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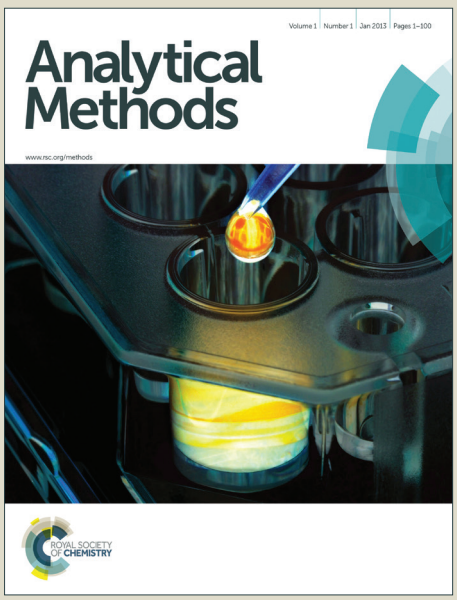

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ARTICLE TYPE

Simultaneous determination of niclosamide and its degradates in water by LC-MS/MS

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A new method for the analysis of niclosamide (NIC) and its primary degradates 2-chloro-4-nitroaniline (2C4NA), aminoniclosamide (AN), hydroxyniclosamide (HN) and 5-chlorosalicylic acid (5CSA) in water was developed using direct injection LC-MS/MS. Methanol and acetonitrile mobile phases were compared. Methanol was superior for both separation and sensitivity for all chemicals. LLoQs for all chemicals were 3-50 times better in methanol than in acetonitrile, and baseline separation was observed for HN and 5CSA in methanol but not acetonitrile. The LLoQ for NIC in the current study was approximately 20 times lower than that previously reported using LC-MS/MS methodology, and 10-250 times lower for all chemicals than obtained by HPLC-UV/visible detection. The method reported in the current study relies upon smaller injection volumes and direct injection of water, eliminating time consuming clean-up steps and increasing sample throughput. The current method is also more selective for all four chemicals than existing HPLC-UV/visible methods, and is not susceptible to spectral interferences from DOM. The use of a shorter LC column with core shell particles resulted in shorter run times and lower mobile phase consumption than previously reported methods for NIC, which rely on traditional porous particle columns.

1 Introduction

Niclosamide [5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxy-benzamide] is an anthelmintic chemical released in 1960 to treat infection by parasitic worms in human and animal hosts. More recently, niclosamide (NIC) has found applications as a drug to combat cancer[1-4], viruses[5] and tuberculosis[6]. It is active against the cercarial stages of *Schistosoma* in aquatic environments[7], and is also active against a range of aquatic molluscs including the snail vectors of schistosomiasis[8], invasive pests such as zebra mussels[9] and the golden apple snail, a serious pest of rice in SE Asia[10, 11]. Studies have shown its potential for controlling the pestiferous snail *Isidorella newcombi* in Australian rice crops, which is currently achieved using copper sulfate. However, NIC is advantageous over copper sulfate in that only low application rates are required to achieve a high mortality rate at all growth stages, and accumulation in the environment is less likely to occur than with copper sulfate[12, 13].

Degradation of NIC can occur by a variety of mechanisms[14], but its primary degradates are 2-chloro-4-nitroaniline (2C4NA), aminoniclosamide (AN), hydroxyniclosamide (HN) and 5-chlorosalicylic acid (5CSA) (Fig. 1). Reduction of NIC to AN was originally linked to reductive degradation[15], but more recent work indicates that both light and reducing conditions are crucial to its production[16]. While the primary degradate in moist soil was AN, no degradates were recovered in dry soils in the presence or absence of light. Some studies indicate that metal catalysis of pesticides such as alachlor does not tend to occur in the water column and depends on clay surfaces [17], whereas pesticides such as chlorpyrifos may freely undergo metal catalysis in the water column by copper(II)[18]. Hydrolysis of the amide bond in NIC results in 5-CSA and 2C4NA, whereas the acid hydrolysis of NIC results in HN.

