Talanta 82 (2010) 668–674

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Detection of s-triazine pesticides in natural waters by modified large-volume direct injection HPLC

David J. Beale[a,c,1], Sarit L. Kaserzon[a], Nichola A. Porter[a,c,*], Felicity A. Roddick[b,c], Peter D. Carpenter[a]

[a] School of Applied Sciences, RMIT University, GPO Box 2476, Melbourne 3001, Australia
[b] School of Civil, Chemical and Environmental Engineering, RMIT University, GPO Box 2476, Melbourne 3001, Australia
[c] Water Quality Research Australia (Formally the CRC for Water Quality and Treatment), Private Mail Bag 3, Salisbury, South Australia 5108, Australia

ARTICLE INFO

Article history:
Received 26 February 2010
Received in revised form 12 May 2010
Accepted 12 May 2010
Available online 21 May 2010

Keywords:
Atrazine
Direct injection HPLC
Large-volume injection
Monolithic column
Hexazinone
Natural organic matter
Natural waters
Simazine

ABSTRACT

There is a need for simple and inexpensive methods to quantify potentially harmful persistent pesticides often found in our water-ways and water distribution systems. This paper presents a simple, relatively inexpensive method for the detection of a group of commonly used pesticides (atrazine, simazine and hexazinone) in natural waters using large-volume direct injection high performance liquid chromatography (HPLC) utilizing a monolithic column and a single wavelength ultraviolet–visible light (UV–vis) detector. The best results for this system were obtained with a mobile phase made up of acetonitrile and water in a 30:70 ratio, a flow rate of 2.0 mL min^{-1}, and a detector wavelength of 230 nm. Using this method, we achieved retention times of less than three minutes, and detection limits of 5.7 $\mu g L^{-1}$ for atrazine, 4.7 $\mu g L^{-1}$ for simazine and 4.0 $\mu g L^{-1}$ for hexazinone. The performance of this method was validated with an inter-laboratory trial against a National Association of Testing Authorities (NATA) accredited liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) method commonly used in commercial laboratories.

1. Introduction

It is common practice for water utilities to apply a risk approach to pesticide residue monitoring in drinking water catchments, where pesticides are identified and the risk of contamination is calculated (*i.e.*, solubility and mobility of pesticide being applied in conjunction with the proximity and rate of application) [1]. This information is used to inform the water utility's monitoring programme. Current Australian drinking water guidelines do not enforce a sampling program frequency (although it is recommended to sample for pesticide residues monthly), nor do they specify which pesticides are to be monitored, as no single method of analysis is suitable for all the organic compounds that may be present in water. Each compound, or perhaps group of compounds, has specific analytical requirements, so monitoring for all of them would be extremely costly, time consuming, and probably unjustified [2]. To highlight the deficiencies in current monitoring programs, Benotti et al. [3] investigated pharmaceutical and endocrine disrupting compounds (including atrazine) in drinking water from the USA. Their study concluded that the level of tertiary treatment currently applied by 19 water utilities resulted in atrazine and other potentially harmful chemicals passing through to finished drinking water, and in some instances at concentrations as high as 0.9 $\mu g L^{-1}$ (note current US EPA drinking water guidelines for atrazine are set at 3 $\mu g L^{-1}$ [4]). Of greater concern was the presence of atrazine in waters in areas where this compound was not believed to be in use [3].

While current standard methods recommended for the determination of pesticide residues are satisfactory with respect to detection limits and analytical performance, they are often criticized for the time and costs involved. The development of new cost effective and rapid methodologies is becoming increasingly desirable because they enable water utilities to increase the frequency of sampling and broaden the range of pesticides analysed, giving them a better picture of the state of contamination in their system. As such, many researchers are looking for new techniques that address this time and cost problem, and to achieve this, some are considering enhancement and further development of liquid chromatographic techniques, in particular HPLC, ultra performance liquid chromatography (UPLC) and LC as shown in Table 1.

LC–MS methods offer significant reductions in detection limits, and considerable effort has been expended to reduce retention times by employing fast short narrow bore columns and high

* Corresponding author at: School of Applied Sciences (Applied Chemistry), RMIT, University, GPO Box 2476, Melbourne 3001, Australia Tel.: +61 3 9925 1787; fax: +61 3 9639 1321.
E-mail address: nichola.porter@rmit.edu.au (N.A. Porter).
[1] Present address: CSIRO Land and Water, PO Box 56, Highett 3190, Australia.

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doi:10.1016/j.talanta.2010.05.030

Table 1
Summary of recent developments in rapid pesticide analysis by HPLC.

