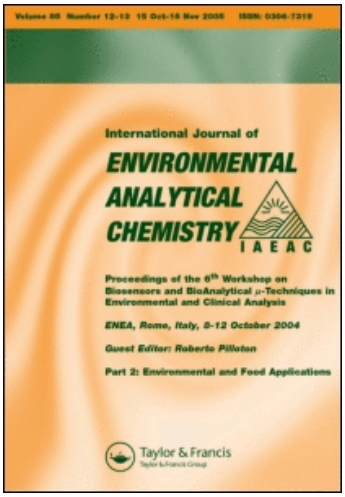

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ANALYSIS OF CATIONIC PESTICIDES BY THIN LAYER CHROMATOGRAPHY/MATRIX—ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY

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This study demonstrates the analysis of pesticides by direct coupling of Thin Layer Chromatography (TLC) with Matrix-Assisted Laser Desorption Ionization mass spectrometry (MALDI). TLC-MALDI takes advantage of the high sensitivity of MALDI while utilizing the relatively quick and inexpensive separation offered by TLC. The optimization of a protocol to analyze cationic pesticides by TLC-MALDI is reported and tested on normal phase, reverse phase, and cellulose TLC plates. Detection limits in the picogram range were found for two analytes, phosphon and avenge. Detection limits for glyodin were in the nanogram range. Consistency in detection limits for each compound is observed on all types of stationary phases investigated. This demonstrates the applicability of this method for use on a variety of TLC plates.

Keywords: Cationic pesticides; thin layer chromatography; laser desorption ionization mass spectrometry; TLC-MS coupling

INTRODUCTION

Pesticides are biologically active substances, which are designed to control the growth or reproduction of one or more species. These substances include a broad range of toxic compounds which, when released into the environment, may have effects far from the point of application, due to either persistence or runoff[1]. By 1994, over two billion pounds of pesticides were used annually in the United States alone; this amount is expected to climb.[2] Many of these substances are controlled by US EPA regulations, and therefore their levels must be monitored

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locally to ensure that the amount present is not harmful to local wildlife or to humans.[3]

Pesticides are traditionally analyzed by gas chromatography, gas chromatography/mass spectrometry, or for non-volatile compounds, high performance liquid chromatography.[4] The objective of the present study is to develop a protocol to analyze cationic pesticides using mass spectrometry (MS) after separating the analytes on thin-layer chromatography (TLC) plates. Such a process would take advantage of the simple yet multidimensional capabilities of TLC separations, while utilizing the specificity and sensitivity of MS detection.[5,6]

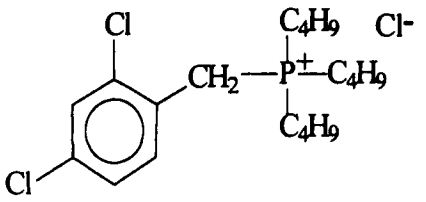
There are two general approaches for combining TLC with MS detection. The first involves scraping the analyte spot from the plate and extracting it into a solvent. The extractant can then be analyzed by conventional mass spectrometric techniques.[7,8] The second involves analysis of the spot directly on the plate; analysis takes place in the presence of the stationary phase.

Fast atom bombardment (FAB), secondary ion mass spectrometry (SIMS) and laser desorption (LD) have been used for direct TLC-MS coupling.[9–15] TLC-FAB and TLC-SIMS normally require that a liquid matrix be deposited directly on the TLC plate. The liquid matrix facilitates extraction of the analyte from the stationary phase and also improves sensitivity. A major drawback of this method is the occurrence of lateral analyte spreading through diffusion after the application of the liquid matrix. Busch and co-workers overcame this problem through the use of a phase transition matrix, which was held in the liquid state for analyte extraction, and then solidified for SIMS analysis. This prohibits further spreading of the analyte.[16–18] While this method exhibits good spatial resolution, its applicability is limited by its requirement for complex custom-made instrumentation. TLC-LD can be performed with high sensitivity and good spatial resolution without the use of liquid matrixes. However this technique suffers from poor reproducibility and significant molecular fragmentation. Fragmentation can be reduced by using IR laser desorption followed by multiphoton ionization,[19–22] but this technique again requires sophisticated instrumentation, and is not applicable to a broad range of compounds.