Methods of analysis for niclosamide and its degradates have primarily been based on HPLC, with UV/visible[13, 15] and scintillation counting, but the latter is restricted to laboratory studies[16, 19]. However, methylation using CH_3I followed by GC analysis has also been reported[15]. Environmental samples contain varying amounts of dissolved organic matter (DOM) which can absorb UV/visible light and interfere with UV/visible detection in HPLC methods if not removed from samples during clean-up, or adequately separated from analytes during the chromatographic process. Detection using mass spectrometry is far less susceptible to interferences such as DOM, is more selective, and can also offer the advantage of improved lower limits of quantitation (LLoQ). LC-MS/MS using Multiple Reaction Monitoring (MRM) offers a highly selective and sensitive method for detecting chemicals at very low concentrations. This capacity is essential when determining the potential impact of a chemical and its degradates on non-target species, particularly when low chemical concentrations still demonstrate considerable toxicity. For example, the insecticide fipronil is used as a seed treatment to prevent plant damage by water weevils in Louisiana rice

production and is applied at extremely low rates, but the degradates of fipronil show toxicities similar to fipronil itself[20]. An application of fipronil in 1999 was identified as unintentionally impacting the 2000-2001 crawfish production when tail-water from rice bays drained into bays containing crawfish[21].

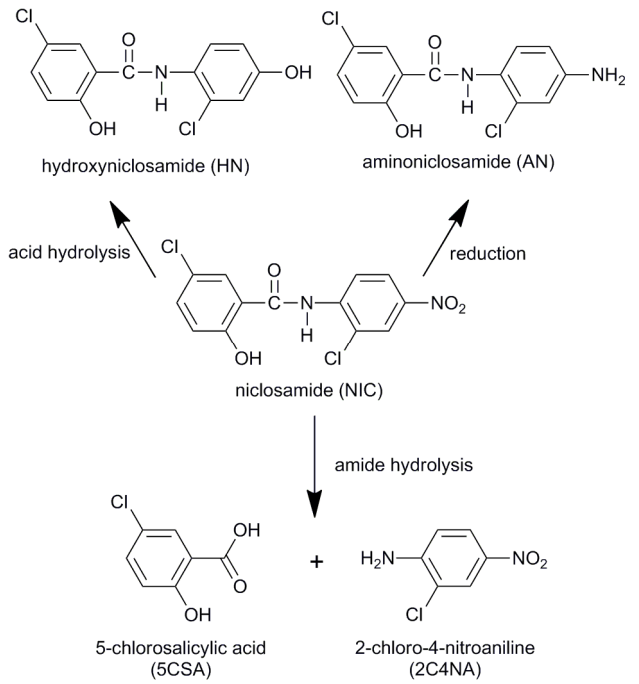

Fig. 1 Chemical structures of NIC and its primary degradates

Flooded crops such as rice provide a continuous connection between surface and underground water, creating a conduit for potentially mobile chemicals to enter the water table. This poses a particular risk to humans in locations where groundwater is used as the primary source of drinking water, as is the case in many towns in New South Wales (NSW), Australia. While release of chemically-treated irrigation water into surface drains is prohibited during the withholding period, unintentional escape may occur when flood events occur due to excessive rainfall. Consequently, environmental monitoring of applied chemicals should be thorough and provide LLoQs as low as reasonably possible to identify the presence of trace contaminants, particularly when seeking registration of new pesticides, such as niclosamide. While MRM has been employed to detect NIC[5, 22-24], it has not been used to detect the common degradates resulting from niclosamide degradation. The following work describes a method for the simultaneous analysis of NIC, 2C4NA, AN, HN and 5CSA by LC-MS/MS in water that improves the LLoQ of current HPLC methods by more than an order of magnitude for all chemicals, and demonstrates its application in a preliminary study of metal photocatalysis of NIC in the water column catalysed by Fe and Mn.

