A multi-component method to determine pesticides in surface water by liquid-chromatography tandem quadrupole mass spectrometry

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Abstract

Pesticide pollution of surface water is a major concern in many agricultural catchments. The development of rapid and accurate methods for determining pesticide concentrations in water samples is, therefore, important. Here we describe a method for the simultaneous analysis of six pesticides (metaldehyde, quinmerac, carbetamide, metazachlor, propyzamide and pendimethalin) in natural waters by direct aqueous injection with liquid chromatography-tandem mass spectrometry. The method validation showed good linearity from 0.2 to 50.0 µg L\(^{-1}\) with correlation coefficients between 0.995 to 0.999. Method accuracy ranged from 84 to 100% and precision (RSD) from 4 to 15%. The limits of detection for the targeted pesticides ranged from 0.03 to 0.36 µg L\(^{-1}\). No significant matrix effects on quantification were observed (\(t\) test). The method was tested on water samples from a small arable catchment in eastern England. Peak concentrations for the determinands ranged from 1 to 10 µg L\(^{-1}\).

Key Words

Pesticide pollution, surface water, direct injection, LC-MS/MS
1. Introduction

Agriculture is generally considered to be the greatest contributor to pesticide pollution in many ground and surface waters, although in some catchments runoff from hard surfaces may be locally important [1]. Pesticide monitoring is a challenging task because a high number of active ingredients is typically used in agricultural catchments with mixed land use (presenting a wide range of physico-chemical properties) which are applied at different times of year and at different rates. This means that several different analytical methods may need to be employed on a single sample in order to detect the compounds of interest. The challenges of detecting target compounds can also be exacerbated by the episodic nature of pesticide transport from land to water (which tend to occur predominantly during storm events) [2]. Hence, high sampling frequencies may be required to capture representative temporal patterns, which results in significant analytical costs.

Most methods for pesticide analysis at the low concentrations generally encountered in natural water bodies require a sample pre-concentration step such as solid phase extraction (SPE), solid phase micro-extraction, or liquid-liquid extraction. Of these techniques, SPE is most commonly employed because it often provides good sample extraction, concentration and clean up[3][4]. However, there are several disadvantages with this technique including potential for low recoveries, long processing times per sample, the high cost of SPE cartridges and differing extraction procedures for different classes of pesticide owing to their polarities.

As an alternative, direct aqueous injection (DAI) methods have been developed for the analysis of a wide range of pesticides in various sample matrices. Applications include analysis of polar organophosphorus pesticides in fruit and vegetables [5][7] and analysis of pesticides in potable water[8][9]. The main advantages of DAI are easy sample preparation/manipulation, low consumable costs and reduced analysis time allowing high sample throughput as well as low limits of detection.

In this paper, we describe a DAI multi-component method for the determination of six pesticides by LC-MS/MS in environmental waters. The specific requirements of the method were to be accurate and rapid so as to allow the efficient processing of a large number of samples. The pesticides analysed were metaldehyde, quinmerac, metazachlor, carbetamide, propyzamide and pendimethalin. Molecular structures and relevant physico-chemical properties are listed in Table 1. With the exception of pendimethalin, all the compounds examined have organic carbon-water partition coefficient ($K_{oc}$) values less than 217 L kg$^{-1}$. 
which suggests that they will be moderately mobile in soil and, hence, prone to leaching losses. All six pesticides are widely used in arable agriculture in Europe and have been previously detected at concentrations of concern in UK water bodies [2][10]. Metaldehyde is a particular problem for the UK water industry and has been responsible for the highest number of compliance failures in recent years [10][11]. It is a selective molluscicide which is widely used to control slugs and snails in several crops. It is only moderately mobile ($K_{oc} = 240 \text{ L kg}^{-1}$) and has been observed to degrade in water-sediment interface with a median dissipation time ($DT_{50}$) of 12.2 days (Table 1) which should, in principle reduce the risk of leaching loss from soil.

Quinmerac is used to control *Galium aparine*, *Veronica* spp and other broad leaved weeds in cereals, oil seed rape and sugar beet. Carbetamide and propyzamide are herbicides used to control black grass infestations predominantly in oil seed rape [12]. Metazachlor and pendimethalin are also herbicides used to control grass and broad-leaved weeds in a range of crops including oil seed rape and Brussel sprouts [12]. Pendimethalin is not expected to be particullary mobile and was included to provide a contrast to the other more mobile compounds.

