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Analysis of glyphosate and glufosinate by capillary electrophoresis–mass spectrometry utilising a sheathless microelectrospray interface

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Abstract

The potential of capillary electrophoresis combined with mass spectrometry for the simultaneous determination of two herbicides (glyphosate and glufosinate) and their metabolites (aminomethylphosphonic acid and methylphosphinicopropionic acid) as the native species is demonstrated utilising a simple microelectrospray interface. The interface uses the voltage applied to the CE capillary to drive separation and generate the electrospray, avoiding sample dilution associated with the use of a sheath liquid interface. The chemistry of the internal walls of the capillary has a marked influence on peak shape, and appropriate choice is essential to successful operation of the interface. A linear polyacrylamide coated capillary, which has no electroosmotic flow, gave best reproducibility, with precision of migration time and peak area in the range 1–2 and 7–12% RSD, respectively, for the four analytes. Limits of detection, low-pg on-column, are substantially better than for previous methods and calibration curves over the range 1–100 μM have R^2 values greater than 0.97. The observed concentration limit of detection for glyphosate in water is 1 μM and for a water–acetone extract of wheat is 2.5 μM, allowing the underivatised herbicide to be detected at 10% of the maximum residue limit in wheat.

Keywords: Interfaces, CE–MS; Instrumentation; Glyphosate; Glufosinate; Pesticides; Aminomethylphosphonic acid; Methylphosphinicopropionic acid

1. Introduction

Glyphosate (GLYP) and glufosinate (GLUF) are two of the world's most widely used non-selective herbicides. Although they are not considered to present a major risk to human health, recent evidence suggests that exposure may cause neurological disorders [1,2]. The Food and Agriculture Organization (FAO) of the United Nations has set a maximum residue limit (MRL) of GLYP in wheat at 5 mg/kg [3] and, although GLUF does not have a specific MRL in wheat, it is generally necessary to be detectable at concentration around one order of magnitude lower than GLYP in most other crops [3]. Thus, precise and accurate methods to quantify these residues at levels encompassing and extending above the MRLs are required to ensure that limits are not breached. Methods for analysing GLYP, GLUF and their metabolites, aminomethylphosphonic acid (AMPA) and methylphosphinicopropionic acid (MPPA) (structures shown in Fig. 1), include liquid chromatography (LC) [4–9], gas chromatography (GC) [10–16] and capillary electrophoresis (CE) [17–20]. These analytes have no UV chromophore, making simple UV detection impossible. Because of this, most methods of analysis developed thus far

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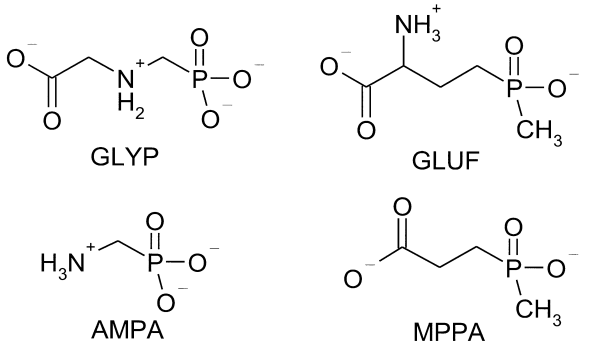

Fig. 1. Structures of GLYP, GLUF, AMPA and MPPA in their ionic forms dominant in aqueous solution at pH 6.3.

employ derivatisation procedures to confer volatility, fluorescence or UV absorption properties to the analytes, adding time, cost and analytical uncertainty to the assay. A method for analysing underivatised GLYP and AMPA using CE with indirect UV detection has been reported [17], but was found to lack specificity and showed poor response with real samples. An isotachophoresis method capable of analysing all four compounds without derivatisation has also been reported, though the use of conductivity detection may preclude its application for complex matrices [20]. Notably, previous CE studies have utilised untreated fused-silica capillaries, as is the norm for the majority of CE methods due to the high electroosmotic flow (EOF) produced in such capillaries.

Mass spectrometry (MS) has the potential to be a rigorous direct detection method for these compounds, particularly in their ionic states. Since the initial application of electrospray ionization (ESI) as an interface for capillary electrophoresis–mass spectrometry (CE–MS) [21,22], the technique has been utilised for the analysis of a wide range of compounds including biopolymers [23], pharmaceuticals [24,25] and agrochemicals [26,27]. The standard CE–ESI-MS coupling technique utilises a sheath liquid [21,22]. The CE capillary outlet sits inside an electrically grounded stainless steel needle within the ES source, and a conducting liquid is pumped between the capillary and the needle so that the capillary outlet is grounded. When a voltage is applied across the CE capillary the current travels in the direction of the MS, spraying the analytes into the detector. A sheath gas can be pumped around the outside of the needle to focus the electrospray and aid desolvation of the analyte ions. A drawback of this technique is that the capillary eluent is continually diluted by the sheath liquid, reducing the MS response. Alternative systems replace the sheath liquid by applying a conductive coating to the CE capillary tip. Although this approach improves response, conductive coatings are often difficult to fabricate and have limited lifetimes [28,29]. Van der Greef and co-workers [30,31] developed a microelecrospray interface which has no physical contact between the capillary outlet and a ground state electrode. The electrical contact is achieved by placing the capillary tip ~1 mm away from the MS inlet and applying a voltage to the injection end of the capillary. Under these conditions the voltage serves the purpose of generating the electrospray and producing the field required to drive the electrophoretic separation [30]. The resulting electrospray aids desolvation and carries the current across the small air junction between the CE capillary outlet and the MS inlet which is at ground potential. A limitation of the technique is that only low concentration, high resistivity background electrolytes (BGEs) may be used. If the BGE concentration is too high, electrical discharges occur, seen as a blue/purple glow between the end of the CE capillary and the MS inlet, and the analytes are not detected. This report describes the development of a reproducible CE–MS method for analysing GLYP, GLUF, AMPA and MPPA as the native species.