2 Results and Discussion

2.1 Selection of ion transitions and ion modes

Negative and positive ion modes were compared for NIC, and negative ion mode was more than an order or magnitude more sensitive, which agreed with the literature[22-24]. This is presumably due to the electron withdrawing chlorine atoms and nitro group attached to the benzene rings in NIC. The *m/z* 325.0→171.0 transition was used to quantify NIC and provided the greatest sensitivity, confirming the results of previous studies[23, 24]. While *m/z* 325→289 has also been reported for quantification of NIC[22], it was shown to be 3.1 times less sensitive than 325.0→171.0 in this study and 2.6 times less sensitive than other studies[23]. ^{35}Cl and ^{37}Cl have relative abundances of 76% and 24%, respectively. NIC exhibited three isotopic peaks in a ratio of approximately 10:6:1 due to the presence of two chlorine atoms in the molecule. The use of the 171.0 daughter ion for the quantitation of NIC reduced this isotope effect because the 3:1 isotope ratio for the 171.0 ion indicated the presence of only one Cl atom.

No ion transitions are currently available in the literature for any of the four NIC degradates, so these were determined in this study. Negative and positive ion modes were investigated for all four degradates, with negative ion mode proving optimal for HN and 5CSA, presumably for the same reason as for NIC. While AN produced a 258.7 daughter ion in negative ion mode, the peak was more than an order of magnitude smaller than the peak for the 154.8 daughter ion detected in positive ion mode, presumably as a result of the presence of the amino group on the benzene ring and its tendency to ionise in an acidic mobile phase. Optimal ion transitions for 2C4NA were identified for negative and positive ionisation modes as *m/z* 170.7→134.7 and 172.5→155.6, respectively. Negative ion mode produced a signal approximately 3 times larger for test solutions. This observation suggests that the influence of the electron withdrawing $–NO_2$ group has a greater influence than the electron donating $–NH_2$ group on stability, possibly as a result of resonance via the $–NO_2$ group.

2.2 Comparison of LC mobile phase solvents

Methanol and acetonitrile were compared as mobile phases for the separation of NIC from its four primary degradates, AN, HN, and 5CSA. Figs 2 and 3 show typical chromatograms for the four chemicals using acetonitrile and methanol gradients, respectively, and Table 1 shows retention times under the two regimes. The most obvious differences between the two solvent systems are the reversal of elution order for HN and 5CSA, and the relative height of all peaks. All peaks were 2-2.5 times larger when using methanol than when acetonitrile was used as the mobile phase. Baseline resolution was achieved for HN and 5CSA using methanol but not using acetonitrile, making quantitation more difficult, but this could be overcome by extracting the MRM transitions. The LLoQs of all chemicals for direct injection are shown in Table 1. The use of methanol as a mobile phase resulted in LLoQs that were 3-4 times lower for AN and NIC, and 35-50 times lower for HN and 5CSA compared to when acetonitrile was used. These observations are consistent with a study involving fenpropathrin sensitivity when acetonitrile and methanol were compared, however, there was no mention of the same observation for NIC in that study, even though NIC was also analysed[22]. The current study demonstrates that considerable suppression occurred when using acetonitrile, particularly for 2C4NA, 5CSA and HN, and for AN and NIC to a lesser extent.

Table 1 Comparison of the lower limits of quantitation for NIC and its primary degradates for methanol and acetonitrile mobile phases using MS/MS and UV/visible detection methods. All concentrations are reported in ng/mL and are based on direct injection of water samples

		2C4NA	AN	HN	5CSA	NIC
Acetonitrile	UV/visible LLoQ (ng/mL)	25	5	50	50	5
	MS/MS LLoQ (ng/mL)	50	2	25	50	0.4
	Calibration Range (ng/mL)	150-2200	10-2500	50-600	100-2000	1-600
	(Linear Range) (ng/mL)	(150-1000)	(10-2500)	(50-600)	(100-1000)	(1-100)
	R^2 (*n*)	0.9988 (9)	0.9999 (*11*)	0.9957 (*7*)	0.9967 (*8*)	0.9996 (*12*)
	% Organic solvent at elution	56	59	64	62	80
Methanol	UV/visible LLoQ (ng/mL)	50	50	20	250	50
	MS/MS LLoQ (ng/mL)	10	0.6	0.7	1	0.1
	Calibration Range (ng/mL)	50-5000	2-2500	2-3000	5-5500	1-1000
	(Linear Range) (ng/mL)	(50-2200)	(2-2500)	(2-300)	(5-1000)	(1-100)
	R^2 (*n*)	0.9965 (*14*)	0.9998 (*14*)	0.9967 (*14*)	0.9994 (*13*)	0.9990 (*12*)
	% Organic solvent at elution	80	80	80	80	90

