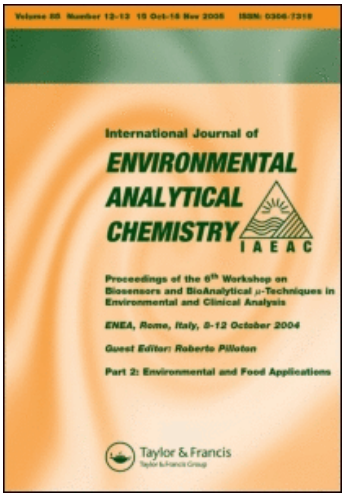

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ANALYSIS OF DIQUAT AND PARAQUAT BY SOLID PHASE EXTRACTION DIRECTLY COUPLED WITH MATRIX ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY

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The herbicides diquat and paraquat have been successfully analyzed through a process which combines solid phase extraction (SPE) with Matrix Assisted Laser Desorption/Ionization (MALDI) mass spectrometry. Ion pair interactions are utilized to selectively load the herbicides onto a solid phase extraction disc. A MALDI matrix is then applied and analysis takes place directly from the disc, eliminating the need to elute the sample.

This SPE-MALDI combination produced detection limits comparable to those obtained from conventional analysis with U.S. EPA Method 549.1. The SPE-MALDI method was tested on a variety of water samples and found to be suitable for "real world" analysis.

Keywords: Diquat; paraquat; solid phase extraction; MALDI mass spectrometry

INTRODUCTION

Diquat (1,1′-ethylene-2,2′-bipyridylium ion) and paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) are dipyridyl herbicides and plant growth regulators. Although available commercially as methylsulfate, bromide and chloride salts, solutions of the diquat dibromide[1] and paraquat dichloride[2] are the only formulations with wide agricultural use in both the United States and abroad. The structures of their dications appear in Figure 1.

Diquat is classified as a general use herbicide by the U.S. Environmental Protection Agency. It is used as a non-crop weed killer, a general aquatic herbicide

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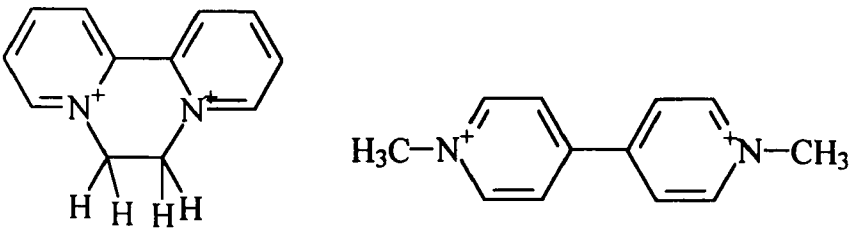

FIGURE 1 Structures of the dications of diquat and paraquat

and as a pre-harvest desiccant of seed crops.[1] It is considered moderately toxic if swallowed, inhaled or absorbed through the skin.[3] It is currently regulated in drinking water at a concentration of 20 mg/L.

Paraquat is classified as a restricted use pesticide by the U.S. EPA; under normal conditions it can only be purchased and used by certified applicators. Similar to diquat, paraquat is used as a desiccant, defoliant and as an aquatic herbicide. It is considered highly toxic to humans[3] and is well known to cause damage to human lung tissue.[4] Paraquat has been reported as possibly being the restricted use pesticide exported in the greatest volume by the United States.[5] While not currently regulated in drinking water, its biological hazards and environmental uses make the analysis of this compound of interest.

In order to analyze these compounds successfully, sample preconcentration through liquid/liquid extraction or solid phase extraction (SPE) is usually required. The dicationic nature of these salts presents experimental difficulty during both concentration and analysis. Low recovery amounts and irreproducible retention times are observed during the reverse phase chromatography of these compounds.[6] While the addition of an ion-pair reagent improves reverse phase separations, several steps are added to the method because of the need to prevent irreversible interactions with any residual free silanol groups on the stationary phase.