2. Experimental

2.1. Instrumentation

Experiments were carried out using a SpectraPhoresis Ultra CE instrument (Thermo Finnigan, San Jose, CA, USA) coupled to a Finnigan MAT LCQ ion-trap MS detector (Thermo Finnigan) via the CE–MS interface described below. Fused-silica capillaries (Composite Metal Services, Hallow, UK) of 60 cm×75 μ*M* I.D. were used for separation.

Sheathless interface CE–MS was performed by completely removing the CE–MS interface supplied with the LCQ and using a device manufactured in

house, adapted from a design described in detail by Mazereeuw et al. [30]. A polyetheretherketone (PEEK)–glass fibre (30% reinforced) composite material (RS Components, Corby, UK) was used to fabricate the outer case of the device (Fig. 2), designed to fit over the end of the heated capillary inlet to the MS. The outer case contains a short PTFE sleeve through which the CE capillary is passed. The capillary outlet tip was tapered by holding a section of a length of capillary in a butane flame and pulling laterally with tweezers. The end was cut to produce a tip ~15 μm I.D.: there may be variations in diameter between capillaries. The interface was manufactured from a PEEK–glass fibre composite rather than Delrin [30] due to the higher maximum working temperature of the former. The capillary can be moved horizontally or vertically using four alignment screws (also made from the PEEK–glass fibre composite), which grip the PTFE sleeve, for accurate positioning of the capillary; for optimal response the capillary should aim directly into the MS orifice. Notably, the sheath liquid interface supplied with the LCQ does not have the facility to manoeuvre the capillary horizontally or vertically, meaning the capillary tip is fixed in these two dimensions. Movement in the axial direction is achieved using a micrometer attached to the capillary at the rear end of the device. For optimisation of axial positioning of the tip the capillary was filled with a solution containing the analytes in methanol–water (50:50, v/v), positioned ~1 cm from the MS inlet and −20 kV applied. Movement of the capillary tip towards the MS resulted in a steady increase in analyte ion current from 6 to 2 mm distance from the heated capillary, reaching a plateau within the last 2 mm from the MS inlet. This was followed by a sudden disappearance of the ion current due to shorting as the tip entered the heated capillary inlet to the MS system. For optimum response the capillary was positioned within the ion current plateau region, ~1 mm from the heated capillary.

When using the LCQ for atmospheric pressure chemical ionization (APCI) or ESI, an interlock ensures that high voltages on the corona discharge or electrospray needle are not exposed if the source is opened. When activated, the interlock disables volt-

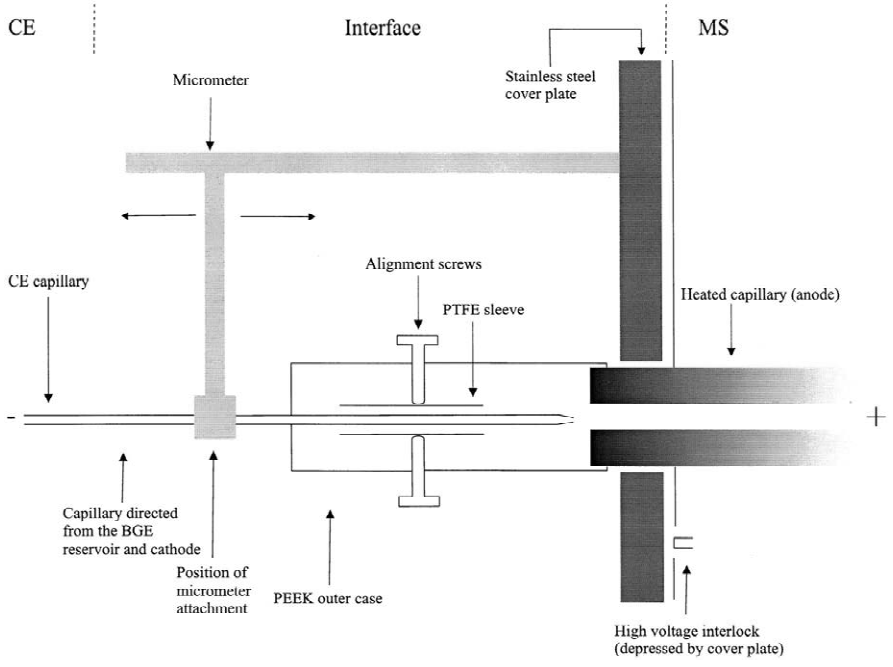

Fig. 2. Schematic diagram of the sheathless microelectrospray interface. Alignment screws allow capillary tip to be positioned horizontally and vertically whilst micrometer allows lateral movement. A cover plate attached to the front of the LCQ depresses the interlock and holds the micrometer in place.

ages within the MS, giving a loss of signal. As the microelectrospray system described in this paper does not have an ionisation source requiring high voltage supply by the MS, the interlock is not required. However, caution must be taken as the CE capillary is still at high potential, the value of which is controlled by the CE instrument. The interlock was depressed using a cover plate (stainless steel), made in-house, attached to the front of the LCQ by fixing screws. The plate also served to hold the micrometer onto the mass spectrometer (see Fig. 2).

2.2. Reagents

Glufosinate was purchased from Qm_x (Saffron Walden, UK) and methylphosphinicopropionic acid from Riedel–de Haën (Seelze, Germany). All solvents were of HPLC grade and were purchased from Fisher Scientific (Loughborough, UK). All other chemicals were obtained from Sigma (Poole, UK) or Aldrich (Poole, UK). Electrolytes and standards were prepared using ultrapure 0.45-μm filtered water with a resistivity of 18.2 MΩ from an Elgastat UHQII system (Elga, High Wycombe, UK).