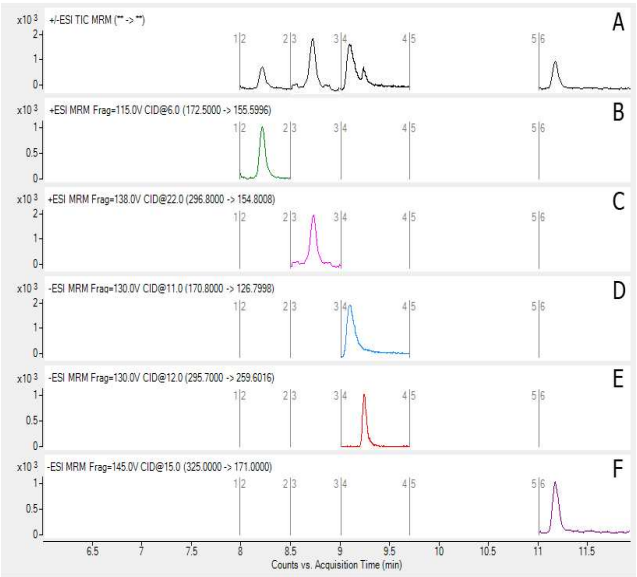

Fig.2 Typical LC-MS/MS of NIC and degradates (A), and extracted MRM transitions for 2C4NA (B, 250 ng/mL), AN (C, 10 ng/mL), 5CSA (D, 250 ng/mL), HN (E, 120 ng/mL) and NIC (F, 2 ng/mL) in anaerobic water using an acetonitrile gradient

The percentage of organic solvent present at elution for each chemical was calculated, taking into account the dead volume of the column (Table 1). The percentage solvent at elution was 15-20% higher for all degradates under methanol than acetonitrile, allowing more complete ionisation in the MS. Increasing the vaporisation gas flow and temperature in the MS/MS did little to improve the response under acetonitrile.

The LLoQ of NIC using a methanol mobile phase has been reported to be 40 pg/mL[22]. However, this value was achieved using a complex liquid/liquid extraction process to concentrate the analyte 50-fold, indicating the LLoQ of the instrumentation for NIC was 2 ng/mL, which is more than an order of magnitude larger than the 0.1 ng/mL obtained using methanol in the current study. While triple quadrupole mass spectrometry was used in our study, it should be noted that a linear ion trap was used in the literature study[22]. Lower sensitivity would be expected from the linear ion trap due to ion depletion resulting from ejection or fragmentation from the ion trap, often as a result of overloading from complex matrices and co-eluting peaks[25]. In the current study, the low collision energy required to fragment the NIC precursor demonstrated the greater tendency to produce a smaller *m/z* 171 daughter ion than the larger *m/z* 289, reported in the comparable study[22].

