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FUNDAMENTAL STUDIES WITH A MONODISPERSE AEROSOL-BASED LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY INTERFACE (MAGIC-LC/MS)

~~FINAL~~ Progress REPORT

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ACCOMPLISHMENTS DURING GRANT PERIOD SEPTEMBER 1, 1985 to NOVEMBER 30, 1989

Note: This report attempts only to summarize the accomplishments made during the term of the present DOE grant. More detailed descriptions of many projects are contained in publications in the open literature. Research material relevant to ongoing and proposed research projects is also described in the attached proposal requesting renewal of support.

A number of accomplishments have been made during the term of this grant. These include both technical aspects of the project, and developments associated with commercial development of the MAGIC-LC/MS interface.

Technical advances have been made through a number of detailed studies of fundamental processes occurring within the interface. These have led to substantial improvements in the performance of the interface, many of which are detailed below. Of particular importance has been a major improvement in detection capabilities. At beginning of this grant period, sensitivity for many compounds was in the high μg to low mg range. Presently, full-scan (40-400 daltons) electron impact mass spectra may be obtained for many compounds with typically 10-50 ng of material injected on column. Selected ion monitoring gives detection limits in the range of 100-1,000 pg. These figures are particularly noteworthy because they represent values which fall between 3-10x the lowest detection limits possible with the same instrument when used in a GC/MS mode.

From a practical perspective, a licensing agreement signed between Hewlett-Packard and the Georgia Tech Research Corporation led to the development of a commercial version of the interface, which was introduced to the market on July 1, 1988. To date, there are approximately 100 interfaces installed worldwide. The research leading to the original MAGIC-LC/MS patent preceded the DOE research support for this project. However, the metamorphosis of the interface from a laboratory concept to a working reality has taken place entirely under DOE support, during the current grant period. The main thrust of this research has been the study of fundamental aspects of the interface, in order to better understand basic processes occurring in the interface operation.

The MAGIC interface was specifically designed to overcome a number of the functional problems experienced with other LC/MS approaches. MAGIC-LC/MS can be used to generate either EI and/or CI spectra of compounds that are either too involatile or too thermally labile to allow their direct analysis by GC/MS. While early designs of the interface have proved successful in generating EI spectra of involatile compounds and in producing detection limits in the nanogram range, a number of features were in need of improvement.

Problems with earlier models of the interface included: (1) difficulty of maintaining accurate nozzle/skimmer alignment (2) poor reproducibility (3) inadequate sensitivity for many separations, and (4) undesirably large dead volumes in both aerosol generator and aerosol evaporation chamber. Based on this need, and following principles of aerodynamics and particle dynamics, an improved MAGIC-LC/MS interface has been developed. This interface overcomes many of the limitations of earlier models.

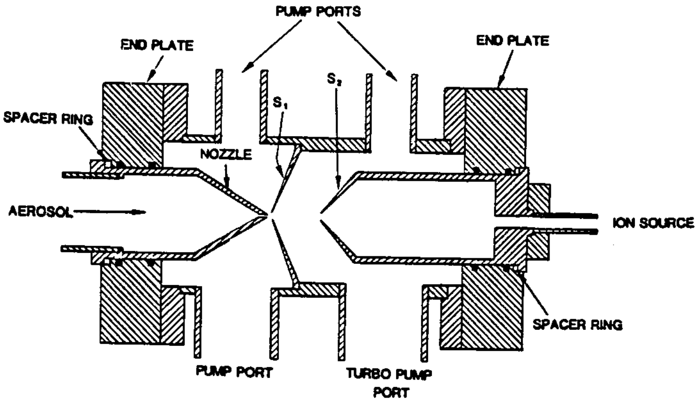

Figure 1: Schematic of Redesigned MAGIC Momentum Separator

Improvements in Interface Design

Aerosol generator. The newly designed aerosol generator uses cylindrical bore fused silica capillary tubing in place of the conical glass tip of the earlier aerosol generator. The fused silica capillary is of the type used routinely for capillary GC applications, and has a polyimmide coating. In the new aerosol generator design, a short length of 25 μm i.d. fused silica is connected to a length of 200 μm i.d. fused silica, using a zero dead volume fused silica connector (Valco). The 200 μm capillary tube is used to connect the aerosol generator with the LC column. The liquid jet is formed at the tip of the 25 μm i.d. tube. This aerosol generator is much simpler than the previous design, and seldom clogs.