There are few published papers that report on the analysis of more than one of our target pesticides. In general, these protocols only included 2 or 3 pesticides at the most with fruits and vegetables being the studied matrices. Analysis in food stuffs requires an extraction step before any determination can take place. A popular method is QuEChERS which includes SPE followed by LC-MS/MS. Pesticides detected by this method include metazachlor, pendimethalin and quinmerac [13][14][15]. Others used homogenisation followed by evaporation or supercritical fluid extraction as the extraction step followed by GC-MS or GC-NPD (Nitrogen, Phosphorus Detection). Pesticides detected following these methods included carbetamide, propyzamide and pendimethalin [1][15]. Other protocols dealt with several of our target pesticides in water samples, namely carbetamide, metazachlor, propyzamide [14] metazachlor and pendimethalin [15]. These protocols involved SPE followed by LC-MS and GC-MS retraspectively, although the method by Irace-Guigand (2004) required additional UV-DAD detection.

Of the six target pesticides, metaldehyde appears to be one of the more difficult compounds to detect in complex samples containing several analytes. To the best of our knowledge, no method has been previously reported for the combined rapid determination of these particular six pesticides with minimal sample preparation approach in environmental water samples.
The method improves upon existing knowledge in order to produce a robust value analytical tool in which minimal sample preparation is needed to monitor pesticide concentrations from agricultural runoff.

**Table 1.** Physico-chemical properties for the pesticides considered in this method.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Type</th>
<th>Molecular mass (g mol⁻¹)</th>
<th>Chemical structure</th>
<th>Chemical formula</th>
<th>DT₅₀ (days)¹</th>
<th>KOC (L kg⁻¹)²</th>
<th>Log Kow³</th>
<th>Solubility (mg L⁻¹)⁴</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaldehyde</td>
<td>Molluscicide</td>
<td>176.21</td>
<td><img src="image" alt="Metaldehyde structure" /></td>
<td>C₈H₁₆O₄</td>
<td>5.1</td>
<td>12.2</td>
<td>11.5</td>
<td>240</td>
<td>0.12</td>
</tr>
<tr>
<td>Metazachlor</td>
<td>Herbicide</td>
<td>277.75</td>
<td><img src="image" alt="Metazachlor structure" /></td>
<td>C₁₄H₁₆ClN₃O</td>
<td>8.6</td>
<td>20.6</td>
<td>216</td>
<td>54</td>
<td>0.03</td>
</tr>
<tr>
<td>Propyzamide</td>
<td></td>
<td>256.13</td>
<td><img src="image" alt="Propyzamide structure" /></td>
<td>C₁₂H₈Cl₂NO</td>
<td>47</td>
<td>94</td>
<td>21</td>
<td>840</td>
<td>0.002</td>
</tr>
<tr>
<td>Quinmerac</td>
<td>Herbicide</td>
<td>221.6</td>
<td><img src="image" alt="Quinmerac structure" /></td>
<td>C₁₁H₈ClNO₂</td>
<td>30</td>
<td>179.4</td>
<td>88.7</td>
<td>86</td>
<td>0.039</td>
</tr>
<tr>
<td>Carbetamide</td>
<td></td>
<td>236.27</td>
<td><img src="image" alt="Carbetamide structure" /></td>
<td>C₁₂H₁₆N₂O₃</td>
<td>12.4</td>
<td>55.5</td>
<td>9.1</td>
<td>89</td>
<td>1.78</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td></td>
<td>281.21</td>
<td><img src="image" alt="Pendimethalin structure" /></td>
<td>C₁₃H₁₉N₃O₄</td>
<td>90</td>
<td>16</td>
<td>4</td>
<td>17581</td>
<td>5.2</td>
</tr>
</tbody>
</table>

¹DT₅₀ – Median dissipation time in different test systems; ²KOC – organic carbon-water partition coefficient (L kg⁻¹); ³Log Kow – octanol-water partition coefficient; ⁴Solubility in water (mg L⁻¹) [16]
2. Experimental

2.1. Chemicals and reagents

Pesticide standards were purchased from QMX laboratories (UK), methanol (HPLC grade) and acetic acid (HPLC grade) were obtained from Sigma-Aldrich (UK). Ultra pure water was produced by PURELAB® ultra, Elga.

2.2. Standards and stock solutions

Pesticide stock solutions (100 µg L\(^{-1}\)) were prepared by dissolving the neat pesticides in methanol. Working standards were prepared by diluting with ultra pure water with concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 8.0 and 10.0 µg L\(^{-1}\) for each pesticide. All standards were stored at 4 °C for a maximum of one month.