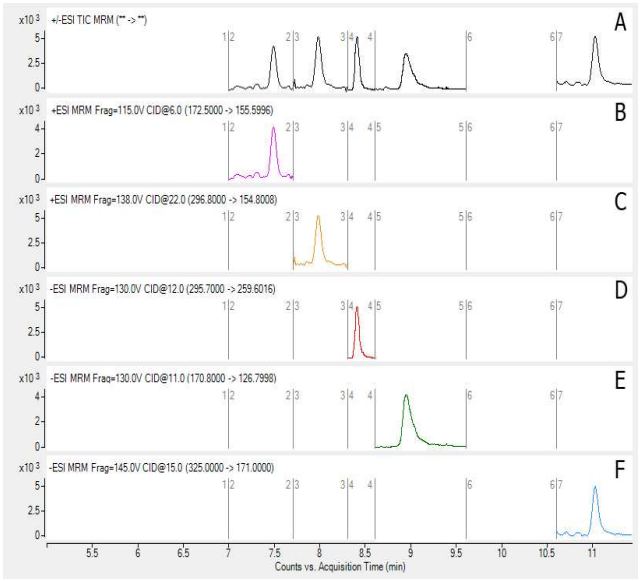

Fig.3 Typical LC-MS/MS of NIC and degradates (A), and extracted MRM transitions for 2C4NA (B, 50 ng/mL), AN (C, 3 ng/mL), HN (D, 4 ng/mL), 5CSA (E, 5 ng/mL) and NIC (F, 3 ng/mL) in anaerobic water using a methanol gradient

Since no literature is available regarding the analysis of the four degradates of NIC by LC-MS/MS, no comparison can be made with values obtained in our study. Our results indicate better sensitivity can be obtained for all four degradates using methanol, which also offers the additional advantage of a lower flow rate, thereby reducing solvent consumption and cost.

The operational MS calibration ranges for all five chemicals are shown in Table 1. Only AN showed linearity over its entire

operational range under both solvent regimes, whereas NIC, 5CSA and HN all required a quadratic regression line. The linear ranges for all four chemicals are shown in brackets in Table 1. While no comparison can be made for 2C4NA, AN, 5CSA and HN with the literature, a linear range of 2-100 ng/mL has been reported for NIC using LC-MS/MS[22] which is comparable to the range of 1-100 ng/mL in the current study. Other work has reported a quadratic operational range of 0.5-1000 ng/mL for NIC [5], which is comparable to the 1-1000 ng/mL reported here.

2.3 Comparison of UV/visible and MS/MS detection

Existing methods for the analysis of NIC and its degradates generally rely on HPLC with UV-visible detection. However, these methods are susceptible to spectral interferences by other chemicals and often lack the sensitivity that may be required for environmental persistence studies, therefore requiring sample clean-up and pre-concentration prior to analysis. While UV-visible detection is reported for NIC and its degradates, LLoQs and operational ranges for all five chemicals have not been supplied[15, 16, 19], with the exception of one report of a LLoQ of 10 ng/mL for NIC[26]. As a result, LLoQs for 2C4NA, AN, 5CSA, HN and NIC using UV/visible and MS/MS detection under both solvent regimes were determined in the current study simultaneously (Table 1).

Generally, acetonitrile provided better LLoQs and a smoother baseline than methanol by UV/visible, as the chromatographic baseline in methanol showed more drift during the solvent gradient. While there was only a small gain in performance between UV/visible and MS/MS using acetonitrile, the LLoQs for MS/MS with methanol were significantly lower ($P<0.05$) than those for corresponding compounds measured using all other solvent/detector combinations evaluated in this study. The lowest LLoQs for NIC and its four main degradates were observed using MS/MS and a methanol mobile phase. To determine the matrix effect on ionisation in the MS, UV wavelengths of 210, 230, 280, 310 and 330 nm were monitored during the chromatographic run. Chromatograms indicated that interfering DOM eluted within 4 minutes, more than 3 minutes prior to the first analyte. Additionally, inorganic interferences are unlikely to be retained by the C18 column and are therefore unlikely to have interfered with ionisation. Additionally, spiked instrument grade and anaerobic waters were spiked with NIC and degradate standards. No significant difference between the two sample types was detected ($P>0.05$). While, the use of prolonged aqueous conditions at the beginning of the chromatographic run extended run times, the lack of difference between results from instrument grade and anaerobic waters supports the hypothesis that it also assisted in separating NIC and its degradates from possible matrix interferences.