The U.S. EPA approved method of analysis of diquat and paraquat in drinking water (Method 549.1) involves preconcentration of the analytes (SPE) followed by ion-pair high performance liquid chromatography (HPLC) with ultraviolet absorption detection.(UV)[7] The sample (250 mL) is first adjusted to pH 10.5. It is then extracted using either a C-8 solid sorbent cartridge or C-8 solid phase

extraction disc, which has been specially prepared for use in the ion-pair mode. This preparation involves seven steps that include the addition of conditioning reagents to prevent adhesion of the pesticide to free silanol groups. Also included in the conditioning is the addition of an ion-pair reagent to allow loading of the relatively hydrophilic dications onto the hydrophobic C-8 disc. After conditioning, the sample is applied to the disc in a manner similar to vacuum filtration. The absorbed pesticides are then eluted from the disc, ion pair reagnets are again added and the sample is adjusted to a final volume of 5mL. HPLC analysis with UV detection is then performed.

Due to the complexity of the approved method and the importance of the two herbicides, several other methods have been proposed for cleanup and analysis of samples containing these compounds. A few examples include simple colorimetric determinations after addition of sodium borohydride,[8] immunoassays,[9] GC-MS after derivatization,[10] and more recently CE-MS.[11–13]

In the current work, we propose to simplify the approved procedure and reduce the sample size needed by performing direct analysis of these dications from a solid phase extraction disc onto which the herbicides have been absorbed. The disc preparation steps are reduced and the need to elute the sample from the disc after loading is eliminated. The procedure is related to the technique performed by Bristow et al.[14], but utilizes ion-pair interactions to selectively isolate the compounds of interest from the sample matrix for analysis.

EXPERIMENTAL

MALDI Instrumentation and data analysis

A Voyager DE-STR time-of-flight laser mass spectrometer (PE Biosystems, Foster City, CA), with a nitrogen laser emitting at 337 nm, was employed for all studies. Data acquisition and mass calibration were performed utilizing PerSeptive GRAMS/386 software. (version 3.04 Level III, Galactic Industries Co.)

Materials

Samples of diquat dibromide monohydrate (diquat) (purity: 99%) and paraquat CL tetrahydrate (purity: 99%) were purchased from Chem Service (West Chester, PA) and used without further purification. Ammonium laurylsulfate solution (30% T) was purchased from Fluka Chemical Corp. (Ronkonkoma, NY), and a-cyano-4.-hydroxycinnamic acid (α-CHCA) was obtained from Aldrich. (Mil-

waukee, WI) ACS certified grade sodium hydroxide, as well as HPLC grade solvents were obtained from Fisher Scientific (Pittsburgh, PA). Tap water representing the Metropolitan Nashville water supply was obtained from a laboratory sink faucet. Veryfine Balsams Spring Water (source: Balsams Spring, Dixville Notch, NH) was purchased from the Balsams Spring Water Co. Inc. (Westford, MA).

METHODS

MALDI Analysis of Diquat and Paraquat

Stock samples of diquat (87.4 mg/L) and paraquat (85.4 mg/L) were prepared in HPLC grade water. A 50 μL aliquot of each sample was diluted to a volume of 500 μL with water and mixed 1:1 v/v with a saturated solution of α-CHCA in methanol. A 2 μL sample was deposited onto the sample plate, allowed to dry and analyzed by MALDI-MS.

SPE-MALDI

The SPE MALDI process depicted in Figure 2, takes advantage of the special features of the Ansys® Diagnostics, INC. Toxi•LAB® SPE•C18•I extraction cartridge system. The 10 mL sample reservoir contains a glass fiber filter to allow removal of particulate matter from the sample, eliminating the need for particulate filtration of the sample before analysis. The disc is manufactured from a glass microfiber which is impregnated with silica. This silica is then covalently bonded with C-18 material to produce a reverse phase disc. It is encased in a disposable plastic cartridge, which separates to allow removal of the disc from the housing. One additional advantage of the disc system is its small size (80mm dia. × 0.3 mm thick) compared to other SPE discs. This allows for the use of less sample while still providing adequate surface coverage for MALDI analysis.