2.3. Capillary coating

Silica capillaries were coated with linear polyacrylamide (LPA) using the method described by Baba et al. [32] to produce a neutral inner wall. To create a capillary with a positively charged capillary wall, the method of Bateman et al. [33] was utilised to provide a [3-(methacryloylamino)propyl]trimethylammonium chloride (MAPTAC) coating. In both cases a Harvard Apparatus syringe pump (Holliston, MA, USA) fitted with a 25-ml Hamilton plastic syringe was set up to pump the reagents through the capillary at 0.1 ml/min.

2.4. Capillary conditioning and use

Prior to use, the capillaries were conditioned daily using various wash steps performed at 100 p.s.i. (1 p.s.i.=6894.76 Pa). Underivatised silica capillaries were conditioned by washing for 1 min each at 60 °C with 1 *M* NaOH, 0.1 *M* NaOH and water, allowing 5 min between each wash step. The capillary was then washed for 1 min at 30 °C with BGE. After use the capillary was washed for 1 min with water at 30 °C. Before use, LPA-derivatised capillaries were washed first with water and then with BGE for 1 min each at 30 °C, allowing 5 min between each wash. After use the capillary was washed for 1 min with water at 30 °C. MAPTAC-derivatised capillaries were washed for 10 min with BGE at 30 °C before use.After use, the capillary was washed for 5 min with water at 30 °C then purged with nitrogen for 5 min.

Unless otherwise stated, all separations were carried out using the same run cycle; capillary wash for 1 min with water then 1 min with BGE, sample injection for 5 s at 3 p.s.i. (70 nl), separation by pressurising at 2 p.s.i. in the direction of the MS whilst applying a potential of −20 kV.

2.5. Extraction of wheat sample

Wheat (5 g) was mixed with water (5 ml) for 30 min using a magnetic stirrer. Acetone (5 ml) was added to the wetted wheat and the mixture was stirred for 1 h. The solids were allowed to settle and the liquid was decanted and vacuum-filtered using a Buchner funnel. The filtrate was spiked to produce a final solution containing each of the analytes at the specified concentration.

2.6. Conductivity and pH measurement

The pH of BGEs and wheat extracts were measured using a Corning ion analyzer 150 (Medfield, MA, USA) calibrated using aqueous buffer standards at pH 4 and 9. The pH values given for BGEs in methanol–water (50:50, v/v) mixtures cannot be regarded as the true pH, as this is restricted to measurement in purely aqueous media. However, as all BGEs have the same methanol:water ratio the values act as a relative guide to hydrogen ion concentration [34]. Conductivity measurements were carried out using a Jenway 4310 conductivity meter (Princeton, NJ, USA).

3. Results and discussion

3.1. Comparison of BGEs for use with the sheathless interface

The sheathless interface introduced by Mazereeuw

et al. [30] improves response due to the elimination of sheath liquid dilution. One advantage of a sheath liquid interface is the ability to add a volatile organic solvent to the capillary eluent, enhancing the stability of the electrospray. Accordingly, in this study, all BGEs used with the sheathless interface contained methanol (50%). BGEs with ionic concentration greater than 1 m*M* showed electrical discharges between the capillary tip and the MS inlet at an applied voltage of −20 kV, whilst those with a concentration less than 1 m*M* provided insufficient conductivity, gave poor peak shape and exhibited coelution.

Applied pressure can be used to maintain a constant flow from the capillary tip, resulting in a more stable electrospray [25]. At 0 p.s.i. (zero added pressure) the electropherogram showed pulsing of the ion current due to the cycle of the capillary tip being evacuated, refilled and evacuated again. The driving pressure was varied over the range 0–3 p.s.i. in steps of 0.5 p.s.i., and 2 p.s.i. was found to give the best response for all analytes.

CE experiments with indirect UV detection (data not shown) suggested that all four analytes have a net negative charge in an aqueous BGE of pH ≥5. To find the appropriate pH and BGE conditions for CE–MS analysis using a bare silica capillary, 1 m*M* solutions of ammonium acetate and ammonium formate were adjusted with the corresponding acid or base to give pH values of 4, 5, 6 and 7 in methanol–water (50:50, v/v). Each BGE was retained in the capillary for 5 min prior to analysis to allow the silanol groups on the capillary wall to equilibrate. Triplicate injections of a standard solution containing all four analytes at 25 μ*M* shows the sensitivity to pH of their response (Table 1). With the exception of AMPA, pH 6 gave the highest signal-to-noise ratio (*S*/*N*) and, therefore, response for acetate and for formate as BGE. AMPA exhibited the highest response at pH 7 with acetate BGE and no signal with formate BGE. Accordingly, ammonium acetate was chosen as the BGE for subsequent work. The optimum ammonium acetate BGE concentration was determined using solutions at concentrations 1, 2 and 5 m*M* in methanol–water (50:50, v/v), and the optimum pH for the BGEs using solutions at 0.2 pH unit intervals over the range 5.5–6.5. These were prepared by adjusting the pH of 1 m*M* acetic acid in methanol–water (50:50, v/v) with ammonia solution. Standard solutions containing all four analytes at 25 μ*M* were analysed in triplicate. The highest response was obtained for the BGE adjusted to pH 6.3; all future references to 1 m*M* ammonium acetate refer to 1 m*M* acetic acid, adjusted to this pH using ammonia solution, in methanol–water (50:50, v/v).