Method	Analyte	Separation column	Extraction type	Retention time (T_R) (min)	Limit of detection (LOD) ($\mu g\,L^{-1}$)	Reference
HPLC–UV	Atrazine Simazine (33 pesticides analysed in 90 minutes)	RP-C18	SPE	34 23	–	[5]
HPLC–UV	Atrazine Simazine	RP-C18	Liquid	10 12.6	0.1 0.04	[6,7]
LC–ESI-MS	Atrazine Simazine	ACQUITY BEH C18	SPE	2.37 1.91	0.0006 0.00008	[8]
UPLC–ESI-MS/MS	Atrazine Simazine	BEH C18 column	SPE	2.3 1.6	0.006 0.01	[9]
HPLC–UV	Hexazinone	RP-C18	Direct injection	7.9	0.3	[10]
LC–EIS-MS	Atrazine Simazine	XDB-C18	Direct injection	4.39 4.36	0.3	[11]

mobile flow rates operating under increased pressure (*i.e.*, retention times commonly achieved between *ca.* 2 and 5 min for atrazine and simazine [8,9,11]). Shortening the analytical run time is an important step towards high sample throughput often required in commercial laboratories conducting routine pesticide monitoring. Run time of several minutes (*e.g.*, up to 10–15 min) is not acceptable and emphasis has been directed towards ensuring maximum chromatographic resolution in a significantly reduced time. However, LC–MS methods are still considered to be highly sophisticated and expensive pieces of equipment requiring specialized personnel to operate and interpret MS data [12]. In contrast, conventional HPLC–UV methods are typically more robust, cheaper and easier to operate, but fall short in terms of required detection limits and the time required for analysis. Recent advances in monolithic column technology have lead to an improvement in peak resolution, and combined with HPLC, provide an affordable option for fast screening of samples prior to confirmation by LC–MS if required. In general, resolution between solute bands depends on the square root of column efficiency (*i.e.*, the number of theoretical plates (NTP)), which in turn is proportional to the reciprocal of particle diameter ($1/d_p$) of the column packing material. Concurrently the pressure drop across the column is inversely proportional to the square of d_p. Attempts to obtain greater NTP by decreasing particles size, results in significant increases in instrument operational pressure, often exceeding the instrument specifications. The structure of monolithic columns overcomes this problem [13].

Monolithic columns are prepared by in situ polymerization of monomers in a column, providing greater flexibility than densely packed columns, and a wider range of monomers can be used with integrated structures that can increase the overall porosity. The higher porosity leads to an increase in permeability which consequently results in a decrease in the required operational pressure. Coupled with the presence of small-sized monostructure skeleton, higher efficiencies can be expected. Up to now, monolithic columns have been used mainly for the determination of biological amino acids and drug residues [14], although there have been some instances of monolithic chromatography for pesticide analysis (see Table 2); however, its application to natural waters without pre treatment is limited [15].

While monolithic columns have been used previously for the determination of pesticides; the application has involved specialised expensive equipment (*e.g.*, HPLC–MS/MS) or has been applied to sample matrices comprising concentrated formulations [16,17]. Similarly, more conventional methods (utilising packed columns) have relied on specific extraction techniques, increased operating pressures and sophisticated detectors to remove interferences, concentrate target analytes and decrease detection limits respectively. The purpose of this research is to devise a simple, affordable, robust HPLC method utilising a monolithic column and UV–vis detection for the determination of pesticides in natural waters without any pre-treatment (*e.g.*, sample extraction). As such, this paper describes the development of a rapid, cost effective, large-volume direct injection HPLC method utilizing a monolithic column with UV detection for the combined determination of atrazine, simazine and hexazinone in natural waters. The development of the proposed method is described and compared to a conventional packed column. The method is applied to natural water samples and cross validated against a commercially operated, NATA accredited, MS method (conducted by SGS Consulting, Australia). An investigation into possible interferences is also presented.

2. Materials and methods

2.1. Solution preparation

A stock solution of atrazine ($9.8\,g\,L^{-1}$; Supelco, Germany, neat analytical standard (NAS)), was prepared in 10 mL acetonitrile and sonicated for an hour. Similar stock solutions of simazine ($10.0\,g\,L^{-1}$; Supelco, USA, NAS) and hexazinone ($10.0\,g\,L^{-1}$; Supelco,

Table 2
Summary of chromatography monolithic separation methods for pesticide analysis.

Method	Analyte	Sample matrix	Extraction type	Retention time (T_R) (min)	Limit of detection (LOD) ($\mu g\,L^{-1}$)	Reference
HPLC–UV	Fenoxycarb Permethrin	Veterinary sprays	SPE	1.8 7.2	2000 1000	[16]
HPLC–MS/MS	Atrazine Simazine (plus 9 additional pesticides)	Natural waters	SPE	*ca.* 2.0 *ca.* 1.5	0.002 0.001	[17]
CEC	17 Different pesticides and metabolites	MilliQ-water	–	<2.1	–	[18]

USA, NAS) were also prepared. Working standard solutions of atrazine, simazine and hexazinone were prepared daily in MilliQ water. Stock solutions were stored at 4 °C in the dark when not in use.

2.2. Dissolved organic carbon (DOC)

After filtration using a 0.45 μm hydrophilic membrane (Durapore PVDF) DOC in water samples was determined in triplicate using a Sievers 820 TOC analyser.

