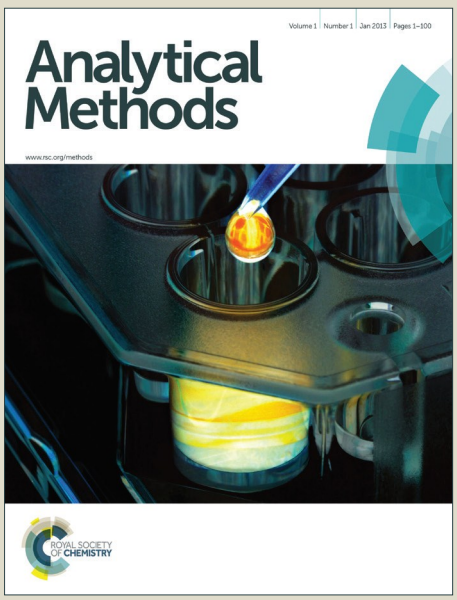
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

ARTICLE

Direct analysis of herbicides by paper spray ionization mass spectrometry

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Steven L. Reeber,[a] Sneha Gadi,[a] Sung-Ben Huang,[b] Gary L. Glish*[,a]

Measurement of herbicide residues in environmental matrices is typically performed using liquid or gas chromatography coupled to mass spectrometry, generally with one or more stages of sample processing or purification prior to analysis. Paper spray ionization enables the rapid mass spectrometric analysis of such samples without the use of chromatography or sample cleanup techniques. Samples are applied to a paper strip and dried, after which they may be stored or transported. By applying solvent and a high voltage to the paper strip, the analyte is extracted from the paper and ionized by electrospray from the tip of the paper strip. Qualitative and quantitative measurement of triazine herbicides and the chloroacetanilide herbicide metolachlor are demonstrated using samples spiked into water and crop extracts at part-per-billion concentrations. The linear dynamic range includes the U.S. statutory maxima for atrazine in crops and human health hazard levels in water, as well as E.P.A. levels of concern and regulatory limits for metolachlor in crops and water.

Introduction

Water monitoring and other widespread screening programs represent a critical tool for ensuring safe drinking water, protecting natural resources, and assessing the impact of herbicide use on our environment. However, conventional methods of water analysis require large volumes of liquid water samples (typically >10 mL per site, per collection, often as much as 1 liter) to be transported from field collection sites to the analytical laboratory at significant expense.[1–6] The cost of transporting liquid samples is particularly problematic for water monitoring programs targeting a wide area. An alternative to bulk liquid sample collection is collection on an absorbent medium such as paper. The sample may then be dried and shipped at reduced cost.

Analysis of samples collected on paper substrates may be performed by conventional extraction-liquid chromatography-mass spectrometry procedures, but this entails additional time and sample preparation steps. Alternatively, ambient ionization techniques may be employed to ionize the sample directly from the paper. A wide variety of ionization techniques have been developed over the past decade, beginning with desorption electrospray ionization (DESI) in 2004[7] and "direct analysis in real time" (DART) in 2005[8] and continuing to include dozens of other methods including plasma, spray, acoustic nebulization, and laser-based techniques, among others.[9,10]

Several of these techniques have been demonstrated for analysis of pesticides, including DART,[11] DESI,[12,13] low temperature plasma ionization (LTPI),[14] and more unorthodox techniques such as leaf spray[15] or liquid extraction surface analysis (LESA).[16] Most of these techniques, however, are used for analysis of samples on relatively non-porous surfaces (e.g., the surface of produce) or from liquid samples.

Paper spray ionization is a simple and robust ambient ionization technique used for analysis of samples applied to paper substrates. This technique has been demonstrated for the analysis of a wide range of analyte classes, such as illicit drugs,[17] amino acids,[18] protein complexes,[19] and therapeutic drugs.[20–23] Paper spray is compatible with very complex matrices including urine,[24] blood,[21–23,25] biological tissue,[26] and foodstuffs[27–30] with little or no sample processing or purification, conditions which generally produce significant interference in conventional electrospray ionization or atmospheric pressure chemical ionization.[17]

Paper spray ionization functions by generating an electrospray from the pointed tip of a wetted piece of paper. The spray is driven by the application of a high voltage to the paper, producing an intense electric field at the sharp tip, from which charged droplets are emitted.[31] This is a "soft" ionization technique, depositing little internal energy into the ions generated and causing minimal fragmentation. Paper spray ionization is an electrospray-based technique, and generally produces protonated or deprotonated molecules as observed in electrospray. The ionization mechanism is a combination of electrospray and field ionization or corona discharge processes, depending on the abundance of solvent and electric field intensity at the tip.[32]

[a] Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill NC, 27599. Email: glish@unc.edu
[b] Syngenta Crop Protection LLC, Greensboro NC, 27409.
Electronic Supplementary Information (ESI) available: Analyte structures, instrument parameters, QC sample measurements, calibration curves in alternative matrices, and equations of linear fits for calibration curves are available in PDF format. See DOI: 10.1039/x0xx00000x

Paper spray has been employed for the direct analysis of several types of fresh samples, including foods, using the paper tip as a spray emitter.[17,20,21,30] The use of paper spray for analysis of dried samples, where the paper substrate is used for both sample collection and ionization, has focused on biological samples such as dried blood spots and dried urine samples.[22,24,33] Applications of dried sample paper spray to food or agriculture analysis include investigation of coffee samples for origin discrimination[28] and detection of azo dyes in chili peppers and anti-inflammatory compounds in olive oil.[29,34] The use of paper spray ionization for measurement of part-per-million levels of fungicides in fruits has recently been demonstrated using both a wiping technique and by applying a homogenate to the paper and drying.[35]

Collection of water samples for paper spray analysis is trivial, requiring only that a set volume (50 or 100 µL) be applied to a paper strip. The paper may be dried under ambient conditions and then packaged for transport by simply placing it in a plastic bag. As noted above, transportation of these dried samples would be much less costly and difficult than shipping samples for conventional methods, which typically call for collection of significantly larger sample volumes.[3–6] Paper spray of dried samples also involves minimal sample handling in the laboratory. Internal standards are applied to the paper strips, dried, and then the strips are analyzed without additional liquid handling or sample preparation.