Table 1
Mean *S*/*N* (*n*=3) for CE–MS of GLYP, GLUF and metabolites analysed over the pH range 4–7 in 1 m*M* ammonium acetate and ammonium formate BGEs (methanol–water; 50:50, v/v)

BGE	pH	Mean *S*/*N*			
		GLYP	GLUF	AMPA	MPPA
Acetate	4	0.0	0.0	0.0	0.0
Acetate	5	2.9	4.3	2.5	2.9
Acetate	6	33.3	45.5	7.4	34.5
Acetate	7	9.5	6.1	9.1	40.0
Formate	4	0.0	0.0	0.0	0.0
Formate	5	10.5	7.1	0.0	14.3
Formate	6	33.3	22.2	0.0	52.6
Formate	7	9.1	6.7	0.0	20.0

The charge state of the analyte plays an important role in determining its electrophoretic mobility and separation. The pK_a values for GLYP are 0.8, 2.2, 5.4 and 10.2 [35] and for GLUF are 2.0, 2.9 and 9.8 [36]. There is some uncertainty in the exact pK_a values of AMPA with pK_{a1}=1.8, 2.4, pK_{a2}=5.4, 5.9 and pK_{a3}=10.0, 10.8 being cited [37]. There are no published data on the pK_a values of MPPA, although values supplied by Sirius Analytical Instruments (Forest Row, UK) are 2.5 and 4.5. Acid dissociation constants depend on solvent composition such that pK_a values typically increase between 0.8 and 1.2 pH units with a change from water to methanol–water (50:50, v/v) [38]. Thus, the dominant form(s) of the individual analytes at the nominal pH 6.3 in this solvent mixture are expected to be: GLYP −1, −2; GLUF −1; AMPA 0, −1; MPPA −2. Given that the analytes have different net charges, it appears that the charge state of the analyte is not the key factor responsible for the maximum response at pH 6.3.

3.2. Comparison of capillaries with negative, neutral and positively charged inner walls

Replicate analyses (*n*=20) using the bare silica capillary showed poor peak shape (Fig. 3a) and reproducibility in peak area and migration time (Table 2). A likely explanation is that the (negative)

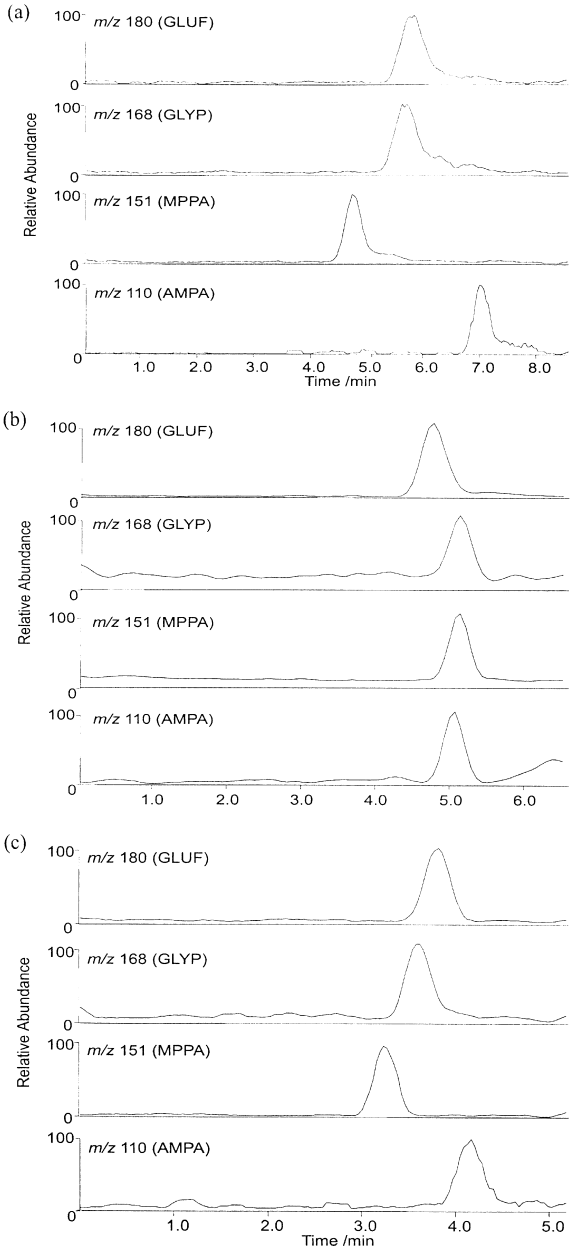

Fig. 3. Electropherograms at constant voltage (−20 kV) of a solution containing all four analytes at a concentration of 25 μ*M* injected for 5 s at 3 p.s.i. (a) Underivatised silica capillary; (b) MAPTAC-derivatised capillary; (c) LPA-derivatised capillary.

EOF operates in the opposite direction to the applied pressure necessary to achieve a stable flow in the sheathless microelectrospray interface. Possible solutions are to use capillaries with positive or zero EOF.

Capillaries with neutral and positively charged inner walls were prepared by derivatisation of the bare silica capillary (LPA and MAPTAC, respectively; see Section 2). The maximum response for all four analytes, in each capillary type, was at pH 6.3, indicating that charge state of the capillary walls is not the dominant factor in controlling response. The four analytes exhibited differing degrees of separation on each of the three capillaries (Fig. 3). In the case of the MAPTAC-derivatised capillary (Fig. 3b), the positive EOF resulted in short migration time and poor separation, necessitating use of a longer capillary. The difference in elution order from that observed with the bare silica capillary is attributed to retention of the analytes with double negative charge at pH 6.3 (GLYP and MPPA) via ionic interaction with the capillary wall. This was established using the sheath liquid interface set to produce a zero potential across the capillary. With the sheathless interface the MAPTAC-derivatised capillary gave poor reproducibility in peak shape and migration time, attributed to the EOF driving towards the MS, as variations in this flow would cause slight changes in the electrospray, introducing variability in migration time and peak area.

The LPA-derivatised capillary gave the best reproducibility in migration time and peak area, attributed to the removal of EOF and consequent reduction in peak tailing and analyte retention associated with the other two capillary types. Clearly, therefore, the charge state of the capillary wall is a crucial factor to consider when utilising this interface. The results indicate that the best performance is achieved by eliminating the EOF completely and providing a suitable applied pressure to maintain a stable electrospray, with electrophoretic separation based on analyte mobility alone. Based on these results it is clear that the MAPTAC-derivatised and underivatised capillaries have unacceptable RSDs in migration time and peak area and cannot be considered useful for the analysis of the herbicides. The LPA-derivatised capillary was used for all subsequent experiments described below.