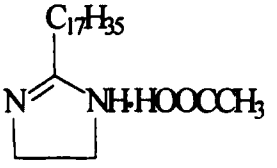
Our laboratory has previously reported the development of a direct TLC-MS coupling protocol, which utilizes Matrix-Assisted Laser Desorption/Ionization (MALDI).[7–8,23] This combination demonstrates several advantages over previous TLC-MS protocols, including simple sample preparation, minimal analyte spreading, and broad utility. One further advantage is that the analysis can take place in commercial MALDI instruments without the need for extensive modifications.

Three model compounds, phosphon, glyodin, and difenzoquat methylsulfate (avenge), all of which are herbicides, were utilized in this study. Their structures

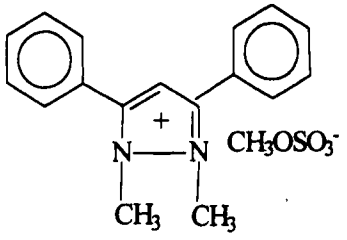
appear in Figure 1. This investigation focused on the optimization of the parameters involved in the TLC-MALDI protocol for these pesticides. The detection limits obtained on each stationary phase are compared to the detection limits obtained from conventional MALDI analysis on stainless steel.



Phosphon (396)
m/z 361



Glyodin (368)
m/z 309, 327



Difenzoquat Methyl Sulfate (360)
m/z 249

FIGURE 1 Pesticides Investigated

EXPERIMENTAL

MALDI instrumentation and data analysis

A Kratos Kompact MALDI III (Kratos Analytical, NJ) time-of-flight laser mass spectrometer, with a laser emitting at 337 nm, was employed for all studies. Standard processing of the spectra included data acquisition, mass calibration and spectral smoothing.

Materials

Samples of difenzoquat methylsulfate (avenge) (purity: 99%) glyodin (purity: 98%) and phosphon (purity: 98%) were obtained from Chem Service (West Chester, PA). Whatman PE SIL G/UV TLC plates were used for all normal phase experiments. The reverse phase experiments were carried out on DC-Alufolien RP-18 F254s TLC plates manufactured by E. Merck. The cellulose plates used were Machery-Nagel Polygram CEL 300 plates. All solvents used were obtained from Fisher Scientific (Pittsburgh PA), and were HPLC grade except for acetic acid and hydrochloric acid which were ACS Plus grade.

Sample preparation

Stock solutions of each pesticide were prepared by dissolving 4 mg of the solid in 1 ml of an appropriate solvent. For phosphon and difenzoquat methylsulfate the solvent used was a 1:1 methanol/water mixture. For glyodin a 1:1 isopropyl alcohol/dichloromethane solution was used. Serial dilutions were performed to reach the desired concentrations for all samples.

TLC separation

Separation protocols were developed to allow separation of the cationic pesticides on normal phase, reverse phase and cellulose TLC plates. In all cases, analytes were deposited in 0.5 µl increments onto the TLC plate using a manual micropippetor. Visualization of the analyte spot was performed by either utilizing a fluorescent indicator irradiated at 254 nm or by placing the developed TLC plate in an iodine chamber for 3–5 minutes. Factors investigated in mobile phase selection included not only suitable analyte separation (as measured by Rf), but also minimization of peak tailing and of analyte spot spreading.

TLC coupling protocol

The TLC-MALDI-MS coupling protocol used for these experiments has been described in detail elsewhere.[7,8] Briefly, a MALDI matrix layer is generated by "fast evaporation" of the MALDI matrix on the surface of a 1 × 1 cm polished stainless steel plate (.015 inch thick) or other inert substrate. MALDI matrices are cast by depositing 25 µL of a matrix solution in a volatile solvent such as acetone on the surface of the stainless steel substrate and allowing the solvent to evaporate. After drying, the developed TLC plate containing the separated analytes is sprayed with an appropriate extraction solvent. A suitable extraction sol-

vent must dissolve the analyte completely, but only partially dissolve the matrix layer. The substrate on which the MALDI matrix was cast is then inverted and placed face-to-face with the sprayed TLC plate. This "sandwich" is then pressed at 2.5 kg/mm^2 –3.0 kg/mm^2 for 10–60 seconds. The squeezing process forces the solvent from the interior of the TLC plate toward the surface. A portion of the analyte is carried with this solvent. On the surface, the solvent contacts the MALDI matrix inducing analyte incorporation into the MALDI matrix layer and effecting the MALDI matrix transfer. The entire TLC plate/MALDI matrix complex can then be analyzed by MS.