Paper spray ionization has several other advantages for analysis of environmental samples. It is immune to clogging, eliminating the need for filtration of samples containing dispersed solids. Even dense suspensions may be analyzed by paper spray; the only consideration is how effectively the analyte will be eluted and ionized. This may make paper spray a viable technique for analysis of herbicide formulations for regulatory or quality control purposes, as these frequently contain particulate matter. Once in the laboratory, analysis is rapid and straightforward, requiring no separation techniques and only approximately two minutes of mass spectrometer time per analysis. All steps relating to preparation of the paper are carried out prior to application of the sample. The ion source described herein is modular and may be implemented on most mass spectrometers designed for atmospheric pressure ionization techniques such as ESI or atmospheric pressure chemical ionization. Alternatively, paper spray has been demonstrated in conjunction with a portable mass spectrometry for *in situ* analysis.[36]

Paper spray has the potential to serve as a complementary tool to conventional LC-MS methods, enabling rapid, low cost, targeted analyses with virtually no sample processing. In this paper we present a method for the detection and measurement of two representative herbicides of different classes by dried sample paper spray mass spectrometry and a brief discussion of the potential of this technique for environmental applications.

Experimental

Chemicals

Environmental matrices (ground water, lake water, soil extracts, and crop extracts) and herbicide standards were provided by Syngenta Crop Protection, LLC (Greensboro, NC). The structures of the analytes used in these experiments are shown in Figure S1 in the supporting material. Crop extracts were prepared by homogenization of 10 g of crop sample using a Polytron homogenizer, followed by extraction with 200 mL of 80/20 acetonitrile/water. Soil extracts were prepared by extraction from 20 g of soil sample with 200 mL of 80/20 acetonitrile/water. LC-MS grade acetonitrile (Fisherbrand Optima Acetonitrile) and glacial acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Deuterated atrazine (ethyl-d_5) was purchased from C/D/N Isotopes (Pointe-Claire, QC, Canada). Deuterated metolachlor (propyl-d_6) and deuterated propazine (isopropyl-d_6) were purchased from Crescent Chemical (Islandia, NY).

The purity of the organic solvent used for paper spray was observed to impact the background ions detected and measurement reproducibility. Even LC-MS grade methanol (Fisherbrand Optima Methanol) generated undesirable background signal in some cases. The presence of organic impurities in high grade methanol has been previously reported in experiments using atmospheric pressure chemical ionization and photoionization.[37–39] Use of LC-MS grade acetonitrile instead of methanol was observed to reduce background signal substantially.

Paper Selection

Whatman 903 paper was used for all analyses. This paper is a standard dried blood spot collection paper used in neonatal testing, similar to the Whatman #31 ETF paper used in several paper spray experiments using blood samples.[21,22] Several paper types were evaluated for use in these experiments, including Whatman #1 chromatography paper, #903 dried blood spot paper, and #3, #4, #40, #41, #43, and #598 filter papers. The ease of preparing suitable paper strips and the quality of the mass spectra generated by paper spray were best for the #903 dried blood spot paper and the #598 filter paper.

The main factors observed to be relevant to selection of paper for paper spray ionization experiments are the tip and edge quality, durability, and sample capacity. Thinner and harder papers, such as #40 filter paper, tend to yield better tip quality, whereas softer papers such as #1 chromatography paper tend to fray, producing fuzzy tips and edges. However, thin papers are not capable of absorbing as large of sample volumes as thicker papers and are generally more easily damaged than thicker papers. #1 chromatography paper is a common choice for paper spray experiments using both fresh and dried samples,[25,27,31,33] and functions well when freshly cut.[40] However, it was observed to be fragile and thus likely to be less suitable for field experiments and larger sample volumes. Both the #598 filter paper and #903 dried blood spot paper are relatively durable and absorbent, and yield reasonably sharp tips when cut with scissors or a razor blade. Although the #598 paper tends to produce a slightly sharper

tip, #903 paper was selected for these experiments as its greater sample capacity allows for greater sample volumes, increasing signal intensity.

Sample Preparation

Whatman #903 filter paper was cut into strips for sample collection using a razor blade and template. Strips were 4 cm by 1 cm with a 1 cm triangular taper at one end, and approximately 0.50 mm thick (±0.03 mm). This tip configuration yields a tip angle of approximately 53°, which represents an acceptable compromise between the higher signal intensities reported with larger angles and the lower spray voltages required at smaller angles.[20]

Washing paper strips with spray solvent prior to addition of analyte to the strips was observed to reduce the intensity of many background ions by an order of magnitude or more. This effect has been previously reported,[41] and is particularly beneficial in work with charge-limited mass analyzers, such as quadrupole ion traps. The origin of the background ions eliminated by this technique is not known, but we hypothesize that they are due to residual chemical contamination from manufacturing, packaging, or transportation of the paper.

Paper strips were washed three times on each side with approximately 1 mL LC-MS grade acetonitrile and dried at ambient conditions for at least 30 minutes prior to application of sample solutions. Herbicides were dissolved in environmental matrices and aliquots were diluted in the same matrix to the desired concentration (0.5 – 25000 ppb). A 50 or 100 µL aliquot of the sample solution was applied to washed, dried paper strips in the center of the strip, 1 cm from the tip. A diagram of the strip design is included in the supporting information (Figure S2). The strips were then allowed to dry under ambient condition. For quantitative experiments an aliquot of equal volume of a solution of an isotopically labeled internal standard in acetonitrile was then applied and allowed to dry for a minimum of 30 minutes. Strips were cut in half 2 cm from the tip and the pointed half inserted into a holder for analysis.