3.3. Limit of detection (LOD) and dynamic range

Standards containing the four analytes were analysed to determine the concentration LOD (cLOD) of the method. GLYP, GLUF and MPPA were detect-

Table 2
Analysis of migration time and peak area reproducibility of CE–MS method for 25 μ*M* replicate samples ($n=10$) of glyphosate, glufosinate and their metabolites using capillaries with different internal derivatisation and charge states

Analyte	Bare silica capillary (negative)	MAPTAC coated capillary (positive)	Linear polyacrylamide coated capillary (neutral)
Migration time/min (RSD, %)			
GLYP	5.07 (6.6)	6.04 (17)	3.77 (2.0)
GLUF	5.19 (6.1)	5.42 (14)	3.90 (1.7)
AMPA	5.84 (8.6)	5.82 (16)	4.40 (1.9)
MPPA	4.19 (5.6)	5.85 (15)	3.43 (1.3)
Peak area/arbitrary units (RSD, %)			
GLYP	$3.0{\cdot}10^6$ (28)	$2.5{\cdot}10^6$ (49)	$3.1{\cdot}10^6$ (7.0)
GLUF	$2.2{\cdot}10^6$ (37)	$1.2{\cdot}10^7$ (24)	$4.1{\cdot}10^6$ (9.2)
AMPA	$0.4{\cdot}10^6$ (19)	$0.7{\cdot}10^6$ (22)	$0.5{\cdot}10^6$ (11)
MPPA	$5.5{\cdot}10^6$ (38)	$1.4{\cdot}10^7$ (32)	$6.9{\cdot}10^6$ (12)

able at a concentration of 1 μ*M* and from this observed value cLODs, calculated as three times the background noise, were determined as 0.4, 0.5 and 0.3 μ*M*. AMPA was observed at a concentration of 2.5 μ*M* giving a slightly poorer calculated cLOD (1.2 μ*M*) than the other three analytes. Corresponding (calculated) mass LODs under the same conditions are 3, 5, 6 and 9 pg on-column for MPPA, GLYP, GLUF and AMPA, respectively. Compared to the previous method for CE analysis of underivatised GLYP using indirect UV detection [16], which had calculated cLODs for GLYP and AMPA at 9 and 12 μ*M*, respectively, this method has greater than 20 times improved cLOD, and is 70 times below the concentration equivalent to the wheat MRL for the extraction procedure detailed in Section 2.

Six standards containing all four analytes over the range 1–100 μ*M* were analysed to determine the dynamic range and LOD for GLYP, GLUF, AMPA and MPPA using the sheathless interface system. One of the data points in the AMPA 100 μ*M* replicates was questionable, having a peak area of $2{\cdot}10^6$ arbitrary units (a.u.), whereas the other two points are approximately $3{\cdot}10^6$ a.u. As the data points at each concentration are independent of each other, a *Q*-test was performed on the residuals of all of the data points for the calibration (actual peak area minus the peak area calculated from standard line) to determine if a data point can be rejected. Q_{calc} was found to be 0.60, indicating that the data point may be rejected with 95% confidence [39]. Standard curves for the LPA coated capillary over the range 1–100 μ*M* (GLYP, GLUF and MPPA) and 2.5–100 μ*M* (AMPA) (Fig. 4) gave good calibration curves for a quadratic regression, with R^2 values of 0.98 for GLYP and GLUF and 0.99 for AMPA and MPPA. MPPA, with dominant charge state −2 under the conditions of the experiment, gave a linear calibration and the highest response of all of the analytes. The lowest response was for AMPA, consistent with a high proportion of the analyte being present in the neutral form at pH 6.3 and therefore not detectable by MS using the conditions and the interface described. The upward curvature of the standard lines is likely to be due to suppression of analytes by components of the running BGE, an effect which is more apparent at lower analyte concentrations. This is evident from loop injections into a stream of ammonium acetate; as the ammonium acetate concentration increases the MS signal of the analytes decreases. Although the standard curves fitted up to 100 μ*M*, the peaks at this concentration showed significant tailing, indicating sample overload. Concentrations higher than 500 μ*M* showed severe overloading, with the peaks becoming broad and flat-topped. Considerable peak distortion is expected when working with a BGE of 1 m*M*, comparable to the concentration of the analytes at highest loading. A sample containing all four analytes at 500 μ*M* will have a total concentration of 2 m*M*, twice that of the BGE. In this instance the electric field in the analyte plug is lower than in the BGE, and destacking occurs upon application of the voltage, resulting in an increase in the effective injection plug length by a factor of ~2 and the observed rectangular peak shape [40].