RESULTS AND DISCUSSION

The successful analysis of pesticides through the coupling of TLC and MALDI involves of three steps: separation of the analytes on TLC plates, the coupling protocol and the MS analysis. In this study the optimization of each step was carried out in order to maximize MS signal intensity and achieve the lowest possible detection limits. To allow a comparison of the effect of the composition of the stationary phase on the signal intensity, this process was repeated on normal phase, reverse phase, and cellulose stationary phase TLC plates.

MALDI analysis and matrix selection

Before an analyte can be analyzed by TLC-MALDI a suitable matrix must be found for use with that sample. An appropriate matrix will provide adequate signal intensity for the analyte while not masking the signal with background peaks. It also must be able to form a homogeneous thin film on the surface of the inert substrate, which can later be transferred to the TLC plate. Several matrices were evaluated to match these criteria. The matrix tested which provided the best signal intensity for all analytes was α-cyano-4-hydroxycinnamic acid (α-CHCA) (Aldrich, Milwaukee, WI) cast from acetone at 25 mg/ml. Figure 2 shows the MALDI spectra obtained for each analyte directly from a stainless steel substrate, onto which matrix and sample had been deposited. Because these substances ionize in solution, the mass to charge ratio obtained for each sample corresponds only to the cationic portion of the analyte. The peaks at *m/z* 249, and *m/z* 361 correspond to the expected *m/z* values for the captions of difenzoquat methylsulfate and phosphon, respectively. The resolution of the instrument used is not sufficient to allow observation of the isotopic abundance pattern which one would expect to observe due to the two chlorine atoms in phosphon. Peaks at *m/z* 309 and *m/z* 327 are observed for glyodin. In this case the $(M+H_2O)^+$ peak at *m/z* 327 is the major peak observed. The peak at *m/z* 309 is observed from the disso-

ciation of the acetate group. The remaining peaks in the spectra correspond to typical background peaks from the α-CHCA matrix. The large variability in the intensity of the background peaks is due in part to the variability inherent in MALDI and to changes in the laser energy needed for ionization of the various samples. Detection limits for each pesticide by MALDI on stainless steel were estimated based on a 3:1 signal to noise ratio, and verified by running the samples at the stated detection limits. These values appear in Table I.