Ion Source Design

A custom paper spray ionization source was employed for all experiments. The source consists of a sample holder mounted on a three axis manual micrometer translation stage, a high voltage power supply connected to the sample holder, and a syringe pump used to deliver spray solvent to the paper strip. The sample holder, shown in Figure 1a, is made of two interlocking aluminum plates with a U-shaped prong at the front. The lower plate of the sample holder is mounted in a Teflon holder attached to the three axis stage. A paper strip is inserted for analysis between the upper and lower plates and the plates are clamped together using a wire clip. A stainless steel needle is attached to the upper plate such that its tip is in contact with the paper strip; spray solvent is delivered through this needle throughout the experiment, continuously replenishing the solvent on the paper. This enables extended experiments by allowing solvent to be continuously added until the analyte on the paper is exhausted. Even for part-per-billion concentrations of triazines, the analyte on a paper strip is not consumed for several minutes, well after a single aliquot of solvent would have been consumed. The assembled ion source, with a paper strip inserted, is shown in Figure 1b. This ion source is modular and can be coupled to most mass spectrometers designed for electrospray ionization or other atmospheric pressure ionization techniques.

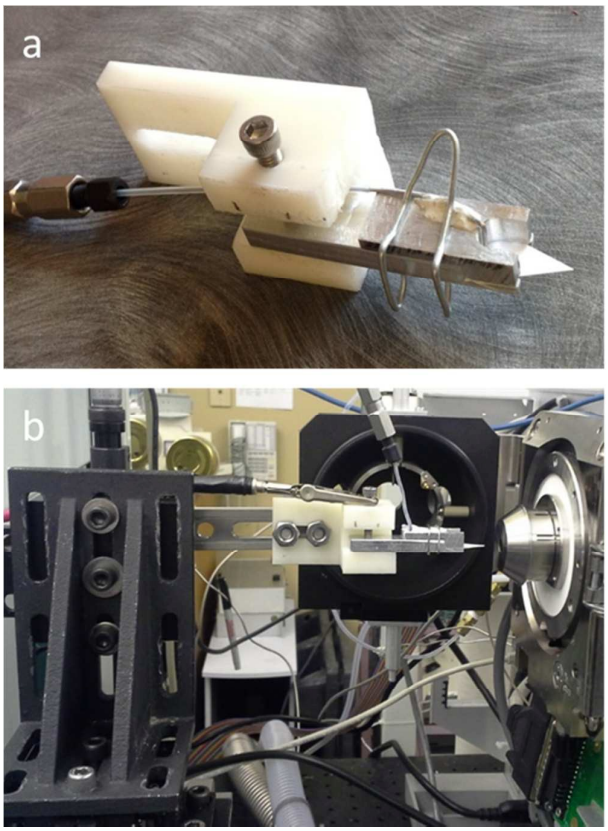


Figure 1. Custom paper spray ion source. a) Dismounted sample holder assembly. b) Complete paper spray ion source positioned at the inlet of a Bruker HCTultra mass spectrometer.

The current source design includes exposed high voltages on both the mass spectrometer inlet and the sample holder. This presents a risk of electric shock to the user; care must be taken to avoid contact with the sample holder and mass spectrometer inlet while the source is energized.

Instrumentation

Experiments were performed on a modified Bruker HCTultra ion trap mass spectrometer. The electrospray ion source was removed and the safety interlock overridden to allow operation with the custom paper spray ion source. Spray solvent was applied using a syringe pump at a rate of 15-35 µL/minute and a voltage (typically 3.5 kV) was applied to the sample holder using a separate power supply. The instrument's ESI desolvation gas (nitrogen) was set to a temperature of 300°C and a flow rate of 1.0 L/min. Voltages applied to the mass spectrometer inlet and ion optics were optimized for each analyte using the automated optimization tool included with the instrument control software. A table of

instrument parameters is available in the supporting information (Table S1).

Results and Discussion

Triazine Herbicides

The triazines comprise a class of synthetic herbicides commonly used for the protection of corn and other crops from broadleaf weeds and grasses. Atrazine is one of the most commonly used herbicides in the United States. The U.S. Environmental Protection Agency (USEPA) limits for triazine herbicides and their metabolites in crops range from 50 ppb in guava to 15 ppm in corn forage for animal feed.[42]

The triazine herbicides atrazine and propazine can be detected in water samples and soil and crop extracts at concentrations in the part-per-billion range using paper spray. Typical paper spray ionization mass spectra of water samples spiked with triazine herbicides and dried are shown in Figure 2. At part-per-million concentrations protonated atrazine and propazine are the dominant ions observed, at *m/z* 216 and *m/z* 230 respectively. A ubiquitous background species is observed at *m/z* 198 in both spectra. Triazine herbicides are also readily detected in more complex matrices, such as crop and soil extracts.

MS/MS of atrazine and propazine, as shown in Figure 3, yields primarily the loss of propylene (-42 Da) from the isopropylamino side chain. The propylene loss product from atrazine is observed at *m/z* 174 and from propazine at *m/z* 188. An ion due to the loss of both side chains (loss of two propylene molecules from propazine, loss of propylene and ethylene from atrazine) is detected in both cases at *m/z* 146. Atrazine and propazine may be detected using MS/MS at concentrations as low as 10 ppb (approximately 5 picomoles per 100 µL aliquot).