Analysis time in our method was reduced by more than half compared to existing HPLC methods[16, 27] by using a shorter column (75 mm compared to 250 mm) with a smaller particle size (2.6 µm Kinetex core-shell column compared to 5-10 µm porous particles) and lower flow rates (0.5 compared to 1.0 mL/min). These enhancements provided greater peak resolution in less time using a smaller injection volume and less solvent, while improving LLoQs by at least one order of magnitude. Additionally, MS/MS was less influenced by interferences such as DOM and provided greater selectivity using MRM, and the absence of extraction and clean up steps dramatically increased sample throughput. While the run time for our method has the potential to be further shortened to increase sample throughput, a more rapid elution resulted in the incomplete separation of HN and 5CSA. Additionally, the possible co-elution of analytes with interfering matrix components was likely. For this reason, the chromatographic run maintained a large water content initially to ensure interfering dissolved organic matter (DOM) and inorganic components were excluded from the mass spectrometer.

2.4 Photolysis of niclosamide

The dependence of niclosamide photodegradation on manganese and iron in the water column was investigated. Concentrations of 10 µg/mL for manganese and 100 µg/mL for iron were used in this study because they have previously been identified as typical concentrations in the water column on Australian rice growing soils[28]. Fig 4 shows that no significant niclosamide degradation occurred in instrument grade water or in 10 µg/mL manganese over the 27 day period ($P<0.05$). However, when instrument grade water was deoxygenated prior to the addition of niclosamide, rapid degradation occurred, but none of the four common degradates were observed. A similar enhancement in degradation was observed when dissolved O_2 was removed in a study on the degradation of a toxin produced by dinoflagellates[29]. This observation can be attributed to dissolved molecular oxygen quenching the excited triplet state in a polar solution, resulting in a less electronically excited singlet state[30]. Likewise, niclosamide degraded rapidly when iron was present in the water column even when kept in the dark, but no degradates were observed in either case. Both dark and photo-assisted iron-catalysed free hydroxyl radical degradation of organic pollutants has been reported[31, 32]. The results in the current study (Fig 4) suggest that iron-catalysed degradation of niclosamide in the water column occurs via both mechanisms, with the greatest change occurring in the first seven days. After the initial drop in concentration, the NIC concentration plateaued, which may be as a result of the available iron being consumed. However, a subsequent experiment was performed in which additional iron was added on day 7 of the incubation. No further decrease in the NIC concentration was observed, indicating that another mechanism was involved in the preservation of the NIC concentration in the water column.

Table 2 Distribution and recovery of NIC and AN in the soil and water microcosms (mean±SD)

Time (days)	Water		Soil		Total Recovered (ug)	% Recovered
	NIC (ug)	AN (ug)	NIC (ug)	AN (ug)		
0	155	0.00	0.00	0.00	155	97
1	132	32.4	ND[3]	ND[3]	164	103
3	77.3	64.5	ND[3]	ND[3]	142	89
7	11.6	40.7	ND[3]	ND[3]	52.3	33
17	3.84	5.25	ND[3]	ND[3]	9.09	6
27	1.14	6.16	0.090	52.5	59.9	37

[3] AN was only determined in soil at the conclusion of the study

Table 3 Acquisition parameters for MS/MS NIC and its primary degradates

MS/MS Parameter	2C4NA	AN	HN	5CSA	NIC
Ion mode	ESI -	ESI +	ESI -	ESI -	ESI -
Ion transition (*m/z*)	170.7→134.7	296.8→154.8	295.7→259.6	170.8→126.8	325.0→171.0
Fragmentation voltage (V)	110	138	130	130	145
Collision energy	12	22	12	11	15
Methanol RT (min)	7.49	7.96	8.49	8.87	11.04
Acetonitrile RT (min)	8.27	8.84	9.36	9.14	11.27

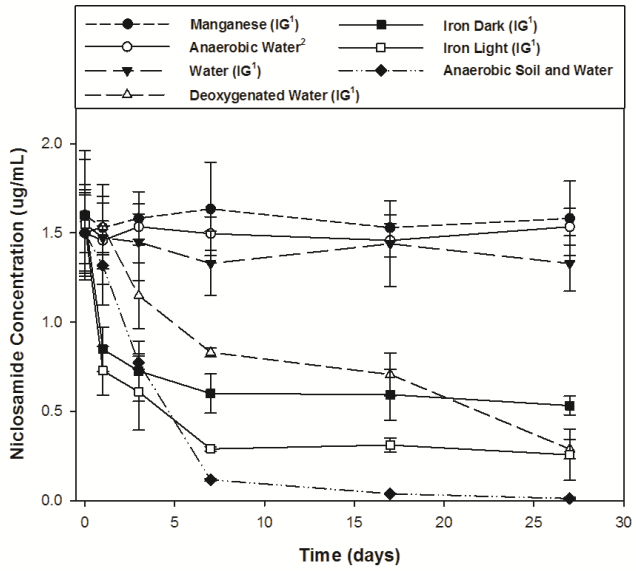

Fig.4 Degradation of NIC in the water column.