This SPE-MALDI process relies heavily on the ability to selectively load a compound or a set of compounds onto the solid phase extraction disc. The dicationic nature of diquat and paraquat in solution, which leads to difficulty for traditional HPLC and SPE techniques, provides the means for selective loading during this particular application.

Figure 2 illustrates the steps required for the successful coupling of SPE with MALDI. In step A, the disk is first conditioned by adding 1mL of methanol to the sample reservoir. One-half of this amount is pulled through under vacuum

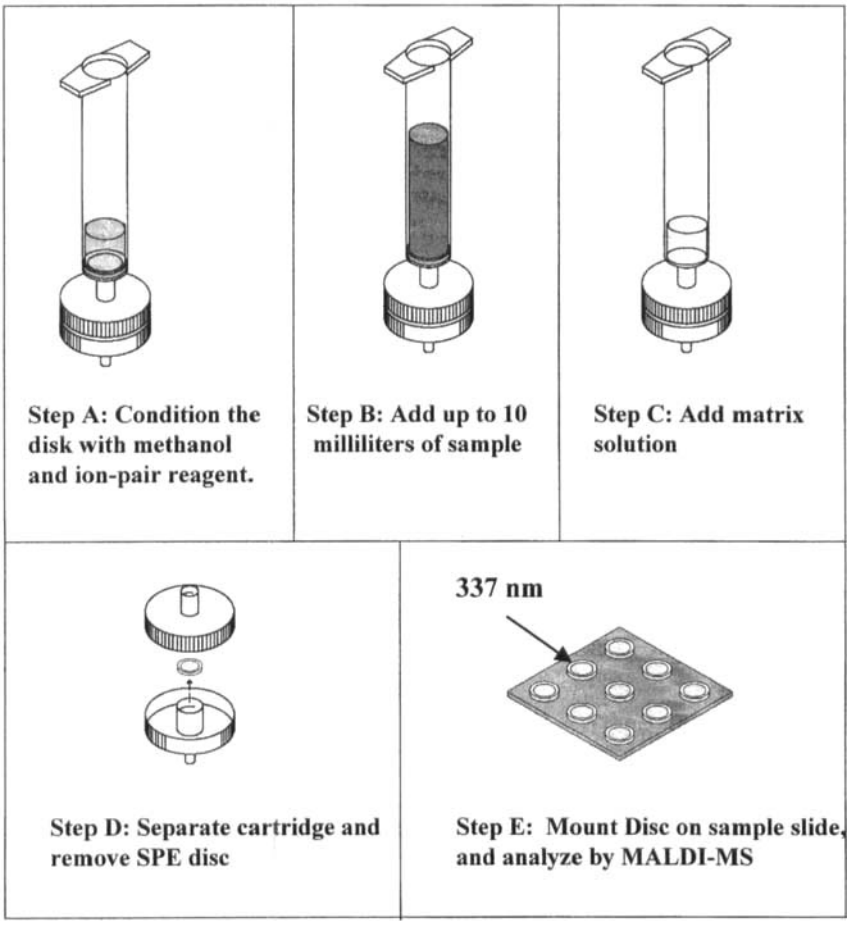

FIGURE 2 Illustration of the SPE-MALDI coupling technique

and the disk is allowed to soak for two minutes. Next 1 mL of ion-pair reagent, which consists of 25mL of ammonium laurylsulfate solution diluted to 250 mL with de-ionized water, is added to the sample reservoir. Again approximately half of this volume is pulled through and then the disk is allowed to soak for another two minutes. The non-polar tails of the ammonium laurylsulfate interact with the C-18 groups on the disc surface, effectively preventing non-polar interferences in the sample from being absorbed. The more hydrophobic ammonium laurylsulfate is used as an ion pair reagent, instead of 1 hexanesulfonic acid specified in Method 549.1 (conditioning solution B). This substitution allows for more inter-

actions between the ion-pair reagent and the hydrophobic tails on the C-18 disc, and ultimately more complete sample loading.