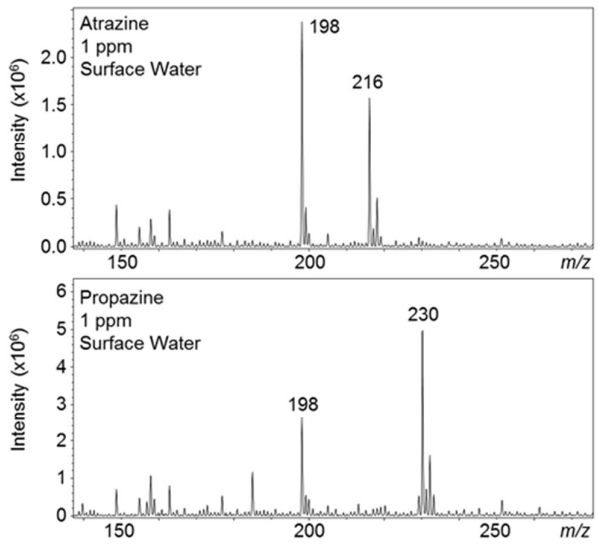


Figure 2. Paper spray mass spectra of 1 ppm atrazine/propazine in surface water. Protonated atrazine and propazine are observed at m/z 216 and 230, respectively, while an ubiquitous background ion is observed at m/z 198.

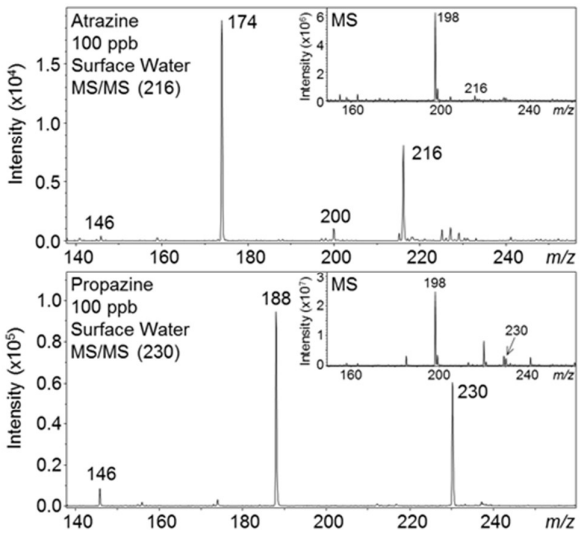


Figure 3: MS/MS spectra of triazine herbicides (Top: atrazine, Bottom: Propazine) in surface water at a concentration of 100 ppb. Mass spectra are inset.

MS and MS/MS spectra of atrazine and propazine in surface water at a concentration of 100 ppb are shown in Figure 3. The inset mass spectra indicate that the protonated triazine herbicide is a minor component in the mass spectrum, and in some cases is even difficult to observe visually in the mass spectrum. However, as the MS/MS spectra show, both herbicides are readily isolated and dissociated to yield their characteristic product ions. The presence of a background species isobaric to atrazine is indicated in the atrazine MS/MS spectrum by the presence of a product ion at *m/z* 200 which is not observed at higher atrazine concentrations. Because this species produces different product ions than atrazine it does not interfere directly with atrazine measurement, although if such ions are present in large quantities they may be a limiting factor in mass analyzers limited by charge capacity.

Metolachlor

Metolachlor is readily detected by direct paper spray ionization-mass spectrometry from dried samples on paper strips at sub-part-per-billion concentrations. Like the triazines, metolachlor is detected in positive mode as the protonated molecule, at *m/z* 284. MS/MS spectra of metolachlor samples at 1, 10, and 100 ppb concentrations in lettuce extract are shown in Figure 4. The sole product ion produced from MS/MS of protonated metolachlor is the methanol loss product at *m/z* 252, consistent with data from previously published methods for LC-MS/MS analysis of metolachlor.[43,44] At lower concentrations, near 1 ppb, the presence of isobaric background ions is evident due to product ions not derived from metolachlor, observed at *m/z* 248 and 266 in the MS/MS spectrum shown in Figure 4. As in the case of the triazine herbicides, the presence of an isobaric species does not directly interfere with measurement as different product ions are produced, confirmed using metolachlor-free control samples. Metolachlor can be detected using this method at concentrations as low as 100 ppt (35 femtomoles in a 100 µL aliquot).

Quantification of Herbicides

Quantification of herbicides may be performed using this method with the addition of a suitable internal standard. The signal intensity ratio for a ladder of herbicide concentrations is then used to generate a calibration curve. Several methods have been employed for the addition of internal standards to samples for paper spray ionization. Ideally, the internal standard would be added to the sample prior to any sample processing, and thus compensate for inefficiencies in extraction or transfer. One approach to preparing paper spray samples in this manner utilizes sampling capillaries pre-coated with internal standard.[45] This approach has the advantage of mixing the internal standard with the analyte before application to the paper, but requires that the analyte of interest be known at the time of application and is designed for very small sampling volumes (approximately 1 µL).[45] Additionally, if the samples are applied to paper strips in the field, this technique would require all relevant internal standards to be prepared and taken into the field with the technician (or farm hand) collecting samples. Internal standards may also be pre-applied to the strips and the analyte applied afterwards.[21,22] This avoids the need for internal standard preparation by the field technician, but still requires that the identity of the analyte of interest be known in advance. Both the coated capillary and pre-applied internal standard approaches involve transporting the internal standards (often expensive isotopically labeled compounds) into the field, with the attendant hazards due to non-ideal storage conditions and limited shelf life.

The following experiments were conducted using the most general approach, in which the internal standard is added to the paper strips after application of the sample. In the case of field collection, the technician need only apply the sample to the paper strip, allow it to dry, and ship the samples to the analytical lab. Internal standards may then be applied as needed for whatever analysis is desired.

Herbicide standards were dissolved in environmental matrices (surface water) to yield the desired concentration, applied to paper strips in 100 µL aliquots, and allowed to dry completely at room temperature in air (minimum drying time 30 minutes). After the samples were completely dry, a solution of isotopically labeled internal standard (atrazine-d_5, metolachlor-d_6, propazine-d_6 as appropriate) was applied in LC-MS grade water and allowed to dry completely before the samples were analyzed by paper spray mass spectrometry.