2.3. Instrumentation

All analyses were performed with a Waters Alliance 2695 liquid-chromatography system coupled to a Quattro premier XE tandem quadrupole. A Kinetex C18 column (5µm 150 × 2.1 mm, Phenomenex, UK) thermostated at 60 °C was used for chromatographic separation. The flow rate was 0.3 mL min\(^{-1}\) and the injection volume was 50 µL. The mobile phase consisted of ultra-pure water with 0.1% acetic acid (A) and methanol with 0.1% acetic acid (B). The elution started at 10% B and was linearly increased to 98% over 12 min, then maintained for 3 min before returning to the intital composition. The total time of analysis per sample was 18 min.

Operating conditions of the mass spectrometer were optimized by infusion of each individual pesticide at a concentration of 1 mg L\(^{-1}\) in a solution of 70% A and 30% B. Electrospray ionization (ESI) was performed in positive mode. The mass spectrometer was operated under multiple reaction monitoring (MRM) with two reactions monitored for each analyte (Table 2), with the exception of metaldehyde, which forms a Na\(^+\) adduct and its fragmentation [M+Na]\(^+\) showed a reaction whose precursor and fragment ions were \(m/z\) 198.9 and \(m/z\) 66.9, respectively. The UK Environment Agency recommends this reaction for quantitative purposes [12].
Table 2. SRM transitions used for target compounds.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>1st transition – quantification</th>
<th>2nd transition – confirmation</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percursor ion (m/z)</td>
<td>Product ion (m/z)</td>
<td>cone</td>
</tr>
<tr>
<td>Metaldehyde</td>
<td>198.9</td>
<td>66.9</td>
<td>25</td>
</tr>
<tr>
<td>Quinmerac</td>
<td>222.3</td>
<td>204.3</td>
<td>30</td>
</tr>
<tr>
<td>Carbetamide</td>
<td>237.1</td>
<td>191.9</td>
<td>15</td>
</tr>
<tr>
<td>Metazachlor</td>
<td>278.1</td>
<td>133.8</td>
<td>15</td>
</tr>
<tr>
<td>Propyzamide</td>
<td>256.0</td>
<td>189.9</td>
<td>15</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>282.1</td>
<td>212.0</td>
<td>25</td>
</tr>
</tbody>
</table>

2.4. Sample collection and Analysis

The method was tested on samples collected from a monitoring study in a small headwater stream at Hope farm in Knapwell, Cambridgeshire, UK (Figure 1). The stream drains a low relief catchment (elevation range 41-78 m above mean sea level) of approximately 3.9 km², which is dominated by arable land.
The predominant crop rotation is wheat-oil seed rape and most of the soils belong to the Hanslope Soil Association, which is a typically under-drained. Stream discharge is low (but usually perennial) in summer, which suggests minimal baseflow contributions and is flashy in winter with flows often exceeding 150 L s\(^{-1}\) during storm events. The stream was monitored for five months between August and December 2014. Discharge was measured with a 90° v-notch weir, equipped with an ISCO AV2150 water level and a velocity sensor. Samples were collected with an ISCO 6712 automatic water sampler at constant sampling intervals of 8 h, with a sample volume of 250 mL. Sample bottles were changed approximately every 7 days and replaced with fresh bottles which had been thoroughly pre-cleaned before each change-over using water and methanol. Pesticide concentrations in field bottle blanks, prepared with ultra pure water, were always less than the limits of detection (LOD) and often not detectable.
Samples were refrigerated immediately upon arrival to the laboratory (typically less than 2 h after sample collection) and filtered through 0.2 μm syringe-mounted disc filters (Milipore Millex™, Fisher Scientific, UK) within 24 h of collection.

2.5. Sample injection and data processing
Sample runs consisted of eight working standards, followed by five unknown samples with solvent blanks and continuing calibration checks (5 μg L⁻¹) in between. Runs never exceeded 80 determinations including analytical standards, blanks, calibration checks and samples. Peak areas of target pesticides were obtained with Quantlynx v.4.1. Weighted (1/x) linear least-squares regression curves were fitted to the observations and not forced through the origin.
3. Results and discussion

Figure 2 shows an example total ion chromatogram (TIC) for the six pesticides in positive ion mode analysed over 18 min from a 10 µg L\(^{-1}\) standard of each pesticide in ultra-pure water.