[1] IG = instrument grade. [2] previously incubated over soil

When anaerobic water was removed from above soil after 2 months of incubation, niclosamide did not degrade even though Fe(II) was present in the water column at approximately 100 µg/mL. This may be as a result of the DOM binding either the Fe(II) or niclosamide, preventing degradation. The addition of humic acid to soil has been reported to increase the half-life of niclosamide in soil and was attributed to the light absorbing properties of humic acids, effectively shielding the NIC from photodegradation[16]. However, the current study suggests the latter explanation is unlikely because iron solutions incubated inthe dark still exhibited niclosamide degradation in the absence of DOM.

When applied to flooded soil, niclosamide degraded faster than in any of the other treatments. AN, the reductive degradate of niclosamide, was detected as the only degradate in soil or water (Table 2). AN appeared in the water column within 1 day of application and peaked in concentration on day 3. Its concentration declined over the next 3 weeks. Sampling of soil only occurred at the conclusion of the incubation study, but approximately 30% of the applied material was recovered at the conclusion of the experiment, suggesting either further degradation to other degradates or irreversible binding by the soil occurred. Given that no degradation of niclosamide occurred in anaerobic water in the absence of soil, but occurred in the presence of soil, this preliminary study suggests that the soil contained microflora/microfauna required for degradation to AN, or non-biological components that hastened degradation.

3 Experimental

3.1 Reagents and chemicals

NIC, 5CSA, 2C4NA and formic acid were supplied by Sigma-Adrich, methanol was supplied by Mallinckrodt, and acetonitrile by LabScan. HN was synthesised by Natland International Corporation (Morrisville, NC) and AN was synthesised by Derse and Schroeder Associates Ltd (Madison, WI).

3.2 Instrumentation

Analysis was performed using an Agilent Technologies 1200 Series LC with binary pump, degasser, column oven, autosampler, DAD (330 nm) and 6410 quadrupole tandem mass spectrometer. A Phenomenex Kinetex XB-C18 (75 x 4.6 mm x 2.6 µm) column was used at 35 °C and the injection volume was 10 µL. The MS/MS used ESI and was operated in both negative and positive ion modes, depending on the chemical. Ion transitions, retention times and parameters varied for each chemical and are shown in Table 3. Additional MS/MS parameters were dwell (500 ms), gas temperature (350 °C), vaporiser temperature (250 °C), gas flow (4 L/min), nebuliser (60 psi) and capillary voltage (3000 V).

3.3 Optimisation of chromatographic conditions

Methanol and acetonitrile were compared as mobile phases for their ability to separate NIC from its four main degradates for analysis via LC-MS/MS. Initial work was conducted using ammonium acetate buffer in accordance with existing HPLC-UV/visible methods[22], but this was abandoned in favour of formic acid due to poor performance and to minimise the amount of salt entering the MS/MS. The mobile phases tested were instrument grade water (Phase A) and either methanol or acetonitrile (Phase B), all containing 0.2% formic acid (v/v). The flow rate was 0.5 and 0.6 mL/min for methanol and acetonitrile, respectively. Gradient programming is shown in Table 4, with each program completed in 13 minutes.