Unlike in LC-MS experiments, paper spray ionization with continuous replenishment of the spray solvent does not produce a discrete peak in time. Rather, the analyte is eluted from the paper over a period of several seconds to tens of minutes, depending on the quantity present. At the concentrations and aliquot volumes employed in these experiments, the analyte signal stabilized within a few seconds after the application of solvent and high voltage, and a nearly constant signal could be observed. Quantitative experiments were conducted by integrating the signal for two minutes beginning immediately after the signal was stable, analogous to a direct infusion experiment.

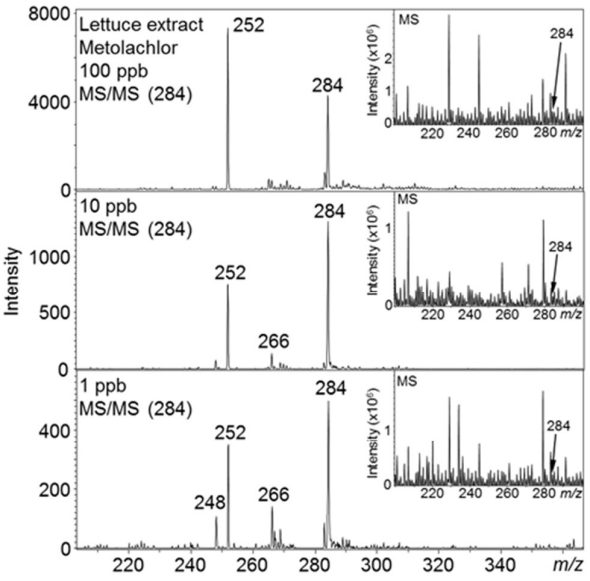


Figure 4. Lettuce extract containing 100 (top), 10 (middle), and 1 ppb (bottom) metolachlor. MS/MS spectra of protonated metolachlor (m/z 284) with mass spectra inset.

Samples were analyzed using MS/MS, switching between the analyte and internal standard after each scan. Collision induced dissociation was used for all experiments. To generate calibration curves, the ratio of the signal intensity for the dominant product ion from the analyte to the intensity of the dominant product ion from the internal standard was plotted against the analyte concentration. As shown in Figures 5 and 6, linear calibration curves for both atrazine and metolachlor can be readily constructed over two or more orders of magnitude. Due to the sequential application of the analyte and internal standard to the paper strip, a differential response is observed, reflected in the slope of the calibration curves. The second compound applied is observed with 1.5-2 fold greater intensity than the first compound applied. An order-of-application dependent response factor is observed consistently for multiple analytes, concentrations, and matrices. This has not been previously reported, and differs from a prior report of similar response factors for pre- and post-application of internal standards in the case of pharmaceuticals in dried blood spots.[33]

A preliminary investigation of signal intensity as a function of time did not indicate any significant changes due to storage on paper in dried form for up to one month, suggesting that this method may be viable for work with samples collected in the field and transported to the analytical laboratory after drying. However, additional study of suitable storage conditions is recommended prior to use in regulatory applications.

A calibration curve for quantification of atrazine in surface water is shown in Figure 5, ranging from 1 ppb to 750 ppb. Atrazine-d_5 (ethyl-d_5) was used as an internal standard at a

concentration of 250 ppb. A linear response is observed over the entire range, with increasing imprecision as the concentration of atrazine increases. Because the variability observed increases with the concentration of analyte, 1/x and $1/x^2$ weighted linear least-squares fits were investigated (see Table S3 for equations of linear fits). Quality control (QC) samples were tested at 3, 60, 150, and 400 ppb. QC results are tabulated in Table S2 in the supporting materials, showing results for the unweighted, 1/x, and $1/x^2$ weighted fits. Absolute error values are in the range of 1 ppb for all but the highest concentration QC samples, calculated using a $1/x^2$ weighting. Relative standard deviations (RSDs) of QC samples (n = 3) are below 15 % except for the lowest concentration (3 ppb). The limit of detection for atrazine, calculated as three times the standard deviation of the signal ratio in the blanks (n = 3) using $1/x^2$ weighting is 3.53 ppb. This limit is just above the concentration of the most dilute QC sample, explaining the high RSD for that measurement. However, this measurement is both precise and accurate between the limit of quantitation (five times the standard deviation in the blank, 9.78 ppb) and several hundred parts per billion, with accuracy and precision falling off at higher concentrations. The error at high concentrations may be remedied by the use of a higher concentration of internal standards; calibration curves for atrazine at concentrations up to 100 ppm in soil and crop matrices are included in the supporting material, along with calibration curves for another triazine herbicide, propazine (Figures S3 and S4).

While the limits of detection and quantitation for atrazine are greater than the USEPA maximum contaminant limits in drinking water,[46,47] they are below the USEPA health advisory limits for both 7 year and single day exposures for children (50 ppb and 100 ppb, respectively).[47] Coupled with the low cost, minimal sample processing requirements, and short analysis time, this suggests that this method may be suitable for rapid response analysis in the case of contaminated water supplies to ensure water is safe for short-term human or livestock consumption. Additionally, this technique may be suitable for measurement of atrazine in post-application runoff, where concentrations are likely to exceed the year-round average (to which the USEPA limits apply). This method involves collection of far less liquid than comparable conventional EPA methods, and requires significantly fewer liquid handling steps.[6,48]

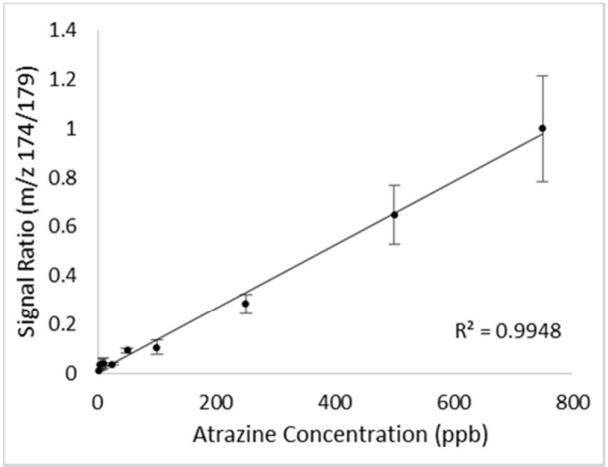


Figure 5: Calibration curve for quantitation of atrazine in surface water with unweighted linear least-squares fit.