In step B, up to 10mL of sample is placed in the sample reservoir and pulled through under vacuum completely. The positively charged analyte molecules are attracted to the negatively charged sulfate groups present on the ammonium laurylsulfate. The complex formed from this attraction is extremely hydrophobic and therefore favorably binds to the C-18 material. Because the analyte is to be analyzed directly from the disc, and is not eluted off, the increased attraction of the ammonium laurylsulfatelanalyte complex (compared to the complex formed in method 549.1) is not a concern. A stronger hydrophobic interaction leads to increased analyte loading, and ultimately lower detection limits.

For step C, the sample reservoir is replaced with a paperless reservoir, and 0.5 mL of saturated α-CHCA matrix solution (in acetone) is pulled through the disc. The paper must first be removed to allow for matrix deposition on the disc as opposed to the paper. The discs are dried under vacuum for 2–3 minutes, and in step D, removed from the cartridge holders. They are finally mounted on sample slides and analyzed directly by MALDI-MS as shown in step E.

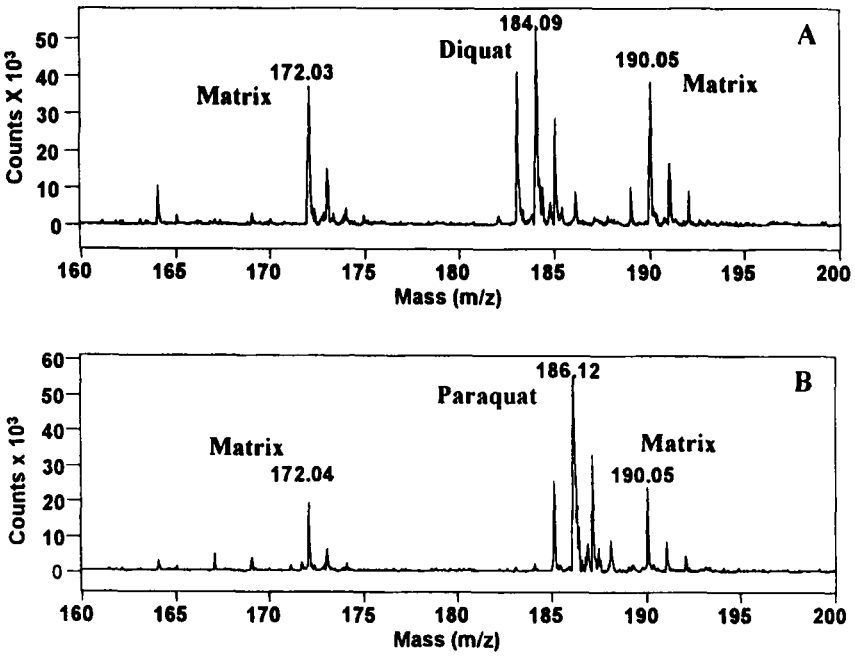

FIGURE 3 MALDI spectra of: A) diquat and B) paraquat

RESULTS AND DISCUSSION

The MALDI spectra of diquat and paraquat with α-CHCA from *m/z* 160–200 are shown in Figure 3. Although the compounds are dicationic salts with a mass to charge ratio of 92 and 93 respectively, no peaks appear in this region of the spectra. Instead, three major peaks centered around m/z 184 for diquat and m/z 186 for paraquat are observed. These peaks correspond to the $[M^{2+}\text{-}H^{+}]^{+}$, $[M]^{+}$ and $[M+H^{+}]^{+}$ species for each of the herbicides. A similar $[M^{2+}\text{-}H^{+}]^{+}$, peak was noted by Budde et al. during the capillary electrophoresis/electrospray (CE-ESI) mass spectra of diquat and paraquat.[11] It was hypothesized that the relatively strong ammonium ion could abstract a proton from the $[M^{+2}]$ species leading to the formation of the *m/z* 183 and *m/z* 185 peaks for diquat and paraquat respectively. The major peaks at *m/z* 184 and *m/z* 186 would occur with a single electron reduction of the herbicide dications. Again, one electron reduction was observed in the CE-ESI mass spectra of these compounds by Budde, but was attributed to interactions during the capillary electrophoresis due to its absence in direct infusion ESI. A two electron reduction followed by protonation of the herbicides from the matrix would account for the appearance of the $[M+H]^{+}$ peaks at *m/z* 185 and *m/z* 187 for diquat and paraquat respectively. Such reductions are often observed for the matrix during MALDI analysis, but less commonly seen for analytes.