2.3. Direct injection HPLC

HPLC with direct injection was carried out with a Waters HPLC pump (M-6000A, Waters Associates Inc., USA) operated isocratically. Aqueous samples (500 μL) were injected via a Waters HPLC injection valve fitted with a 500 μL loop using a 2 mL glass barrel syringe. The injected sample was passed through a monolithic column RP-18e, 50–4.6 mm (by Chromolith, Merck); for comparison, a second column was used, a C8, 5 μm, 250 mm × 4.6 mm (model 831815 Spherisorb, Phase Separations, USA) packed column that was substituted for the monolithic column. The HPLC system was connected to a UV–vis detector (SPD-10AV, Shimadzu, Japan) set at 230 nm, coupled to a chart recorder (Model 3395, Hewlett Packard, USA) and a personal computer operating ChemStation (Agilent, USA). Where noted, chromatograms were digitised from paper chromatograms and reproduced using GraphClick software (Mac OS X, Arizona Software, Switzerland).

2.4. Solid phase extraction (SPE)

SPE cartridges (0.5 mg Bond Elute C18) were pre-conditioned with 5 mL methanol followed by 5 mL MilliQ water (Millipore MilliQ Water System) prior to sample introduction (1 L aliquot, filtered via 0.45 μm hydrophilic membrane) at 2–4 mL min^{-1} using a 12 port vacuum SPE manifold (Varian, Australia) and Visiprep SPE tubing (Varian, Australia). The SPE cartridges were then air dried under a vacuum and eluted using 4 mL of 90:10 methyl-tert-butyl-ether:ethyl acetate. Samples were then evaporated to dryness under nitrogen and reconstituted in 40:60 (v/v) MeOH:H_2O, to final eluent volume of 5.0 mL.

2.5. LC–MS/MS

LC–MS/MS analysis was carried out at SGS Australia Pty Ltd. Instrumentation comprised an LC (Agilent 1200 Series) with a Waters Atlantis T3 column and a MS/MS system (Applied Biosystems API 3200). A mixed 100 μL sample volume was injected into the LC using an auto-sampler. Samples were analysed without pre-concentration using a buffered mobile phase (MeOH:H_2O). Quantification ions used were 216/174 *m*/*z* for atrazine, 202.1/132.1 *m*/*z* for simazine, 253.2/171.2 *m*/*z* for hexazinone. The full method cannot be entirely disclosed due to intellectual confidentiality but it is NATA accredited and follows ISO 9001 QC protocols (SGS 2009).

2.6. Statistical methodology

The analytical performance was assessed by determining the limit of detection (LOD), limit of quantification (LOQ) and practical method detection limit (MDL), where LOD was calculated using a signal to noise ratio (S/N) of 3, LOQ was calculated using S/N of 10, and the MDL was calculated using the lowest standard ($n = 8$), where the SD was calculated and multiplied by the student *t*-value at a 95% confidence level [19].

3. Results and discussion

3.1. Mobile phase optimisation

A mobile phase method development triangle was created according to Harris [19]. Method development triangles are a systematic process applied in HPLC to develop a mobile phase suitable for the separation of the target analytes using a combination of solvents: in this case MeOH, methanol; ACN, acetonitrile; and H_2O, MilliQ water. Solvents were varied from 10 to 90% (v/v) at intervals of 10, 30, 50, 70, and 90% for combinations consisting of two solvents; and at 10, 15, 25, 33.3, 85, 75, 67.7% intervals for mobile phases comprising three solvents until the best separation was achieved. While combinations of MeOH, ACN and water mobile phases were all effective for analysing the individual triazine compounds, hexazinone and simazine co-eluted when all three compounds were present; however, this was overcome utilising a 30:70 (v/v) ACN:H_2O mobile phase. The HPLC column was maintained under stable standard laboratory conditions (*ca.* 22 °C) and all working solutions were brought to room temperature prior to analysis. Since it was the intention to develop a cheap robust HPLC method, no attempt was made to control the temperature using a column heater.

3.2. Effect of mobile phase flow rate

The effect of the mobile phase flow rate on the direct injection HPLC analysis of atrazine, hexazinone and simazine was investigated over 0.25–3.00 mL min^{-1} in 0.25 mL min^{-1} increments. However, the mobile phase flow rate when using the monolithic column did not significantly influence the instrument operating pressure or the quality of the chromatography (*i.e.*, peak width and resolution). Consequently, a flow rate of 2.0 mL min^{-1} was selected for all subsequent experiments because it was the flow rate which achieved baseline separation between all analytes selected and was the fastest flow rate that could be used with the packed C8 column enabling a comparison between the two columns under the same conditions to be made.

3.3. Effect of injection volume

The effect of the sample injection volume on the direct injection HPLC analysis of atrazine, hexazinone and simazine was investigated over 100–1000 μL. It was found that the analyte peak area steadily increased as the volume increased from 100 to 1000 μL; however, the best peak shape was achieved using 500 μL. Injection volumes larger than 500 μL distorted the symmetry of the peak, causing peaks to become broader. All subsequent analyses were performed using a filled 500 μL sample injection loop.