The working range for this technique also includes the US regulatory limits for atrazine in crops (50-15000 ppb, depending on varieties),[42] and little impact on quantitation or signal intensity is observed when working with more complex matrices such as crop extracts. Since this method requires only the most rudimentary preparation from crop or soil samples (crude extraction, no filtration) it ought to be suitable for routine crop testing as well.

A direct comparison to infusion electrospray ionization using the same instrument was carried out using atrazine spiked into surface water samples. To each 200 µL surface water sample containing atrazine, 20 µL 500 ppb atrazine-d_5 in acetonitrile was added, along with 2 µL glacial acetic acid. The source gases were optimized manually to yield the most stable signal for protonated atrazine. Ion optics were optimized using the automated tuning method included with the instrument software. The limit of detection for atrazine in surface water using this technique was determined to be 30.3 ppb.

A similar calibration curve for metolachlor at concentrations from 100 ppt to 500 ppb in surface water is shown in Figure 6. A linear response is observed over the full concentration range. Metolachlor-d_6 was used as an internal standard at a concentration of 75 ppb. QC samples at 750 ppt, 15, 100, and 250 ppb concentrations were measured and used to evaluate the accuracy and precision of the calibration. Measured values, RSDs, and absolute and relative errors for QC samples (n = 3) are tabulated in Table S4 in the supporting material (equations for weighted and unweighted linear fits are listed in Table S5 in the supporting material). Error values are similar to those observed for atrazine, but are more consistent across the concentration range. Relative standard deviations, however are generally higher for metolachlor than atrazine.

Inter-day variation was assessed by analyzing three 250 ppb metolachlor QC samples over several days. The measured values (n = 3 for each day) were 195, 239, and 258 ppb with RSDs of 11.7 %, 15.9 %, and 18.9 % respectively. The inter-day variation (RSD = 14.1 %) is comparable to the intra-day variation, suggesting that the majority of the imprecision in these measurements is due to factors such as strip shape and edge variation or imprecise strip positioning.

The limit of detection for metolachlor, calculated in the same fashion as for atrazine, using the unweighted linear fit, is 1.38 ppb. The limit of quantitation is 1.70 ppb, calculated in the same manner (values calculated using the weighted fits are below zero due to the imperfection of the fit, although the 1/x weighting yields better accuracy overall for QC samples). These values are well below any relevant regulatory limits for metolachlor, such as the USEPA residue tolerances in crops and food commodities (20-20000 ppb)[49] as well as lifetime human health advisory limits for metolachlor in drinking water.[46]

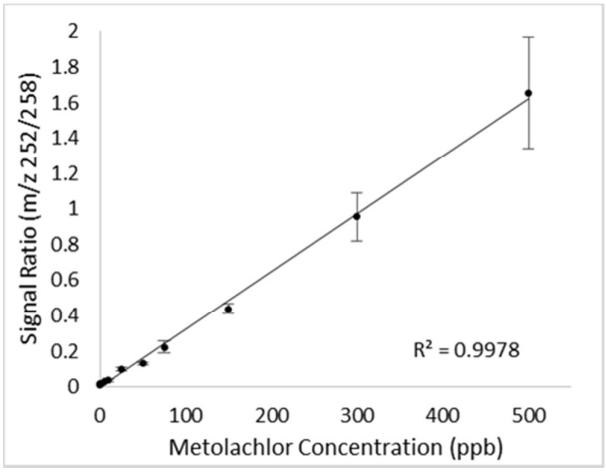


Figure 6. Calibration curve for quantification of metolachlor in surface water with unweighted linear least-squares fit.

The low limit of detection obtained by this method for metolachlor suggests that this technique ought to be suitable for most tasks with this analyte, though it is as yet too irreproducible for regulatory use. However, for routine investigative or other non-regulatory analysis of water, crop, or soil samples, or any other task where fast analysis, minimal sample processing, and low cost are important, paper spray ionization mass spectrometry appears to be a suitable tool for the measurement of metolachlor.

Conclusions

Paper spray ionization mass spectrometry presents an alternative to conventional LC-MS/MS for targeted analysis applications, eliminating the need for sample cleanup and preparation. It is suitable for sample collection in the field, where it has the potential to reduce the mass and volume of samples to be transported to the analytical laboratory, thus reducing costs. Initial results with triazine herbicides and metolachlor indicate that the quantitative measurement of these herbicide residues in environmental and agricultural matrices is feasible at regulatory levels. Additionally, current generation ion trap mass spectrometers are approximately an order of magnitude more sensitive than the Bruker HCTultra used in these experiments, which will likely enable measurement of residues at lower concentrations than achieved here. The robust, rapid, and low cost nature of paper spray ionization make it an attractive alternative for high volume tasks such as quality monitoring of pesticide sprays, analysis of herbicide-damaged crops, and other field collection tasks where low cost and rapid response are high priorities. For compounds with low limits of detection, such as metolachlor, paper spray may be a suitable technique for the monitoring of contaminated water in cases of runoff or spills, although reproducibility is not yet suitable for routine regulatory drinking water testing.

Paper spray ionization has, until recently, been a subject of primarily academic investigation, as it requires a custom designed ion source. However, recently the first commercial paper spray ion source, manufactured by Prosolia, Inc., has become available. These devices differ from the ion source employed in these experiments in several particulars, but the overall assay design should be readily adaptable to the commercial source. It is likely that the more precise paper preparation methods used with this source (laser cut paper mounted in single-use cartridges, rather than manual cutting with a razor) may provide improvements in reproducibility. The availability of a commercial source may enable routine use as it answers the problems of standardization and support that arise from the use of custom instrumentation. It remains to be seen if the new commercial design will prove cost-effective. Challenges remain in the application of paper spray ionization to environmental analysis – improvement is needed in both sensitivity and reproducibility – but its potential as a complement to conventional analytical techniques is excellent.