The unique isotopic distribution patterns, which are produced during the MALDI-MS analysis of these compounds, can be used to increase the specificity of the method. Interference peaks from any co-absorded analyte with an identical mass to charge ratio as the herbicides would easily be recognized by the change in the isotopic patterns.

SPE-MALDI

The SPE-MALDI spectra of 50 μg/L of diquat and paraquat are shown in Figure 4. Higher laser power is needed to desorb the analyte from the surface of the SPE disc than is required for conventional MALDI-MS. Peaks for both the matrix and the analyte are broader than those obtained from direct MALDI-MS of the same compounds. Peaks at *m/z* 184 and *m/z* 186 are again the dominant peaks in the spectra. A similar relative abundance ratio as was seen by conventional MALDI analysis is observed.

Standard samples prepared at a concentration of 2 μg/L were used to estimate the detection limits for diquat and paraquat by SPE-MALDI. Table I presents the detection limits calculated from these samples based on a 3:1 signal to noise ratio. The calculated detection limits for the analysis by SPE-MALDI are very

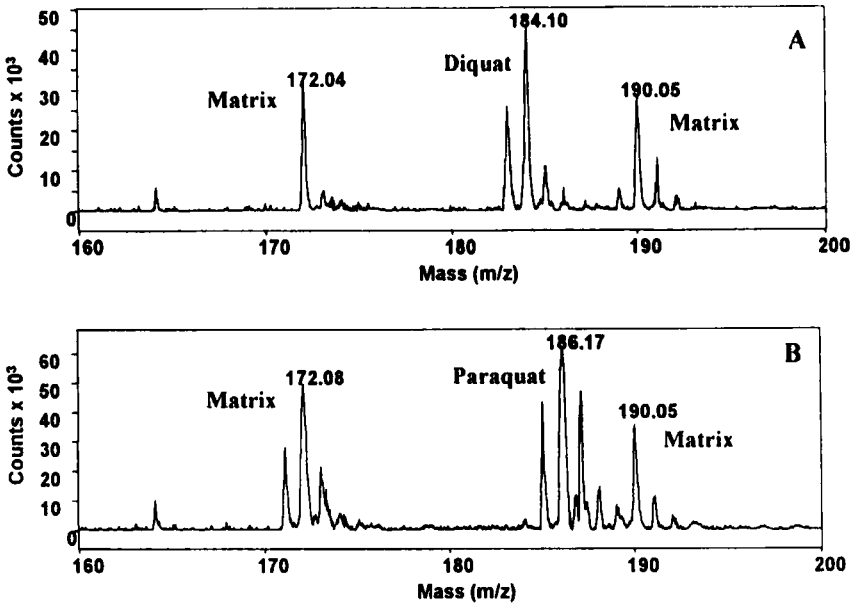

FIGURE 4 SPE-MALDI spectra of 50mg/L:A) diquat and B) paraquat in water

similar to those expected by the U.S. EPA Method 549.1. These low detection limits show that analysis of diquat and paraquat by SPE-MALDI should be a reasonable alternative to this time intensive method.

TABLE I Detection Limits for Diquat and Paraquat by Method 549.1 and SPE-MALDI

	Method 549.1	*SPE-MALDI*
Diquat	0.51 μg/L	0.64 μg/L
Paraquat	0.59 μg/L	0.32 μg/L

The spectra in Figure 5 were obtained by applying the SPE-MALDI method to "real" drinking water samples. Figures 5b and 5c show peaks at both *m/z* 184 and *m/z* 186 corresponding to trace quantities of diquat and paraquat being detected in both bottled spring water and tap water respectively. Figure 5a was obtained by using a sample of 10mL HPLC grade water. The absence of peaks at either *m/z* 184 or *m/z* 186 confirms that the peaks in the previously mentioned spectra are not artifacts of the method.