Momentum separator. Transport efficiency studies have indicated that analyte loss in the MAGIC interface occurs primarily in the momentum separator. The most important analyte loss processes are the result of particle sedimentation, poor nozzle/skimmer alignment and turbulence loss. The earlier MAGIC interface incorporated two cylindrical-bore tubes in the nozzle/skimmer design, with each tube capable of adjustment in the x-y plane at one end only. This approach suffered from two major disadvantages. First, accurate alignment was difficult, because only one end of the tube could be moved during the adjustment process. This tended to cause the axial alignment of the skimmer and nozzle tubes to be disturbed during adjustment. Second, the tubes were typically several centimeters long, which resulted in substantial particle loss due to particle impaction from the expanding beam onto the tube walls.

The improved MAGIC momentum separator (Figure 1) is substantially shorter than the original version, and the nozzle and skimmers are fixed in place radially for proper alignment. The momentum separator body is made from stainless steel. Nozzles and skimmers are machined from 6010 grade aluminum. The nozzle has a 0.5 mm orifice at the apex of a 60^{0} cone. The separation between the nozzle and first skimmer is continuously variable from 0 to 10 mm through the use of shims. Vacuum seals between the various chambers are with Viton O-rings. The first skimmer has a 100^{0} exterior angle and a 95^{0} interior angle, with a 0.5 mm orifice at the tip. The second skimmer has a 45^{0} exterior angle and a 30^{0} interior angle, with a 1.0 mm orifice. The separation between the skimmers is adjustable from 0 to 10 mm. Both vacuum chambers are pumped with mechanical vane (hot oil) pumps of 21.6 m^3/h capacity. A tubular vacuum inlet, with the same dimensions as a direct inlet probe (DIP) or standard GC interface probe, is bolted directly to the second skimmer. This allows the interface to be connected rapidly and simply to the mass spectrometer directly through the DIP vacuum lock. Accurate alignment of the probe to the ion source is then provided by locating the probe tip against the ion source inlet.

The improved design has many advantages over the previous design: (1) it is easier to set up and operate, resulting in less down time of the mass spectrometer (2) the alignment between nozzle and skimmers is fixed and its accuracy depends only on the quality of the component machining (3) the skimmer design is aerodynamically superior, resulting in a less turbulent gas flow through the interface (4) the new momentum separator has higher transport efficiency than the previous design, which results in improved detection limits (5) the lower dead volume in the aerosol evaporation chamber causes less peak broadening, which improves both interface chromatographic resolution and analyte detection limits.

Interface Characterization

Substantial progress has been made on characterization of the interface, in terms of the aerosol generation and transport mechanisms that control the throughput of the system. The relationships that exist between analyte transport and signal magnitudes have also been studied, in an attempt to generate a preliminary understanding of the relationship between the three variables of aerosol transport, analyte volatility, and mass spectrometer ion count. This is of key interest because of the need to characterize the response sensitivity of the mass spectrometer to compounds of widely different volatilities.

Aerosol Generation. The characteristics of the aerosol generated by the monodisperse aerosol generator (MAG) have been determined, using laser Fraunhofer scattering, in order to determine the influence of important parameters, such as solvent flow, He gas dispersal flow, and solvent type on the properties of the generated aerosol. The detailed studies have demonstrated that while there is a significant difference in the primary (unevaporated) drop size between pure aqueous and pure organic solvents, there is very little difference between the primary drop sizes of aerosols generated from different organic solvents, even those of widely different volatilities, such as acetonitrile and hexanes. This is attributed to the predominance in the aerosol formation process of surface tension-related properties. The primary difference between the solvent types is therefore between water, with a surface tension around 80 dyne/cm, and organic solvents with surface tensions in the region of 30-40 dyne/cm.