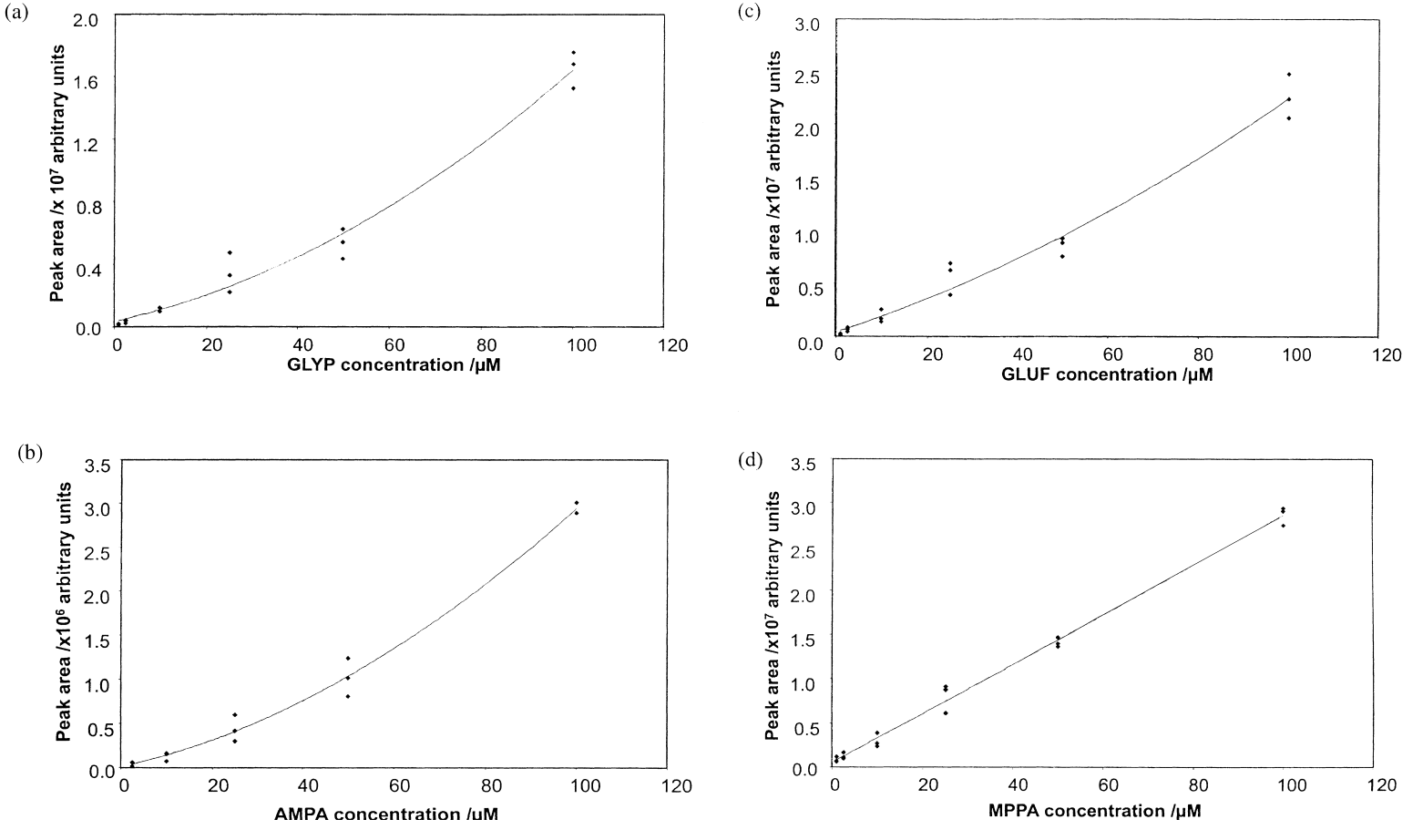

Fig. 4. Standard curves for (a) glyphosate, (b) glufosinate, (c) AMPA and (d) MPPA over the range 1–100 μ*M* (*n*=3).

3.4. Wheat extract

Initial attempts to analyse wheat extracts filtered and spiked with the four analytes employed water as the extraction medium as used previously [17]. Under these conditions the analytes were not detectable using the CE–MS method described in Sections 3.1–3.3. Conductivity and pH values were determined for a range of extraction media and their corresponding wheat extracts (Table 3). Extract conductivities were generally very high when compared to the BGE conductivity (52 μS). The water–acetone extraction described in Section 2.5 gave a wheat extract conductivity which was a factor of 50 lower than with water alone, presumed to be due mainly to a lower extraction efficiency for proteins and salts. The conductivity was, however, almost twice that of the BGE, resulting in poor peak shape due to destacking and failure to resolve the analytes (Fig. 5a). To overcome this the BGE concentration was increased to 5 m*M*. CE is usually performed at a constant voltage so that the migration velocity of an analyte remains constant. To prevent electrical discharges associated with use of a high BGE concentration, separation was carried out at a constant current (1.5 μA). Under these conditions, satisfactory peak shapes were obtained (Fig. 5b) though analysis time increased (Fig. 3b) as a result of the lower voltage (−8 increasing to −11 kV) over the run compared with −20 kV for the 1 m*M* BGE.

Three of the analytes (GLYP, GLUF and MPPA) were detectable in the water–acetone extracts and their ions appeared at the usual m/z values. No ion corresponding to AMPA (m/z 110) was detected. It was considered likely that AMPA, the only one of the analytes for which there is a substantial proportion of the neutral form at pH 6.3 in 50% methanol, may have ionised by capture of an anion present in the wheat extract to give a negatively-charged adduct with m/z >110. Anzalone et al. [41] determined chloride levels in the range 0.5–0.9 g/kg of crop for a wheat sample. To examine possible adduct formation, a standard solution of 10 mg/ml ammonium chloride containing AMPA at a concentration of 50 μ*M* was analysed using the CE–MS method described for wheat extracts. The mass-selected electropherogram (Fig. 6a) revealed a low intensity peak at 5.26 min in the m/z 146 trace but nothing in the m/z 110 trace; the increased migration time indicates the binding of an anion to AMPA, thus increasing its mass and lowering its mobility. The m/z 146 ion was, however, too weak to obtain a full mass spectrum. To overcome this, 5 μl of the solution was loop-injected directly into a 0.4 ml/min stream of methanol infusing into the MS system under electrospray conditions. Although the resulting signal was still weak, a mass spectrum obtained by averaging over three scans showed the expected 3:1 ratio in m/z 146 and 148 due to chlorine isotopes (Fig. 6b). However, the presence of a large peak at