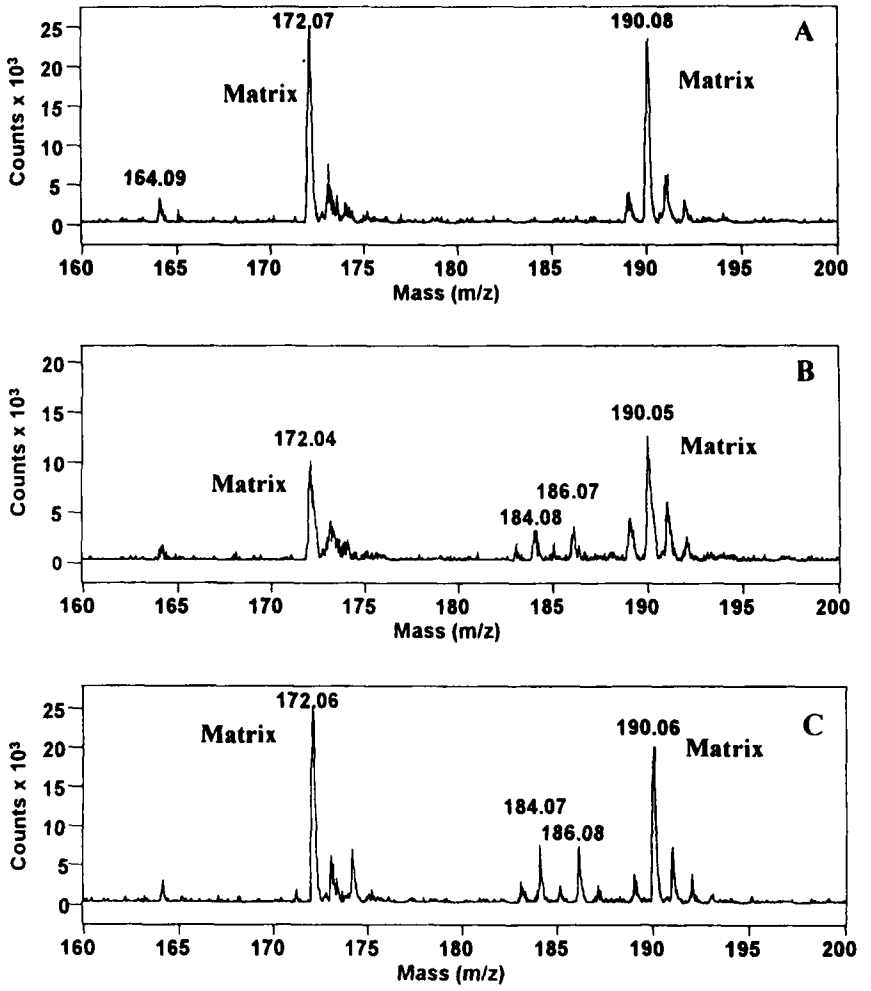

FIGURE 5 Detection of trace quantities by SPE-MALDI of diquat (m/z 184) and paraquat (m/z 186) in: A) an HPLC grade water blank, B) spring water and C) tap water

The spectra in Figure 6 were obtained by spiking tap water with the pesticides. The main peak at *m/z* 184 in Figure 6a corresponds to addition of diquat to the sample of tap water. The peak at 186 in this spectrum arises from the amount of paraquat already in the water, combined with the C-13 contribution from the peak at 185 $[M+H^+]^+$. The peaks present in Figure 6b arise from spiking the sample with paraquat to a concentration of 50 μg/L above the tap water background. Figure 6c shows the relative distribution pattern that arises from a sample spiked

with equal amounts (50 μg/L) of diquat and paraquat. Again, the relative abundance patterns will be helpful in detecting compounds of a similar mass that might interfere with this method of detection.