3.1. Optimisation of the MS/MS parameters.

For the MS operation, only ESI in positive mode was evaluated for the determination of the six pesticides. The optimum cone voltage and collision energies are reported in Table 2. Good peak shape and suitable signal-to-noise ratios were obtained with a dwell time of 0.25 s.
3.2. Optimisation of the LC conditions

Optimisation of mobile phase composition and elution gradient was very important to achieve good separation, high sensitivity, good ionization and resolution, particularly for trace analysis. Results (see example in Figure 2) showed that higher sensitivity and good peak shape could be achieved with 0.1% acetic acid in both eluents. The gradient was optimised to obtain improved resolution and shorter analysis time.

3.3. Validation procedures

The analytical method was validated according to the performance criteria established by ICH guidelines [20]. The validation parameters evaluated were linearity, accuracy, precision, LODs, limits of quantification (LOQs) and matrix effect.

3.3.1. Linearity

Method linearity was evaluated by analysing the response for the seven concentration levels prepared from the working standard solution described in Section 2.2 (0.2, 0.5, 1.0, 2.0, 5.0, 8.0 and 10 µg L\(^{-1}\)). Linear regression analysis of calibration data was performed by plotting the peak areas of the quantitative ion versus the corresponding standard concentrations. Good linearity was achieved with coefficients of determination between 0.994 to 0.999 (Table 3). The method provided acceptable precision, accuracy and linearity over the range of 0.2 to 10.0 µg L\(^{-1}\).

3.3.2. Accuracy and Precision

Inter-day and intra-day accuracy and precision (RSD) were assessed. Inter-day comparisons express within laboratory across-day variations while intra-day comparisons express within laboratory within-day variations. The intra-day test consisted of five consecutive analyses, while the inter-day variations were assessed on different days for a 5 µg L\(^{-1}\) standard. Intra-day precision (RSD) varied from 17.4% (pendimethalin) to 3.1% (metaldehyde), while the inter-day precision varied from 11.4% to 24.3% (pendimethalin). Intra and inter-day accuracy values were close to 100%.
3.3.3. Detection and Quantification limits

Limits of detection (Equation 1) and quantification (Equation 2) were calculated using the standard deviation of the response and the slope, as described by ICH validation of analytical procedures [17]:

\[
LOD = 3.3 \times \frac{\sigma_R}{m} \\
LOQ = 10 \times \frac{\sigma_R}{m}
\]

where \( \sigma_R \) is the standard deviation of the response and \( m \) is the slope of the calibration curve.

The standard deviation of the response was calculated from the standard deviation of y-intercepts in the regression lines fitted to the data. Limits of detection and quantification ranged from 0.05 to 0.3 µg L\(^{-1}\) and 0.2 to 1.0 µg L\(^{-1}\), respectively (Table 3).

Table 3. Calibration curves, coefficient of determination (r\(^2\)), limit of detection (µg L\(^{-1}\)) and limit of quantification (µg L\(^{-1}\)).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration curve</th>
<th>( r^2 )</th>
<th>LOD (µg L(^{-1}))</th>
<th>LOQ (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Intercept</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaldehyde</td>
<td>2219.7 ± 15.3</td>
<td>168.9</td>
<td>0.9998</td>
<td>0.09</td>
</tr>
<tr>
<td>Quinmerac</td>
<td>2489.1 ± 17.3</td>
<td>45.9</td>
<td>0.9998</td>
<td>0.08</td>
</tr>
<tr>
<td>Carbetamide</td>
<td>5524.8 ± 33.9</td>
<td>289.9</td>
<td>0.9998</td>
<td>0.09</td>
</tr>
<tr>
<td>Metazachlor</td>
<td>11302 ± 47.1</td>
<td>584.1</td>
<td>0.9999</td>
<td>0.09</td>
</tr>
<tr>
<td>Propyzamide</td>
<td>4544.5 ± 72.9</td>
<td>628.3</td>
<td>0.9987</td>
<td>0.05</td>
</tr>
<tr>
<td>Pendimethalin</td>
<td>4636.1 ±154.8</td>
<td>223.7</td>
<td>0.9944</td>
<td>0.3</td>
</tr>
</tbody>
</table>
3.3.5. Matrix effects

To assess the matrix effect the slopes of the calibration curves for ultra-pure water (1) and stream water (2) were compared using a Student’s $t$ test (95%). The calculated value of $t$, $t_{cal}$, is defined by:

$$t_{cal} = \frac{|b_1 - b_2|}{\sqrt{S_{b1}^2 - S_{b2}^2}}$$  \hspace{1cm} (3)

where $b$ is the slope of the calibration line and $S_b$ is the deviation of the slope.