Table 3
Conductivity and pH values of pre-extraction media and wheat extracts

Extraction medium		Conductivity (μS)	pH
Deionised water	Pre-extraction	4	6.48
	Post-extraction	5460	6.53
50% MeOH (aq)	Pre-extraction	2	6.65
	Post-extraction	1190	6.68
1 m*M* ammonium acetate in 50% MeOH	Pre-extraction	52	6.48
	Post-extraction	985	6.67
10 m*M* ammonium acetate in 50% MeOH	Pre-extraction	462	7.27
	Post-extraction	4430	6.73
25 m*M* ammonium acetate in 50% MeOH	Pre-extraction	1080	7.31
	Post-extraction	10 600	6.83
Water followed by an equal volume of acetone	Post-extraction	97	5.80

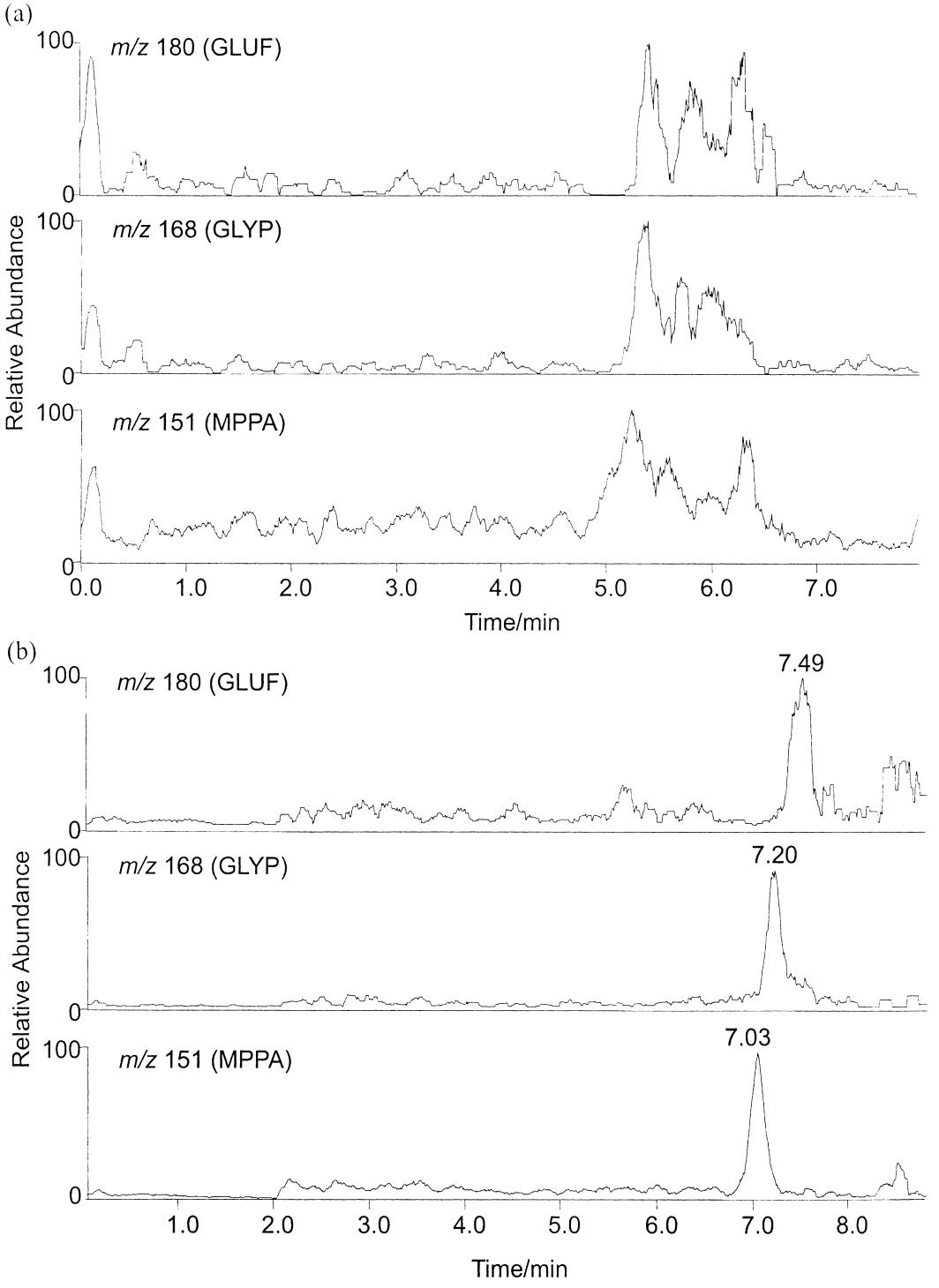

Fig. 5. Electropherograms at constant current (−1.5 μA) of milled wheat water–acetone extract spiked with GLYP, GLUF AMPA and MPPA to give a final concentration of 50 μ*M* for each analyte. 5 s injection at 3 p.s.i. (a) 1 m*M* BGE and (b) 5 m*M* BGE.