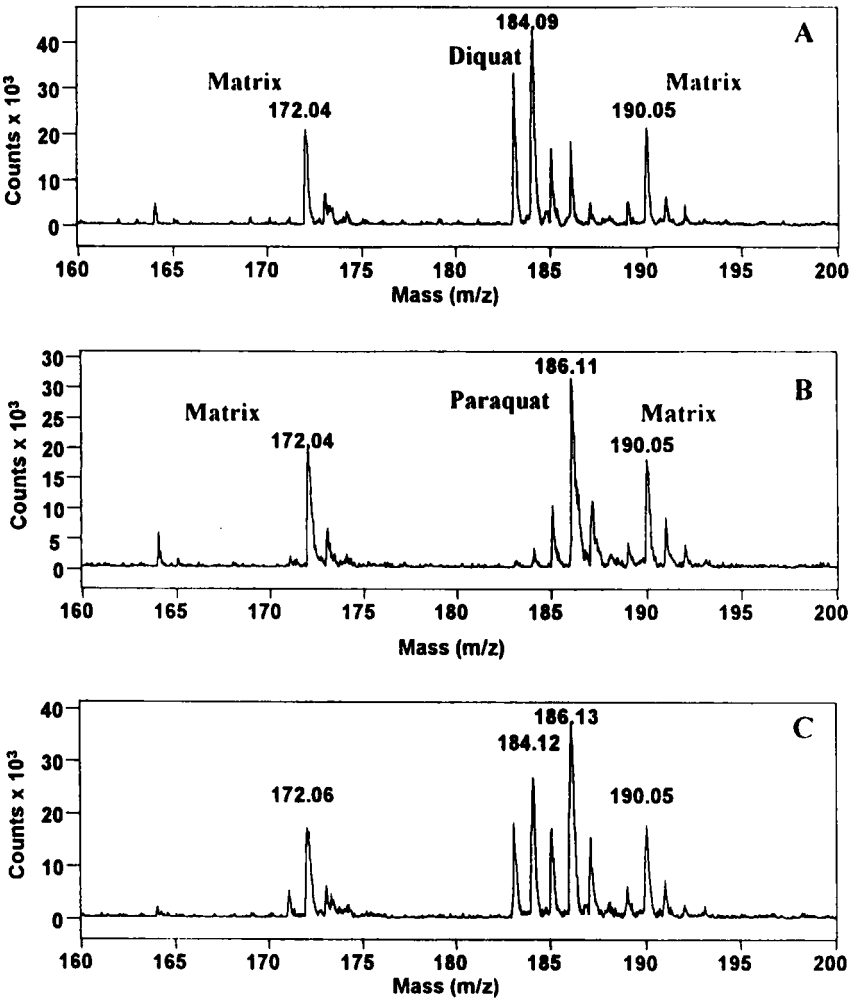

FIGURE 6 Analysis of tap water spiked with A) 50μg/L of diquat B) 50 μg/L of paraquat and C) 50 μg/L of diquat (m/z184) and paraquat (m/z 186) by SPE-MALDI

Table I shows that the detection limits obtained by SPE-MALDI are very similar to those obtained by method 549.1. However, the SPE-MALDI analysis has fewer steps and requires less time. The sensitivity of MALDI-MS detection is

combined with selective loading of the analyte on a disc to provide a reasonable alternative for the analysis of diquat and paraquat in drinking water.

Future work will focus on quantification of diquat and paraquat by SPE-MALDI, as well as extending the usefulness of the technique to other pesticides. This method will also be optimized and applied to other types of samples (ie lakes, streams) to conduct environmental fate studies.

CONCLUSIONS

A successful alternative to the current EPA method for the determination of diquat and paraquat in drinking water by SPE-HPLC has been developed. The dicationic analytes are loaded onto a solid phase extraction disc using ion-pair interactions. The high affinity of MALDI for pre-charged compounds, combined with this selective analyte loading allows separation of the analyte from the sample matrix. The unique abundance ratios obtained from these compounds provide further confirmation of peak identification. The SPE-MALDI process is much less time intensive than the currently approved EPA Method 549.1. It also permits analysis with a lower sample volume and appears suitable for both ideal and "real" drinking water samples.

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