3.4. Effect of detector wavelength

A number of researchers have investigated atrazine, hexazinone or simazine at wavelengths between 220 and 223 nm for atrazine and simazine, as well as hexazinone at 244 nm [21,22]. However, when applying these wavelengths for the simultaneous determination of atrazine, hexazinone and simazine (*e.g.*, either 220–3 or 244 nm) there is a decrease in analyte sensitivity for either atrazine and simazine or hexazinone (depending on the wavelength selected) unless a diode-array detector (DAD) is employed. In the absence of a DAD, the analysis has to be performed utilizing a common wavelength. As illustrated in Fig. 1, the UV spectra of atrazine and simazine intersect with the spectra of hexazinone at *ca.* 230 nm. Fig. 1 also shows that the influence of DOC at 230 nm relative to 220 nm, which potentially poses a problem if present at high concentrations and is

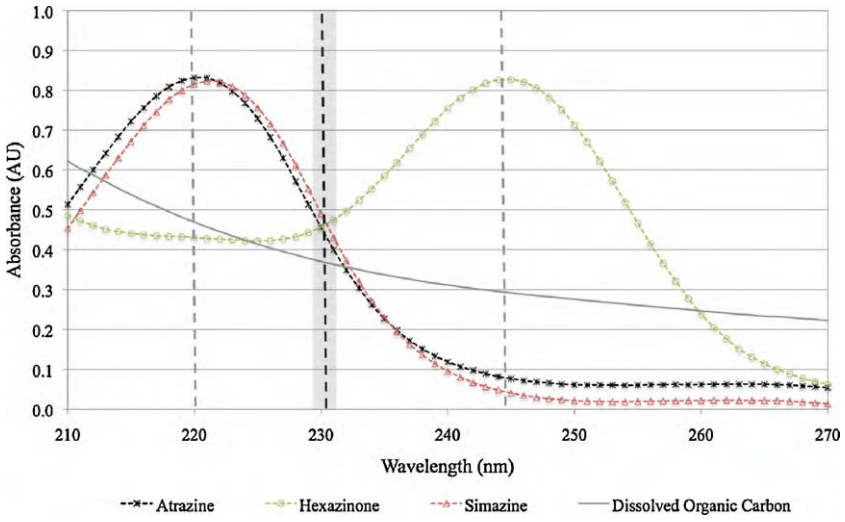

Fig. 1. UV spectra of atrazine, hexazinone, and simazine (500 μg L^{-1}) in MilliQ water and a natural water sample containing 11.1 mg L^{-1} dissolved organic carbon.

found to co-elute with the target analytes in natural water samples.

3.5. Limit of detection

Pesticides at standard concentrations over the range 5–50 μg L^{-1} were prepared in MilliQ water and analysed using the best operating conditions determined: sample injection volume of 500 μL, mobile phase of 30:70 ACN:H_2O at 2 mL min^{-1} with detector wavelengths of 220, 230 and 244 nm. The analytical and statistical parameters obtained for the determination of the three pesticides by direct injection HPLC are summarised in Table 3. Fig. 2 illustrates the difference in chromatographic separation over the three different wavelengths for the monolithic column.

The use of 230 nm reduced the sensitivity by 35% for atrazine compared to the wavelength for its maximum sensitivity (λ_{max}), 33% for hexazinone and 34% for simazine. Nevertheless the reduction in sensitivity had a minimal effect on the detection limits. Hence, it is possible to analyse a range of triazines using a constant wavelength with a simple mono wavelength UV detector while still achieving good analytical sensitivity for all three target analytes.

The analytical performances between the packed and monolithic columns are comparable (*i.e.*, the analytical figures of merit (LOD) for the monolithic column are within ±1.4 μg L^{-1} at 220 and 244 nm; and ±5.2 μg L^{-1} at 230 nm when directly compared with the packed column). The great advantage of the monolithic column over the packed column is the decrease in operating pressure and the reduction in retention time for each analyte, resulting in a significantly reduced analysis time; *i.e.*, 2.0 mL min^{-1} for the packed column at an operating pressure of 3500 psi and analysis time of 7 min per sample compared to 2.0 mL min^{-1} for the monolithic column at an operating pressure of 500 psi and analysis time under 3 min per sample; or 3.0 mL min^{-1} and 600 psi and analysis time under 2 min per sample.

3.6. Application to natural samples

To test the effect of dissolved organic matter (as DOC) on the analytical performance of the described direct injection HPLC method, a series of natural water samples with various DOC concentrations were collected throughout Victoria, Australia (see Table 4). Samples were collected using a 1 L grab glass bottles (pre-cleaned with Pyroneg, Johnson Diversey Australia, and triple

Table 3
Summary of analytical figures of merit for the determination of atrazine, simazine and hexazinone in MilliQ water by direct injection HPLC.