Table 4 Solvent programming for methanol and acetonitrile gradients for LC

Time (min)	% Water	% Methanol	Time (min)	% Water	% Acetonitrile
0	90	10	0	90	10
1	90	10	2	90	10
6	20	80	10	20	80
8	20	80	10.5	20	80
8.5	10	90	11	90	10
10.5	10	90	13	90	10
11	90	10			
13	90	10			

3.4 Photolysis of niclosamide in solution

Seven treatments were compared in a preliminary study to determine the role of common components of floodwater in Australian rice fields on the fate of niclosamide (Table 5). Six treatments were incubated in triplicate in 250 mL Schott bottles with screw cap lids which had both been treated with Coatasil™. Each bottle contained 100 mL of instrument grade water (18.2 MΩ.cm). Deoxygenated water was produced by sonicating instrument grade water for 2 hours, and light excluded samples were covered in 2 layers of aluminium foil. Aerobic treatments were produced by adding 120 mL of water to each of 6 screw cap jars containing 100 g rice growing soil (Birginbigil clay loam) from Yanco Agricultural Institute. The jars were incubated for 2 months prior to the commencement of the photodegradation study, at which point water was removed from 3 jars and transferred to Schott bottles under nitrogen for use as the anaerobic water experimental treatment. The remaining 3 jars were used intact. All 18 Schott bottles and 3 jars were spiked with niclosamide (200 µL, 800 µg/mL) and illuminated for 15 hours per day for four weeks (20 °C). Jars were sampled periodically and then analysed by LC-MS/MS for NIC, 2C4NA, AN, HN and 5CSA.

Table 5 Treatments and conditions for determining the role of metals on photodegradation of NIC

Microcosm	Varied conditions
Instrument grade water	
Instrument grade water	deoxygenated
Iron (100 µg/mL)	
Iron (100 µg/mL)	light excluded
Manganese (10 µg/mL)	
Anaerobic water	
Anaerobic soil and water	

3.5 Statistical analysis

Quantitation of analytes was determined using peak areas. The LLoQ was calculated by performing six injections of a standard mix spiked into anaerobic and instrument grade waters at concentrations for the estimated LLoQ. Provided the % Relative Standard Deviation was less than 20%, the LLoQ was calculated as the mean + (10 × SD). If the %RSD was greater than 20%, a higher concentration standard was injected and the process repeated until the %RSD was less than 20%. Significance was analysed using a t-test. Gradient comparison was performed using a t-test for differences between two independent regression coefficients for treatments in the NIC degradation study[33]. NIC concentration values in each iron solution, the deoxygenated instrument grade water, and the soil/water treatments were logarithmically transformed and then analysed as described for linear treatments.

Conclusions

Aquatic snails are normally controlled in Australian rice crops with copper sulfate, however, continual application may result in soil accumulation. Niclosamide is an organic anthelmintic chemical that has potential to supplement or replace copper sulfate. A new method for the analysis of niclosamide and its degradates 2-chloro-4-nitroaniline, aminoniclosamide, hydroxyniclosamide and 5-chlorosalicylic acid by LC-MS/MS is reported which offers improved selectivity and sensitivity, and shorter preparation and run times to current methods. LLoQs approximately 20 times lower than in previously published niclosamide LC-MS/MS methods were observed, and LLoQs 10-250 times lower for all chemicals by LC-MS/MS compared to those reported for HPLC-UV/visible detection. This improvement in the LLoQ for all chemicals allows a more in-depth study of the environmental fate of all chemicals, which is beneficial when accreditation of new pesticides is being sought. Whilst the LLoQs reported in this study for NIC and its degradates show an improvement over previous studies, the LLoQs could be further reduced by including a pre-concentration step, using techniques such as solid phase extraction or liquid-liquid extraction.

The new analytical method was applied to the degradation of niclosamide in water. Manganese and anaerobic water previously incubated over soil produced no significant differences in the NIC degradation rate relative to instrument grade water, while addition of iron promoted degradation in both light and dark conditions. When niclosamide was applied to aerobic flooded soil, it degraded rapidly and its primary reductive degradate appeared immediately in the water column and then accumulated in the soil. These results indicate that while iron may catalyse NIC degradation, reductive degradation of NIC to AN will occur more rapidly, and AN will be bound by the soil. Degradation of AN may occur in both soil and water to unknown products and this warrants further investigation.

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Notes and references

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