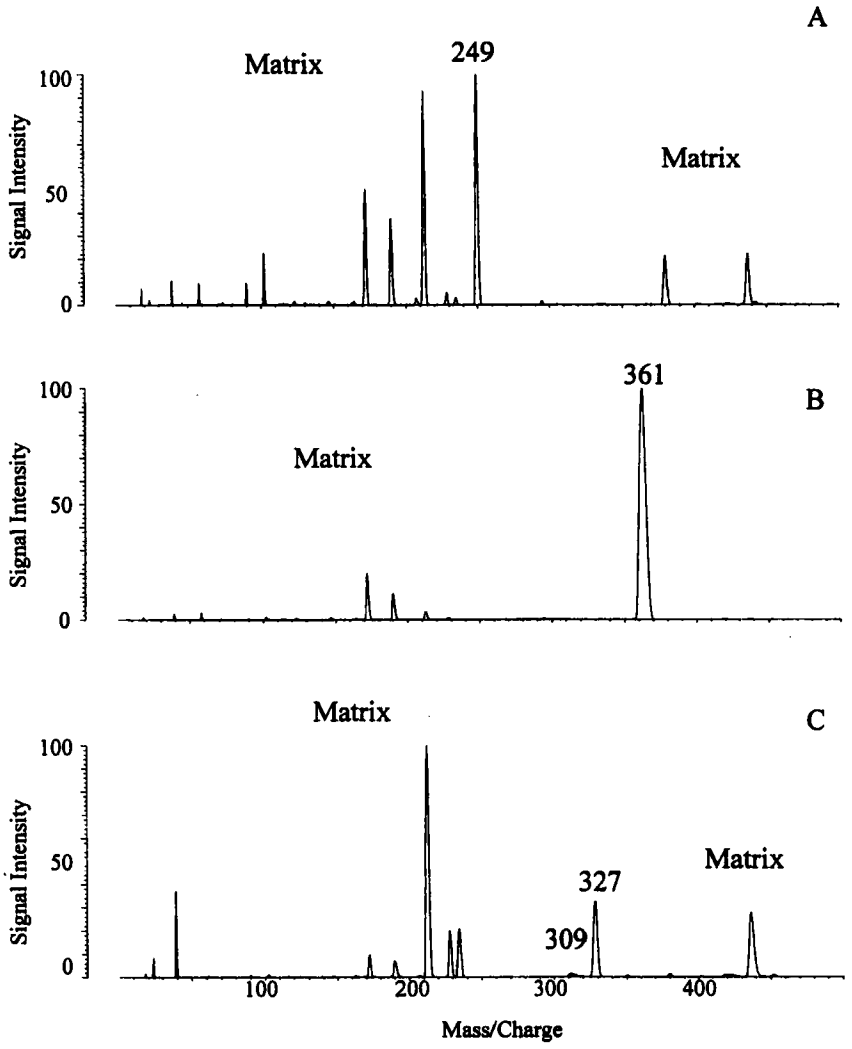


FIGURE 2 MALDI spectra of A) 0.04 ng difenzoquat methylsulfate, B) 0.4ng phosphon and C) 0.4ng glyodin obtained from stainless steel

TABLE I Detection limits of pesticides obtained by MALDI mass spectrometry directly from stainless steel substrates and various types of TLC plates. Masses stated are the total amount of pesticide spotted on the sample slide

		Type of TLC plates		
Compound	*Stainless steel*	*Silica*	*C-18*	*CEL 300*
Avenge	0.1 pg	4 pg	1 pg	0.6pg
Glyodin	10 pg	4 ng	1 ng	1 ng
Phosphon	3 pg	20 pg	20 pg	40 pg

TLC separation

An ideal mobile phase will allow complete separation of all analytes while minimizing spot spreading. For coupling TLC with MALDI the second criterion is the more critical. Although separation schemes were developed to allow complete separation of all analyte spots, the MS detector could also have been used to distinguish analytes provided each analyte displays a different mass to charge ratio. Table II describes the mobile phases used on each type of plate and reports the R_f value obtained for each analyte. Note that for the cellulose plates a two-dimensional scheme was employed. In this scheme, the first dimension provides separation of the analytes from one another, while the second dimension was needed to elute glyodin away from the point of application on the plate. Additionally, all mobile phases contain a relatively high concentration of acid. The addition of the acid minimizes the lateral spreading of the analyte, which effectively increases the concentration of the analyte per unit area. Acetic acid was employed in the separation on normal phase plates because HCl quenched the indicator incorporated into these plates making spot visualization impossible.

TABLE II R_f values obtained from pesticides on various TLC plates

		R_f		
plate	*Mobile phase*	*Avenge*	*Phosphon*	*Glyodin*
Reverse	66% 2-propanol 33 % 10% HCL soln.	0.54	0.31	0.22
Normal	60% methanol 30% ethyl ether 10% acetic acid	0.45	0.87	0.63
Cellulose d1	10% 2-propanol 5% HCl 10% acetic acid 75% water	0.90	0.79	0.00
Cellulose d2	40% 2-propanol 10% HCl 50% water	1.00	1.00	0.23

Extraction solvent selection

The selection of a suitable extraction solvent is the most critical parameter in the coupling of TLC with MALDI MS. The extraction solvent must be able to dissolve the analyte and facilitate its transport to the surface, yet only partially dissolve the matrix layer. Partial dissolution allows the transfer of a homogenous layer to the surface of the TLC plate. The extraction solvent must have a high enough elution strength to transfer the analyte to the surface, but not strong enough to promote lateral spreading of the analyte spot which would lower the concentration of the analyte per unit area.[24] Table III shows the extraction solvents chosen for each type of TLC plate. Again the addition of an acid was found to minimize spot spreading during the extraction and pressing steps.