Statistical figures of merit	UV–vis detector wavelength								
	220 nm				244 nm		230 nm		
	Atrazine		Simazine		Hexazinone		Atrazine	Simazine	Hexazinone
	Mono.[a]	Pack.[b]	Mono.[a]	Pack.[b]	Mono.[a]	Pack.[b]	Mono.[a]	Mono.[a]	Mono.[a]
Slope	6979	6907	7793	7459	8724	9413	519	369	172
Intercept	−3370	−536	−13483	3413	645	502	−1920	−825	−357
Linear dynamic range (μg L^{-1})	5–50	5–50	5–50	5–50	1–50	0.5–50	5–50	5–50	5–50
LOD (μg L^{-1})	0.7	0.6	1.8	0.4	0.1	0.3	5.7	4.7	4.0
LOQ (μg L^{-1})	1.2	1.5	2.1	1.3	0.4	1.2	10.6	10.7	8.5
MDL (μg L^{-1})	1.1	1.4	1.2	3.0	0.9	1.1	4.1	2.5	2.6
SD of the intercept	4916	6180	14221	3891	524	1289	314	272	97
SD of the slope	143	200	280	126	246	245	10	9	3
SD of the regression	6347	9598	19389	6043	719	2412	361	312	111
Correlation coefficient	0.9978	0.9976	0.9921	0.9991	0.9976	0.9973	0.9977	0.9966	0.9980

[a] HPLC system with monolithic separation column (Mono), retention time(s) for 220/244 nm at flow rate 3.0 mL min^{-1}: atrazine 1.77 min; simazine 1.12 min; hexazinone 0.98 min; and for 230 nm at flow rate 2.0 mL min^{-1}: atrazine 2.71 min; simazine 1.71 min; hexazinone 1.53.
[b] HPLC system (C8 column) described by Beale et al. [20] retention time (s) for 220/244 nm at flow rate of 2.0 mL min^{-1}: atrazine 6.77 min; simazine 4.12 min; hexazinone 3.98 min.

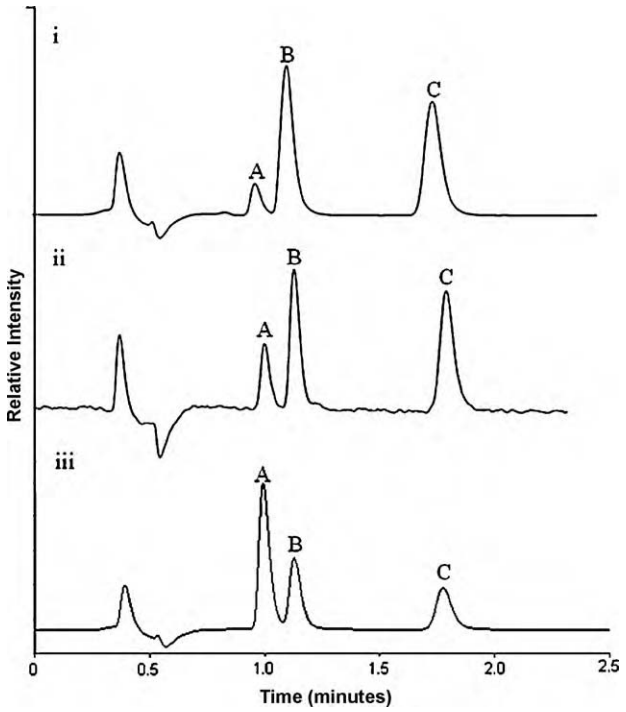

Fig. 2. Monolithic column chromatograms of 50 μg L^{-1} stock solution of atrazine, simazine and hexazinone with UV–vis detection at 220, 230, and 244 nm. *Note*: Peaks identified as (A) hexazinone; (B) simazine; (C) atrazine. Sample solution consisted of 50 μg L^{-1} pesticide in MilliQ water, 500 μL injection. Mobile phase 30:70 (ACN:H_2O) with a UV–vis detector ((i) λ 220 nm; (ii) λ 230 nm; (iii) λ 244 nm), flow rate 2.0 mL min^{-1}.

Table 5
Direct injection HPLC analysis of atrazine, hexazinone and simazine in natural waters.

Concentration (μg L^{-1})	Recovery% (%R_{SD})		
	Atrazine	Hexazinone	Simazine
10.0	105 (6.1)	82 (10.1)	106 (1.5)
15.0	99 (1.3)	89 (8.6)	–
25.0	92 (0.6)	84 (8.2)	95 (0.7)
40.0	82 (0.8)	85 (3.6)	98 (1.4)
50.0	80 (0.3)	80 (4.6)	–
Statistical figures of merit			
Pearson correlation coefficient	0.9965	0.9993	0.9966
p-Value	0.80	0.67	0.97
Correlation coefficient	0.9930	0.9986	0.9933

Note: Each of the 8 water samples at each of the concentrations was analysed in triplicate.

