations of fungicides and water discharge (Fig. 3). This

may be, in part, due to the fact that the river originates at Lake Greifensee that contributes a significant portion of water in the river downstream.

The maximum water concentrations typically coincided with fungicide treatments in the River Glatt catchment area. For example, the occurrence of fluopicolide (approved for use on potato fields with up to four applications per culture) mirrored the potato growing season, which falls within May–September. Typically, fungicide applications are ceased 3 weeks before harvest. This is reflected by lower fluopicolide detections in October. For boscalid, both use and water concentrations increased rapidly in April when some early crops begin to grow. The concentrations peaked in July to September and subsequently decreased in October (Fig. 3). Although the concentrations of boscalid were approximately 50 times lower than those reported in a Californian estuary [15], the concentration pattern of boscalid from March–October was similar.

A detailed comparison to pesticide application in the catchment cannot be made at this point as 2012 pesticide sale and usage data in Switzerland were not yet available. In neighboring Germany, for instance, boscalid was the best-selling SDHI

Fig. 3 Concentration of the fungicides boscalid and fluopicolide in the flow-proportional weekly composite water samples collected at river Glatt, Switzerland in 2012, compared to weekly average discharge (gray line). LOD for both compounds is by a dashed line at the level of 0.5 ng/L. LOQ for boscalid and fluopicolide in surface water were 5 and 2 ng/L, respectively. (Asterisks indicate that there are no samples available)



fungicide with sales reaching 250–1,000 t in 2011 [23]. However, the use of boscalid decreased the following year likely in favor of bixafen, fluxapyroxad, fluopyram, fluopicolide, and flutolanil among others [24]. Meanwhile, the number of approvals of fungicides used in this study increased in Switzerland to six by the end of 2013. It is reasonable to assume that the rapid increase in authorizations of SDHI fungicides on national and European Union levels will be accompanied by increased usage of these compounds and their applications on different crops. Consequently, more frequent detections of these fungicides in natural waters are likely to occur in the future.

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References

- Dehne HW, Deising HB, Gisi U, Kuck KH, Russel PE, Lyr H (2011) Modern fungicides and antifungal compounds VI. *Lebenshilfe gGmbH, Braunschweig*
- Keon JPR, White GA, Hargreaves JA (1991) Isolation, characterization and sequence of a gene conferring resistance to the systemic fungicide carboxin from the maize smut pathogen, *Ustilago maydis*. *Curr Genet* 19:475–481
- Glättli AG, Grote T, Stammler G (2011) SDH-inhibitors: history, biological performance and molecular mode of action. In: Dehne HW, Deising HB, Gisi U, Kuck KH, Russel PE, Lyr H (eds) *Modern fungicides and antifungal compounds VI. Lebenshilfe, gGmbH, Braunschweig*, pp 159–169
- Avenot HF, Michailides TJ (2010) Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot* 29:643–651
- Walter H (2011) New fungicides and new modes of action. In: Dehne HW, Deising HB, Gisi U, Kuck KH, Russel PE, Lyr H (eds) *Modern fungicides and antifungal compounds VI. Lebenshilfe, gGmbH, Braunschweig*, pp 47–54
- Reilly TJ, Smalling KL, Orlando JL, Kuivila KM (2012) Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States. *Chemosphere* 89:228–234
- McCall PJ, Swann RL, Laskowski DA, Unger SM, Vrona SA, Dishburger HJ (1980) Estimation of chemical mobility in soil from liquid chromatographic retention times. *Bull Environ Contam Toxicol* 24:190–195
- Tsuda T, Nakamura T, Inoue A, Tanaka K (2009) Pesticides in water and sediment from littoral area of Lake Biwa. *Bull Environ Contam Toxicol* 82:683–689
- Tanabe A, Mitobe H, Kawata K, Yasuhara A, Shibamoto T (2001) Seasonal and spatial studies on pesticide residues in surface waters of the Shinano River in Japan. *J Agric Food Chem* 49:3847–3852
- Anasco N, Uno S, Koyama J, Matsuoka T, Kuwahara N (2010) Assessment of pesticide residues in freshwater areas affected by rice paddy effluents in Southern Japan. *Environ Monit Assess* 160:371–383
- Anasco NC, Koyama J, Uno S (2010) Pesticide residues in coastal waters affected by rice paddy effluents temporarily stored in a wastewater reservoir in Southern Japan. *Arch Environ Contam Toxicol* 58:352–360
- Oh YJ, Jung YJ, Kang J-W, Yoo YS (2007) Investigation of the estrogenic activities of pesticides from Pal-dang reservoir by in vitro assay. *Sci Total Environ* 388:8–15
- Moreno-González R, Campillo JA, García V, León VM (2013) Seasonal input of regulated and emerging organic pollutants through surface watercourses to a Mediterranean coastal lagoon. *Chemosphere* 92:247–257
- Rasmussen JJ, McKnight US, Loinaz MC, Thomsen NI, Olsson ME, Bjerg PL, Binning PJ, Kronvang B (2013) A catchment scale evaluation of multiple stressor effects in headwater streams. *Sci Total Environ* 442:420–431
- Smalling KL, Kuivila KM, Orlando JL, Phillips BM, Anderson BS, Siegler K, Hunt JW, Hamilton M (2013) Environmental fate of fungicides and other current-use pesticides in a central California estuary. *Mar Pollut Bull* 73:144–153
- Garcia-Ac A, Segura PA, Viglino L, Furtos A, Gagnon C, Prevost M, Sauve S (2009) On-line solid-phase extraction of large-volume injections coupled to liquid chromatography-tandem mass spectrometry for the quantitation and confirmation of 14 selected trace organic contaminants in drinking and surface water. *J Chromatogr A* 1216:8518–8527
- Huntscha S, Singer HP, McArdell CS, Frank CE, Hollender J (2012) Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1268:74–83
- The Swiss Federal Statistical Office (SFSO), Swiss statistics web site, *Arealstatistik 2004/2009*.
- Buerge IJ, Buser H-R, Kahle M, Müller MD, Poiger T (2009) Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. *Environ Sci Technol* 43:4381–4385
- Buerge IJ, Kahle M, Buser H-R, Müller MD, Poiger T (2008) Nicotine derivatives in wastewater and surface waters: application as chemical markers for domestic wastewater. *Environ Sci Technol* 42:6354–6360
- Kahle M, Buerge IJ, Hauser A, Müller MD, Poiger T (2008) Azole fungicides: occurrence and fate in wastewater and surface waters. *Environ Sci Technol* 42:7193–7200
- Reemtsma T, Alder L, Banasiak U (2013) Emerging pesticide metabolites in groundwater and surface water as determined by the application of a multimethod for 150 pesticide metabolites. *Water Res* 47:5535–5545
- Federal Office of Consumer Protection and Food Safety (BVL) 2011, Report on domestic sales and export of plant protection products in 2011 (in German). Available online: http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/meld_par_19_2011_EN.pdf?__blob=publicationFile&v=2
- Federal Office of Consumer Protection and Food Safety (BVL) 2012, Report on domestic sales and export on plant protection products in 2012 (in German). Available online: http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/meld_par_19_2012.pdf?__blob=publicationFile&v=3