MS analysis

The sample slide design of the Kratos Kompact MALDI III required that only a portion of the analyte spot be analyzed by MS. After the matrix layer was transferred to the analyte spot, a portion was cut from the rest of the plate and placed on a slide for MS analysis. The laser energy was adjusted to achieve the highest signal to noise ratio for each sample. In general, more laser energy was required for TLC-MALDI analysis than for the analysis of the pesticides by MALDI directly from stainless steel. In all cases approximately 100 laser shots were averaged to produce a composite spectrum. Figure 3 provides examples of the spectra obtained from each analyte spot after separation on a reverse phase C-18 plate. Again peaks at *m/z* 249, *m/z* 361 and *m/z* 327 correspond to the major peaks for difenzoquat methylsulfate, phosphon and glyodin, respectively. For comparison, Figures 4 and 5 provide examples of spectra obtained from both normal and cellulose TLC plates. For all examples 0.4 ng of difenzoquat methylsulfate, 4 ng of phosphon and 40 ng of glyodin were originally deposited on the TLC plate. Detection limits were determined for each analyte on each type of plate; these values appear in Table I.

TABLE III Extraction solvents chosen for TLC stationary phases. α-CHCA matrix

Stationary phase	*Extraction solvent*
normal phase	2:1 2-propanol: HCl solution (10%)
reverse phase	1:1 2-propanol: 10% HCl solution
Cel 300	3:7 MeOH: HCl solution (5%)