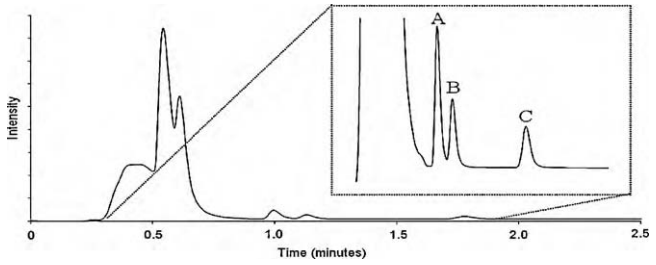

Fig. 3. Effect of natural organic matter (dissolved organic matter; DOC) on monolithic chromatography performance. *Note*: (A) Hexazinone; (B) simazine; (C) atrazine. Sample number three (refer to Table 2); DOC = 6.5 mg L^{-1}) spiked with 10 μg L^{-1} pesticide, 500 μL injection. Mobile phase 30:70 (ACN:H_2O) with a UV–vis detector λ 244 nm, flow rate 2.0 mL min^{-1}.

rinsed with MilliQ water). All samples were stored at 4 °C and allowed to equilibrate at room temperature (*ca.* 22 °C) prior to analysis.

The natural waters spiked with increasing amounts of atrazine, simazine and hexazinone were analysed using the direct injection method at a wavelength (λ) of 230 nm. The position of the DOC peak did not interfere with those of atrazine, simazine and hexazinone in any of the samples analysed ($p < 0.05$ at 95% confidence interval). Statistical analysis of the recovery and relative standard deviation for all natural water samples showed strong correlation between spiked and measured concentrations (Table 5).

Samples directly injected into the HPLC without pre-treatment showed a distinct DOC peak within the first 0.7 min compared with standards as shown in the example chromatogram using 244 nm presented in Fig. 3. The presence of DOC was confirmed by 3D EEM fluorescence spectroscopy as shown in Fig. 4, where distinct humic and fulvic acid fluorophores were observed at 237–260/400–500 and 300–370/400–500 (excitation/emission wavelength) for all of the samples included in this study [23].

3.7. Method validation

Two blind comparison studies were conducted to evaluate the performance of the described multi-analyte (single wavelength; λ = 230 nm) monolithic HPLC method utilising the natural water samples from drinking water catchment waters spiked with all three pesticides. Samples # 7 and 8 in Table 4 were spiked at SGS and presented to our laboratory as unknowns. The first study consisted of samples spiked with concentrations between 10 and 50 μg L^{-1} in order to assess the recovery and reproducibility of the direct injection technique described. The second study involved samples spiked with concentrations between 0.1 and 2 μg L^{-1} to assess and compare the analytical performance of the described HPLC method with SPE pre-concentration. In this case the samples were spiked at SGS, analysed at RMIT and then sent back to SGS for reanalysis as a double blind experiment.

The analytical figures of merit for the LC–MS/MS instrument at SGS are presented below in Table 6.

The first set of spiked samples provided by SGS was analysed by direct injection HPLC after filtration. Apart from a significant

Table 4
Summary of characteristics of natural water samples analysed by direct injection HPLC.

Sample ID tag	Water source	DOC[b] (mg L^{-1})	Location in Victoria	Primary land activity
1	Ground water	3.1	South East	Cattle farm
2	Creek (seasonal)	4.5	South East	Livestock
3	River (metropolitan)	6.5	Central	Metropolitan
4	Drinking water catchment	10.7	South West	Agriculture
5	Drinking water catchment[a]	11.1	East	Natural reserve
6	Drinking water catchment	11.7	South West	Agriculture
7	Drinking water catchment	10.1	North	
8	Drinking water catchment	14.4	North	

[a] Decommissioned drinking water catchment, closed to public access.
[b] Dissolved organic carbon (DOC) measured using a total organic carbon analyser.