Acknowledgements

This research was supported by a grant from Syngenta Crop Protection, LLC.

References

1 Method 619: The Determination of Triazine Pesticides in Municipal and Industrial Wastewater, US EPA, 2007.
2 Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, US EPA, 2007.
3 M. M. Domino, B. V. Pepich and D. J. Munch, Method 523 : Determination of Triazine Pesticides and their Degradates in Drinking Water by Gas Chromatography / Mass Spectrometry (GC / MS), US EPA, 2011.
4 S. Huang, J. S. Stanton, Y. Lin and R. Yokley, Analytical method for the determination of atrazine and its dealkylated chlorotriazine metabolites in water using SPE sample preparation and GC-MSD analysis, *J. Agric. Food Chem.*, 2003, **51**, 7252–8.
5 J. A. Shoemaker and M. V. Bassett, Method 535: Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), 2005.
6 G. A. Smith, B. V. Pepich and D. J. Munch, Method 536: Determination of Triazine Pesticides and Their Degradates in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS), US EPA, 2007.
7 Z. Takats, J. Wiseman, B. Gologan and R. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, *Science,* 2004, **306**, 471–473.
8 R. Cody, J. Laramée and H. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, *Anal. Chem.*, 2005, **77**, 2297–2302.
9 X. Ding and Y. Duan, Plasma-based ambient mass spectrometry techniques: The current status and future prospective, *Mass Spectrom. Rev.*, 2014, DOI 10.1002/mas.21415.
10 M. Monge and G. Harris, Mass spectrometry: recent advances in direct open air surface sampling/ionization, *Chem. Rev.,* 2013, **113**, 2269–2308.