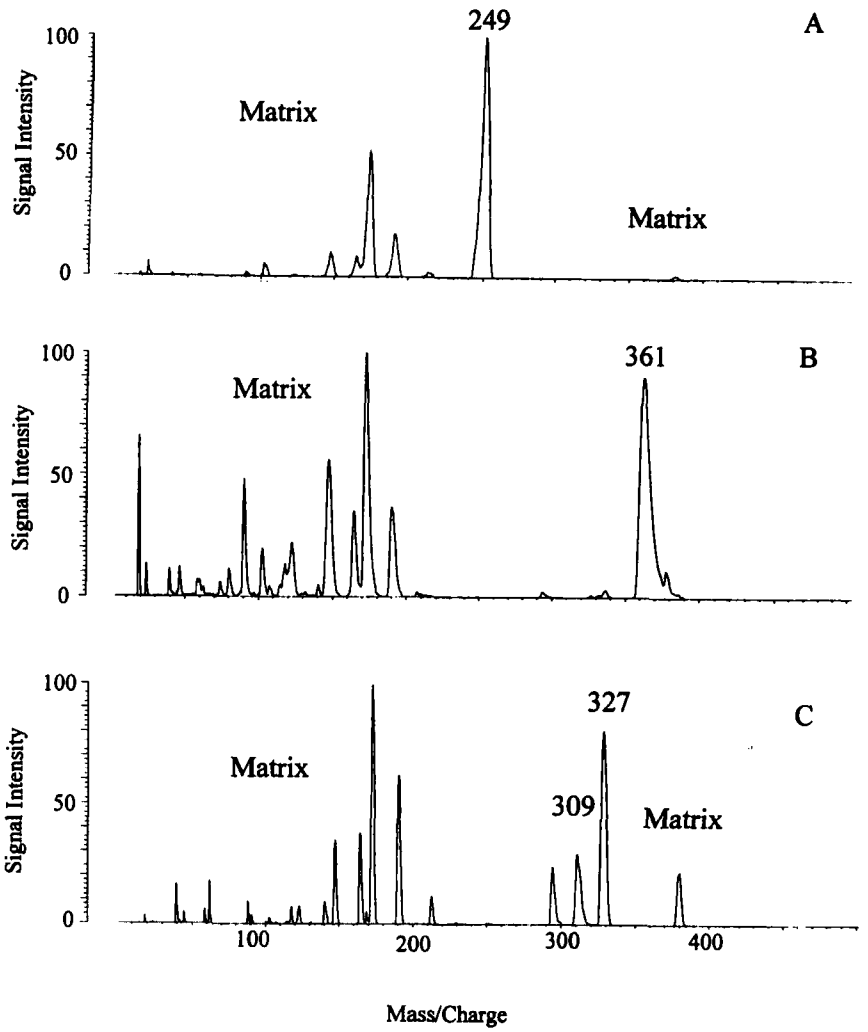


FIGURE 3 MALDI spectra of A) 0.4 ng difenzoquat methylsulfate, B) 4.0 ng phosphon and C) 40 ng glyodin obtained directly from reverse phase TLC plates, after separation

Comparison of the detection limits for each type of TLC plate demonstrates that the TLC-MALDI method is equally effective on the most common types of TLC plates. The increased detection limits obtained for TLC-MALDI vs. MALDI on stainless steel represent the inherent cost of providing separation of the analytes before analysis by this method. While cellulose plates provided slightly lower detection limits, the need for a two-dimensional separation scheme increases both the time needed and the cost involved in the separation of these

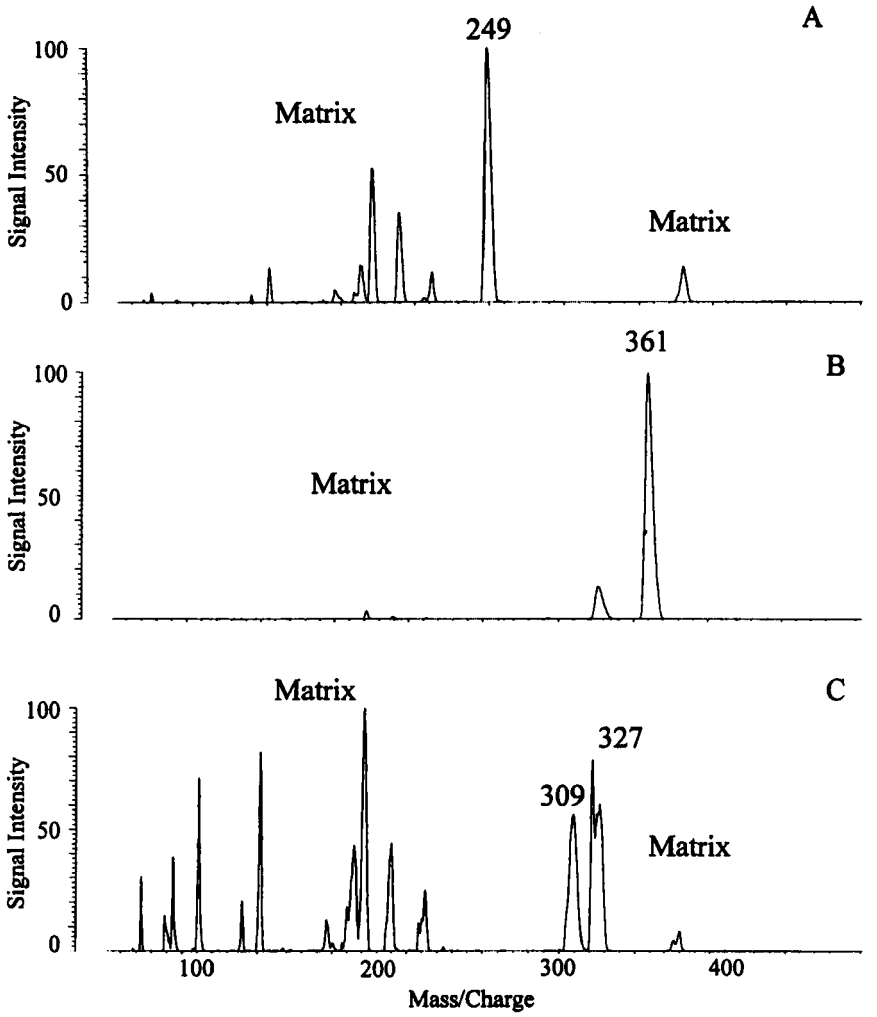


FIGURE 4 MALDI spectra of A) 0.4 ng difenzoquat methylsulfate, B) 4 ng phosphon and C) 40 ng glyodin obtained directly from normal phase TLC plates, after separation

cationic species. In general, due to the similarity of the spectra obtained from all phases tested, the stationary phase selection should be based strictly on desired separation parameters. In this study reverse phase plates provided the quickest and most cost efficient means of separating the compounds while limiting lateral spreading of the analytes.