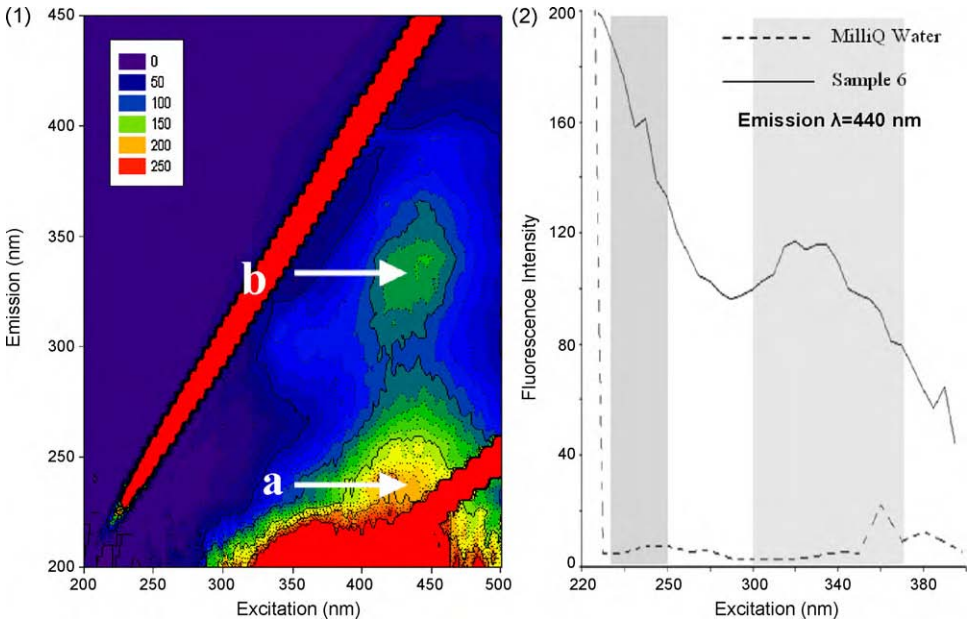

Fig. 4. 3D excitation emission matrix (3D EEM) fluorescence spectroscopy in natural water for the confirmation of DOC. *Note*: (1) EEM spectrum for sample number six (Table 3). Identification of two humic-like flurorphores (a and b) were determined by observing distinct peaks at 237-260/400-500 and 300-370/400-500 respectively (excitation/emission wavelength)[23], which were not present in MilliQ water standards. (2) Fluorescence spectrum for sample 6 at emission wavelength λ = 440 nm over the excitation ranged from 200 to 380 nm. The spectrum indicates the presence of the humic-like fluorphores over a MilliQ blank. The observed peak at 230 nm in the MilliQ sample is an artefact of Raman light scatter.

difference in analysis time, *i.e.*, 12 min per sample by LC–MS/MS compared with <2 min by direct injection HPLC, good correlation between the spiked and measured concentrations was observed as shown in Fig. 5; all of the samples were within 90% confidence intervals for all three analytes.

When using the direct injection method, the concentrations of the second set of samples were below the LOD and MDL limits presented in Table 3. Therefore, prior to analysis, pre-concentration of the samples (by a factor of 25) was performed by solid phase extraction. The HPLC instrumentation used was not altered for the analysis of SPE extracts, *i.e.*, the 500 μL injection loop and volume was utilised.

Table 6
LC–MS/MS analytical figures of merit.

Analyte	Atrazine	Hexazinone	Simazine
Linear dynamic range (μg L^{-1})	0.1–10	0.5–10	2–10
Retention time (min)	11.17	10.56	10.62
LOD (μg L^{-1})	0.05	0.5	0.1
MDL (μg L^{-1})	0.1	2.0	0.5

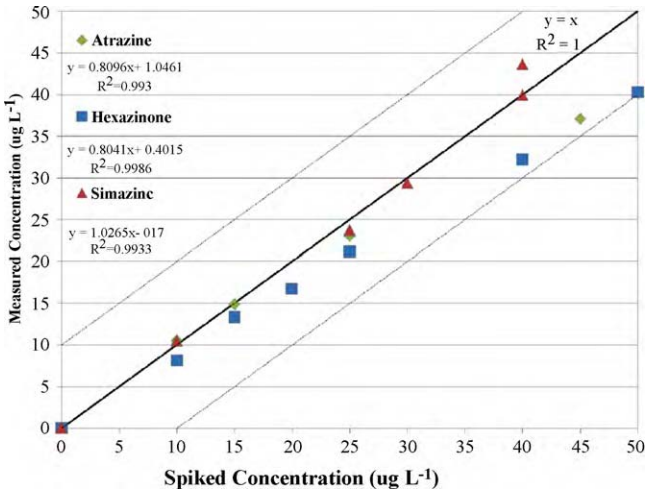

Fig. 5. Blind analysis of spiked natural water samples (10–50 μg L^{-1}) provided by SGS (Victoria, Australia).

Natural samples pre-concentrated with SPE still showed the presence of DOC within sample extracts; however, an 80% reduction in the DOC peak was observed. Although SPE should eliminate the majority of DOC in extracted samples, there is a possibility that during pre-concentration some DOC is retained on and later eluted from the SPE cartridge. This finding is similar to that by Simpson [24], who found that a fraction of DOC can be retained and eluted from the SPE cartridge when performing sample extractions with a sample matrix containing complex DOC. The degree of retention is dependent upon a combination of the SPE material, the sample matrix and chemical characteristics of the DOC, and is relatively independent of concentration.

Linear regression was performed on the results obtained from the two systems. The regression between HPLC and LC–MS/MS systems showed a strong relationship between the instruments for all three herbicides as shown in Table 7. This shows that the HPLC method compared well against the NATA accredited method and has the sensitivity required for triazine and triazinone detection. The reduced recovery observed for hexazinone is probably due to losses during extraction.

3.8. Interferences

The interference of 'like' compounds, in terms of peak resolution and retention time, was investigated utilising known atrazine metabolites, as well as other known triazine pesticide standards. Fig. 6 illustrates the co-elution of peaks between simazine and the

Table 7
Statistical figures of merit from analysis of spiked natural water.