The null hypothesis (there is no significant difference between the two calibration lines) was rejected when $t_{cal}$ was greater than the theoretical value $t_{theo}$ 2.306 ($p = 0.05$). Values of $t_{cal}$ ranged from 0.5 to 1.3 for the different pesticides so that no significant matrix effect was found. After approximately 80 samples, the mass spectrometer sensitivity was observed to gradually decrease over time, probably because of deposition and accumulation of salts on the cone surface. Analytical controls were used to identify when this problem occurred. When sensitivity reduced by 15%, the run was interrupted and maintenance was carried out.

3.3.6. Blanks

Ultra-pure water and methanol were used as solvent blanks during method validation and field sample analysis. No carryover or system peaks were found. Additionally, target analytes were undetected in field blanks.

4. Applications of the method

Data for stream discharge and stream water concentrations of the six pesticides analysed in water samples collected from the study stream are shown in Figure 3, between August and December 2014. Daily rainfall data are also displayed. Pesticide concentrations tended to increase sharply during rainfall events with the highest concentrations typically occurring in the first storm event after application. This is consistent with observations reported elsewhere from catchments with under-drained heavy clay soils [2]. The highest concentrations were observed for metaldehyde over an event in late August which triggered a relatively low hydrograph peak. For quinmerac, which is applied later than metaldehyde, the first peak concentrations occur in an event around the 13th of October. Metaldehyde concentrations also increase in this event but with lower peaks. Other notable increases in concentration...
occur for carbetamide in a series of hydrographs starting on the 14th of November and for propyzamide in the event of the 11th of December, which also resulted in increases in pendimethalin concentrations. Both propyzamide and carbetamide tend to be applied a little later than some of the other herbicides due to the specific requirements of weed control timing for blackgrass on oilseed rape. Concentrations of metazachlor were consistently low, peaking at 0.37 µg L⁻¹ on the 29th of October. The magnitude of peak concentrations will reflect a combination of factors including usage rate and the physico-chemical properties of the compound. Compounds with high values of $K_{OC}$ (such as pendimethalin) will tend to bind to soil solids and hence have a lower propensity to leach than compounds which are more hydrophilic (such as metazachlor, quinmerac and carbetamide). For most compounds, peak concentrations were observed at the same time as the hydrograph peak or slightly after the peak flow (i.e. on the falling limb of the hydrograph), although apparent delays in the appearance of peak pesticide concentration may be artefacts of the relatively low sampling frequency adopted (8 h).

Concentrations for all the pesticide compounds examined tended to decrease in hydrograph recession periods in parallel with falling flow. Again, this is consistent with previous observations of pesticide behaviour during storm events [2]. Clearly, peak concentrations of all six pesticides were periodically greater than the maximum admissible concentration for drinking water. Although this stream is not directly abstracted for water supply, it does feed into the River Great Ouse system, which is used for municipal abstraction downstream. The important point to note for the purposes of this paper is that the temporal pattern and magnitude of observed concentrations is consistent with expectations under the environmental conditions experienced over the study period.
Figure 3. Rainfall (top panel), stream discharge (right axis) and pesticide concentrations (left axis) in the Hope Farm stream from August to December 2014.
5. Conclusions

An LC-MS/MS method for the simultaneous multi-residue analysis of six pesticide active ingredients in natural waters is presented in this paper. This DAI method is rapid and accurate and can be used for quantification and confirmation of metaldehyde, quinmerac, carbetamide, metazachlor, propyzamide and pendimethalin in water samples from ground and surface waters. The omission of a concentration and clean-up step means that sample processing is fast and straightforward. The method showed a good range of linearity ($R^2$ ranged from 0.995 to 0.999), accuracy (84 to 100%) and RSD precision (4 to 15%) and there was negligible apparent matrix effect compared to the same pesticides in ultra-pure water.

The LOQs obtained ranged from 0.2 to 1.0 µg L$^{-1}$. This is acceptable for detecting concentrations in natural water samples from many agricultural catchments where pesticide concentrations are high (edge of field concentrations often exceed 100 µg L$^{-1}$) but would be of limited value in assessing DWD compliance. The use of a multi-residue method with rapid and simple sample preparation reduces analysis time and improves laboratory efficiency. The temporal pattern and magnitude of concentrations in samples from a headwater arable stream were consistent with expectations for the environmental conditions experienced over the study period, suggesting that the method can yield a realistic description of pesticide exposure in natural waters.

Acknowledgements

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6. References