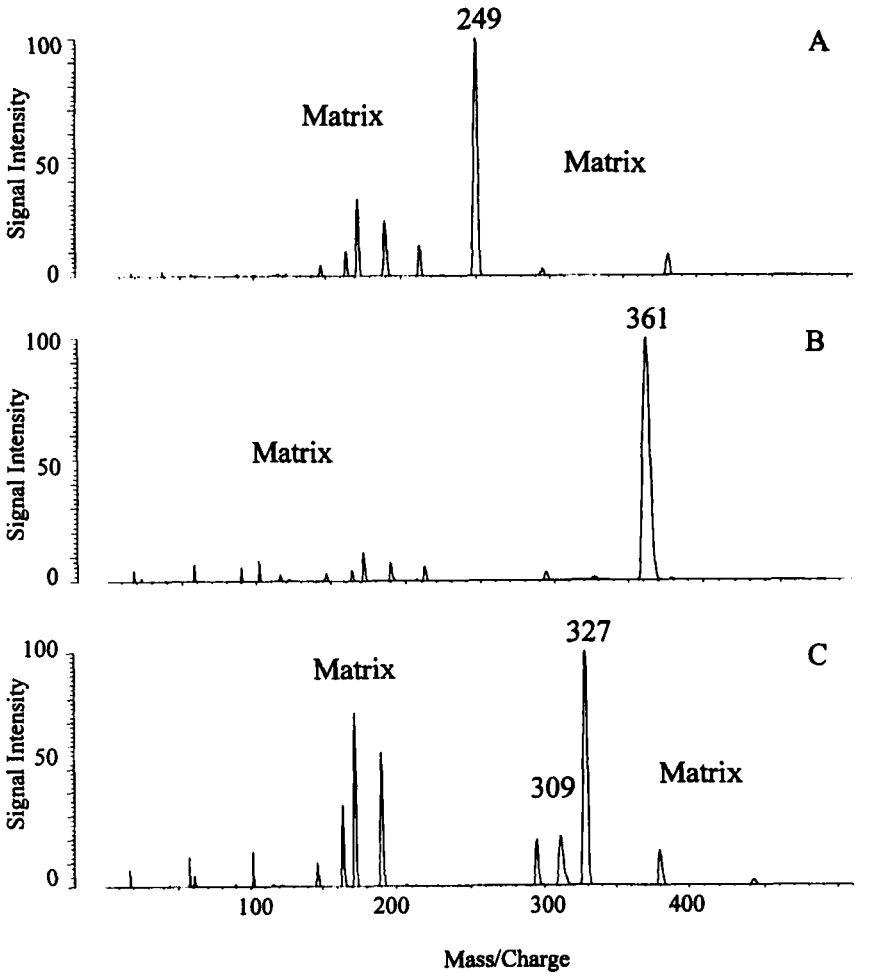


FIGURE 5 MALDI spectra of A) 0.4 ng difenzoquat methylsulfate, B) 4 ng phosphon and C) 40 ng glyodin obtained directly from cellulose TLC plates, after separation

CONCLUSIONS

A protocol to analyze cationic pesticides using mass spectrometry (MS) after analyte separation on thin-layer chromatography (TLC) plates was developed and tested on normal phase, reverse phase and cellulose TLC plates. The key parameter in the successful coupling of TLC-MALDI is limiting the lateral

spreading of the analyte during both the TLC separation and the coupling of the TLC plate with the MALDI matrix. Detection limits obtained were variable from analyte to analyte but were comparable between the different types of stationary phases. The higher detection limit obtained from TLC-MALDI, compared to the limits obtained from stainless steel represents the inherit cost of achieving separation of the analytes before MALDI analysis.

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