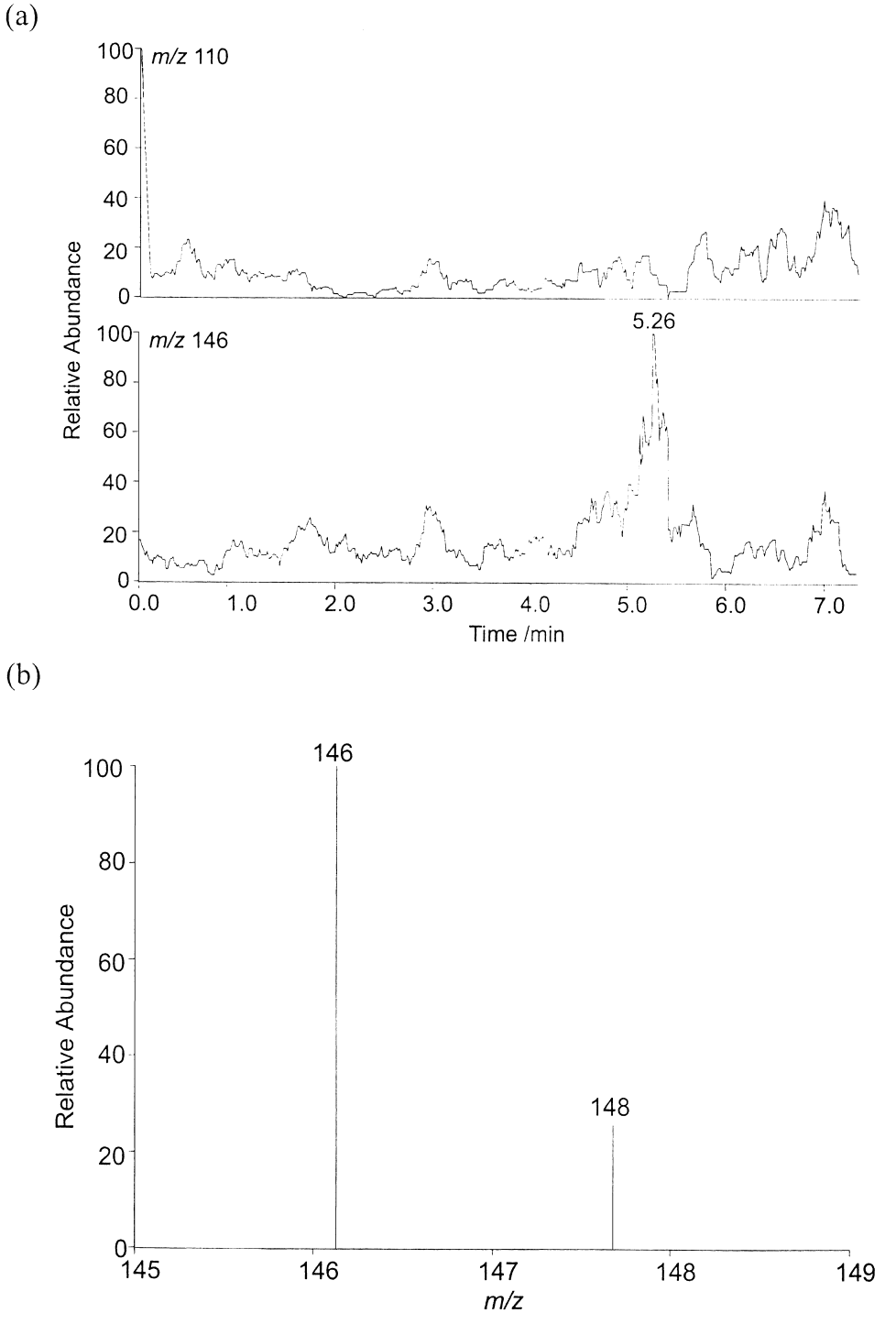

Fig. 6. Analysis of 50 μ*M* AMPA in water containing ammonium chloride at 10 mg/ml; (a) electropherogram of sample analysed by CE at constant current with 5 m*M* BGE (b) mass spectrum of loop injected sample.

m/z 146 in wheat extract, not due to $[AMPA+Cl]^-$, means that AMPA would have to be quantified using the m/z 148 isotope, leading to a poorer LOD.

The two herbicides were observed at a concentration of 2.5 μ*M* in wheat extract. This represents a major improvement over the indirect UV detection CE method [17], where the high conductivity of the matrix precluded analysis of GLYP in spiked wheat extract at any concentration. Use of the water–acetone extraction procedure described here requires detection of GLYP at 28 μ*M* to meet the MRL. Thus, this method allows, for the first time, detection of GLYP in its native form in a wheat extract, and at a concentration greater than one order of magnitude below the MRL. Although there is no MRL for GLUF in wheat in the FAO database, the herbicide would still be detectable at 10% of the GLYP MRL.

Notably, the 100 μ*M* upper limit of the dynamic range is not a major problem in the analysis of analytes in wheat extract, as it is extremely unlikely that wheat extracts will contain the analytes at concentrations greater than this. If there are instances where this occurs, the extract should be diluted to give a sample concentration of less than 100 μ*M*.

4. Conclusion and general comments

This work demonstrates that modification to the sheathless interface introduced by Mazereeuw et al. [30] makes it suitable for CE–MS analysis of GLYP, GLUF, AMPA and MPPA using a combination of electrical and pressure drive. The limitation of this device is set by the need to avoid discharges between the capillary tip and the MS inlet, and operationally the upper limit to the current is ~2 μA. An appropriate ammonium acetate concentration is 1 m*M*, considerably lower than is the norm for typical background electrolytes in CE. This restricts the range of acceptable sample matrices, since the conductivity of the matrix should be less than or equal to that of the BGE in order to provide acceptable peak shapes. BGEs at concentrations higher than 1 m*M* can be used, but the voltage applied for the CE separation should be reduced accordingly. The interface has advantages over typical sheath liquid interface systems in that there is no analyte dilution by the sheath liquid, and, in the case of the LCQ, allows positioning of the capillary tip in three-dimensions.

The interface gives good reproducibility in migration times and peak area when used in conjunction with an LPA-derivatised capillary, and the consideration of capillary performance characteristics has been shown to be critical. GLYP and GLUF were readily detectable in aqueous solutions at 1 μ*M*, thus achieving a level of detection substantially better than that required to meet MRLs and representing more than a 20-fold improvement on the best current method for underivatised GLYP [17]. Analysis of samples from spiked wheat extract shows that use of acetone as a component of the extract medium removes many interfering ions, permitting detection of GLYP down to 2.5 μ*M*, less then 10% of the MRL for the herbicide in wheat. Thus, the technique described represents the most sensitive method for analysis of native GLYP, GLUF, AMPA and MPPA, and is the only method capable of detecting the native analytes in wheat extract. The interface has also been used successfully in the analysis of peptides and organoarsenic compounds, demonstrating the wide range of potential applications for this device.

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