11 L. Wang, P. Zhao, F. Zhang, Y. Li and C. Pan, Direct analysis in real time mass spectrometry for the rapid identification of four highly hazardous pesticides in agrochemicals, *Rapid Commun. Mass Spectrom.*, 2012, **26**, 1859–67.
12 J. García-Reyes and A. Jackson, Desorption electrospray ionization mass spectrometry for trace analyis of agrochemicals in food, *Anal. Chem.*, 2008, **81**, 820–829.
13 N. Hagan and T. Cornish, Detection and identification of immobilized low-volatility organophosphates by desorption ionization mass spectrometry, *Int. J. Mass Spectrom.*, 2008, **278**, 158–165.
14 J. Wiley, J. García-Reyes and J. Harper, Screening of agrochemcals in foodstuffs using low-temperature plasma (LTP) ambient ionization mass spectrometry, *Analyst*, 2010, **135**, 971–979.
15 N. Malaj, Z. Ouyang, G. Sindona and R. G. Cooks, Analysis of pesticide residues by leaf spray mass spectrometry. *Anal. Methods*, 2012, **4**, 1913.
16 D. Eikel and J. Henion, Liquid extraction surface analysis (LESA) of food surfaces employing chip-based nano-electrospray mass spectrometry, *Rapid Commun. Mass Spectrom.*, 2011, **25**, 2345–2354.
17 J. Liu, H. Wang, N. E. Manicke, J.-M. Lin, R. G. Cooks and Z. Ouyang, Development, characterization, and application of paper spray ionization, *Anal. Chem.,* 2010, **82**, 2463–2471.
18 W. Liu, S. Mao, J. Wu and J. Lin, Development and applications of paper-based electrospray ionization-mass spectrometry for monitoring of sequentially generated droplets, *Analyst,* 2013, **138**, 2163–2170.
19 Y. Zhang, Y. Ju, C. Huang and V. H. Wysocki, Paper spray ionization of noncovalent protein complexes, *Anal. Chem.,* 2014, **86**, 1342–1346.
20 Q. Yang, H. Wang and J. Maas, Paper spray ionization devices for direct, biomedical analysis using mass spectrometry, *Int. J. Mass Spectrom.*, 2012, **312**, 201–207.
21 R. Espy, N. Manicke, Z. Ouyang and R. Cooks, Rapid analysis of whole blood by paper spray mass spectrometry for point-of-care therapeutic drug monitoring, *Analyst,* 2012, **137**, 2344.
22 N. E. Manicke, P. Abu-Rabie, N. Spooner, Z. Ouyang and R. G. Cooks, Quantitative analysis of therapeutic drugs in dried blood spot samples by paper spray mass spectrometry: an avenue to therapeutic drug monitoring, *J. Am. Soc. Mass Spectrom.,* 2011, **22**, 1501–1507.
23 Z. Zhang, W. Xu, N. E. Manicke, R. G. Cooks and Z. Ouyang, Silica coated paper substrate for paper-spray analysis of therapeutic drugs in dried blood spots, *Anal. Chem.,* 2012, **84**, 931–938.
24 A. Naccarato, S. Moretti, G. Sindona and A. Tagarelli, Identification and assay of underivatized urinary acylcarnitines by paper spray tandem mass spectrometry, *Anal. Bioanal. Chem.,* 2013, **405**, 8267–8276.
25 Q. Yang, N. E. Manicke, H. Wang, C. Petucci, R. G. Cooks and Z. Ouyang, Direct and quantitative analysis of underivatized acylcarnitines in serum and whole blood using paper spray mass spectrometry, *Anal. Bioanal. Chem.,* 2012, **404**, 1389–1397.
26 H. Wang, N. E. Manicke, Q. Yang, L. Zheng, R. Shi, R. G. Cooks and Z. Ouyang, Direct analysis of biological tissue by paper spray mass spectrometry, *Anal. Chem.,* 2011, **83**, 1197–1201.
27 A. Li, P. Wei, H.-C. Hsu and R. G. Cooks, Direct analysis of 4-methylimidazole in foods using paper spray mass spectrometry, *Analyst,* 2013, **138**, 4624–4630.
28 R. Garrett, C. M. Rezende and D. R. Ifa, Coffee origin discrimination by paper spray mass spectrometry and direct coffee spray analysis, *Anal. Methods,* 2013, **5**, 5944.
29 F. Mazzotti, L. Di Donna, D. Taverna, M. Nardi, D. Aiello, A. Napoli and G. Sindona, Evaluation of dialdehydic anti-inflammatory active principles in extra-virgin olive oil by reactive paper spray mass spectrometry, *Int. J. Mass Spectrom.,* 2013, **352**, 87–91.
30 Z. Zhang, R. G. Cooks and Z. Ouyang, Paper spray: a simple and efficient means of analysis of different contaminants in foodstuffs, *Analyst,* 2012, **137**, 2556–2558.
31 H. Wang, J. Liu, R. Cooks and Z. Ouyang, Paper spray for direct analysis of complex mixtures using mass spectrometry, *Angew. Chemie,* 2010, **49**, 877–880.
32 R. D. Espy, A. R. Muliadi, Z. Ouyang and R. G. Cooks, Spray mechanism in paper spray ionization, *Int. J. Mass Spectrom.,* 2012, **325-327**, 167–171.
33 N. E. Manicke, Q. Yang, H. Wang, S. Oradu, Z. Ouyang and R. G. Cooks, Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots, *Int. J. Mass Spectrom.,* 2011, **300**, 123–129.
34 D. Taverna, L. Di Donna, F. Mazzotti, B. Policicchio and G. Sindona, High-throughput determination of Sudan Azo-dyes within powdered chili pepper by paper spray mass spectrometry, *J. Mass Spectrom.,* 2013, **48**, 544–547.
35 H. Evard, A. Kruve, R. Lõhmus and I. Leito, Paper spray ionization mass spectrometry: Study of a method for fast-screening analysis of pesticides in fruits and vegetables, *J. Food Compos. Anal.,* 2015, **41**, 221–225.
36 F. P. M. Jjunju, A. Li, A. Badu-Tawiah, P. Wei, L. Li, Z. Ouyang, I. S. Roqan and R. G. Cooks, In situ analysis of corrosion inhibitors using a portable mass spectrometer with paper spray ionization., *Analyst,* 2013, **138**, 3740–3748.
37 D. B. Robb and M. W. Blades, Effects of solvent flow, dopant flow, and lamp current on dopant-assisted atmospheric pressure photoionization (DA-APPI) for LC-MS. Ionization via proton transfer, *J. Am. Soc. Mass Spectrom.,* 2005, **16**, 1275–1290.
38 T. J. Kauppila, T. Kuuranne, E. C. Meurer, M. N. Eberlin, T. Kotiaho and R. Kostiainen, Atmospheric Pressure Photoionization Mass Spectrometry. Ionization Mechanism and the Effect of Solvent on the Ionization of Naphthalenes, *Anal. Chem.,* 2002, **74**, 5470–5479.
39 G. Guella, D. Ascenzi, P. Franceschi and P. Tosi, The intriguing case of organic impurities contained in synthetic methanol: a mass spectrometry based investigation, *Rapid Commun. Mass Spectrom.,* 2007, **21**, 3337–3344.
40 Y. Ren, H. Wang, J. Liu, Z. Zhang, M. N. McLuckey and Z. Ouyang, Analysis of Biological Samples Using Paper Spray Mass Spectrometry: An Investigation of Impacts by the Substrates, Solvents and Elution Methods, *Chromatographia,* 2013, **76**, 1339–1346.
41 Y. Su, H. Wang, J. Liu, P. Wei, R. G. Cooks and Z. Ouyang, Quantitative paper spray mass spectrometry analysis of drugs of abuse, *Analyst*, 2013, **138**, 4443–4447.
42 *Atrazine; tolerances for residues*, 40 C.F.R. §180.220, 2012, http://www.gpo.gov/fdsys/pkg/CFR-2012-title40-vol25/pdf/CFR-2012-title40-vol25-sec180-220.pdf.
43 S. Huang, T. Mayer and R. Yokley, Aqueous Injection LC–ESI/MS/MS Analysis of Water for 11 Chloro-and Thiomethyltriazines and Metolachlor and Its Ethanesulfonic and Oxanilic Acid Degradates, *J. Agric. Food Chem.,* 2008, **56**, 2595–2602.
44 A. Kabler and S. Chen, Determination of the 1'S and 1'R Diastereomers of Metolachlor and S-metolachlor in Water by Chiral Liquid Chromatography-Mass Spectrometry/Mass Spectrometry, *J. Agric. Food Chem.,* 2006, **54**, 6153–6158.
45 J. Liu, R. G. Cooks and Z. Ouyang, Enabling quantitative analysis in ambient ionization mass spectrometry: Internal standard coated capillary samplers, *Anal. Chem.,* 2013, **85**, 5632–5636.
46 U.S. Environmental Protection Agency, *Drinking Water Standards and Health Advisories*, 2012,

http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf.

47 U.S. Environmental Protection Agency, *Technical Factsheet on : Atrazine*, 2003, http://www.epa.gov/ogwdw/pdfs/factsheets/soc/tech/atrazine.pdf.

48 J. W. Munch, P. E. Grimmett, D. J. Munch, S. C. Wendelken, M. M. Domino, A. D. Zaffiro and M. L. Zimmerman, Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatograhy/Mass Spectrometry (GC/MS), US EPA, 2012.

49 *Metolachlor; tolerances for residues*, 40 C.F.R. §180.368, 2012, http://www.gpo.gov/fdsys/pkg/CFR-2012-title40-vol25/pdf/CFR-2012-title40-vol25-sec180-368.pdf.

Direct analysis of herbicides by paper spray ionization mass spectrometry

Steven L. Reeber, Sneha Gadi, Sung-Ben Huang, Gary L. Glish*

Table of Contents Entry