Statistical figures of merit	Atrazine	Hexazinone	Simazine
Slope	0.8903	0.5278	0.9097
Pearson correlation coefficient	0.9824	0.9582	0.9512
p-Value	0.92	0.33	0.92
Recovery% (%R_{SD})	96 (2.1)	52 (0.7)	91 (6.9)

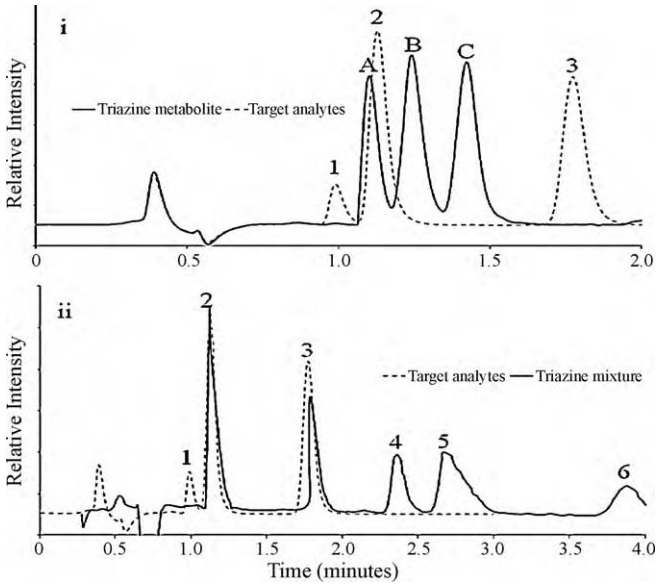

Fig. 6. An investigation into potential interference with other triazine pesticides and their metabolites. *Note*: Peaks identified as (A) 2-hydroxyatrazine; (B) desisopropylatrazine; (C) desethylatrazine; (1) hexazinone; (2) simazine; (3) atrazine; (4) propazine; (5) ametryn; and (6) prometryne (by injecting neat standards of each pesticide and metabolite, and comparing the retention times of each compound with a combined analyte solution). Mobile phase 30:70 (ACN:H_2O) with a UV–vis detector (λ 220 nm), flow rate 3.0 mL min^{-1}. Chromatogram (i) triazine metabolites overlaid with hexazinone, simazine and atrazine standard chromatogram as a point of reference (500 μL injection; 100 μg L^{-1} metabolite stock solution); (ii) triazine mixture (total of five pesticides) overlayed with chromatogram of target analytes as a point of reference (500 μL injection; 100 μg L^{-1} pesticide stock solution). Triazine chromatogram digitised from a paper chromatogram.

metabolite 2-hydroxyatrazine (Fig. 6(i)); this was observed for both the monolithic and packed columns. The other triazines analysed did not co-elute under the described conditions.

4. Conclusion

The analytical performance of two HPLC columns (a conventional packed column and monolithic column) with UV–vis detection at multiple wavelengths were established and compared. It was observed that both columns were able to separate the tested analytes well with sufficient resolution and peak asymmetry, but they differed significantly in analysis time and operating pressure. It was found that the monolithic column was superior in terms of reduced analyte retention times and lower operating backpressure, while limits of detection were slightly better using the packed column. The variation in detector wavelength from 220, 230 and 244 nm was also investigated; it was found that 230 nm was the ideal wavelength for concurrent detection of all three target analytes.

Atrazine, simazine and hexazinone in MilliQ water were concurrently detected in under 3 min per sample using large-volume direct injection HPLC with limits of detection of 5.7, 4.7 and 4.0 μg L^{-1}, respectively, without pre-concentration (validated using LC–MS). The advantage of the described system over more traditional methods and methods described within the literature are three-fold: firstly, large-volume direct injection of the sample enables lower detection limits without preconcentration; secondly, the use of monolithic column significantly reduces the time for analysis of each sample, along with the subsequent affect of limiting operating pressures and flow rates associated with packed columns; thirdly, the use of a low cost detector (in relation to more sophisticated detectors; *i.e.*, MS) using a single wavelength reduces the total cost of analysis, enabling more samples to be analysed.

Cross validation of samples analysed by LC–MS/MS indicated good correlation with samples spiked in the 10–50 μg L^{-1} range, with correlation coefficients of better than 0.9965, supported by statistical analysis. Samples spiked at relatively low concentrations of 0.1–2.0 μg L^{-1} required preconcentration by SPE. Linear regression of the results from the two systems in the double blind experiment correlated well but recoveries were poorer in the lower concentration range, particularly for hexazinone from which we only recovered 52%, suggesting some losses during SPE. Recoveries of 96 and 91% were obtained for atrazine and simazine respectively in the lower concentration range.

Analysis of natural waters showed that various concentrations of DOC from 3.1 to 11.7 mg L^{-1} had no significant affect on the resolution or separation capacity of the described HPLC method. However, while there is some potential for 'like' compounds to co-elute as shown in the case of 2-hydroxyatrazine, the method developed provides a fast, simple, cheap alternative to LC–MS/MS for multi-analyte detection of triazines with similar detection limits, and as such would be an excellent inexpensive screening method.

Acknowledgments

This research was funded by Water Quality Research Australia (formally the CRC for Water Quality and Technology) and Melbourne Water. The authors wish to extend their appreciation to Dr R. Considine and Dr N. Crosbie (Melbourne Water) for guidance, financial support and providing samples. The authors also wish to acknowledge and thank Mr A. Pellegrini and Ms A. Stenta (SGS Australia) for the analysis of water samples by LC–MS/MS and assisting with the blind study.

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