The slightly larger diameters of the aqueous aerosols compared with the pure organic aerosols make efficient solvent removal somewhat harder for high percentage aqueous solutions. This is not very important at low solvent flow rates, but becomes increasingly important at flow rates in excess of 0.5 mL/min. Current studies are centering on means to overcome this limitation, and ensure that solvents up to 100% aqueous buffers may be used routinely under normal liquid chromatography flow conditions.

Interface Transport Processes. The transport of analyte through the interface has recently been studied in detail using a specially designed test device. This has a great advantage over the direct use of the interface with our own mass spectrometer, through allowing unrestricted access to the instrument for mass spectral studies and through avoiding the high possibility of source contamination. The setup is based on a test chamber which is evacuated with a turbomolecular pump to simulate the pressures normally encountered in the ion source volume of a mass spectrometer. It also includes a valve system which allows the collection of analyte test aerosol on a removable probe. The probe can be removed through a butterfly valve and O-ring seals. This allows a number of samples to be collected in sequence, without the need to pump down the chamber between runs.

The performance of the interface has been characterized very precisely using different solvent types, ranging from the highly volatile hexanes to the least volatile solvent used, namely water.

Preliminary data have shown that the current model of the interface is substantially more efficient in analyte throughput than earlier models. The current model gives approximately 17% throughput, compared to 5% with the previous model. This mass transport improvement of 3.5 x leads to corresponding signal enhancements, which is clearly of analytical importance. Test have shown that the majority of the remaining aerosol is lost in the two stages of the momentum separator. Detailed studies have also been made to determine at what precise positions the losses occur in the interface. It is anticipated that further design improvements will allow the further reduction in loss processes in the interface, with resultant signal enhancement.

Chromatographic Studies

A number of solvent and analyte systems have been tried, in an attempt to test the practical performance of the system. These include both reversed phase and normal phase systems. Sample types examined include antioxidants, pesticides, and bis-phenol A dimers, of a type of great interest to the polymer industry. In addition, the interface has been used very successfully with 2.1 mm bore i.d. columns, with no loss of performance, and no detectable peak broadening.

It was previously believed that the factor determining the maximum flow rate would be solvent evaporation rate. If the mobile phase has a high evaporation rate, all solvent droplets would be expected to desolvate before reaching the momentum separator. Heptane (165 $\mu m^3/sec$), for instance, would peak at a higher flow rate than methanol (47.2 $\mu m^3/sec$). This, however, is not found experimentally. It was also predicted that perhaps the total volume of solvent vapor played a key role. Calculations show that the total volume of heptane at 0.5 mL/min would be 0.0766 L/min and methanol would be 0.280 L/min. One can see that the methanol produces a far greater volume of gas phase molecules. In reality, however, the use of heptane affects the response more dramatically than methanol. The total volume of solvent vapor after evaporation does not therefore appear to account for the apparent trend.

Another possible explanation is that those solvents with higher vapor pressures saturate the atmosphere in the desolvation chamber and produce inefficient evaporation of the remaining droplet. The droplets, instead of desolvating, would impact on the nozzle of the momentum separator. The vapor pressure of heptane < toluene < 2-propanol < acetonitrile < benzene < ethyl acetate < methanol. The observed trend of peak flow rates does not follow the vapor pressure order given above. In addition, there was no visible evidence of impacted solvent to confirm this possibility.

Mobile phases utilized with the MAGIC-LC/MS interface have a useful flow rate range from 0.2 ml/min to approximately 1.2mL/min. A flow rate of at least 0.2 mL/min is necessary to form an adequate liquid jet with the nebulizer. At high flow rates, solvent will begin collecting on the glass desolvation chamber and on the inside of the nozzle thus incurring losses.

Gradient Elution. There is not a great change in either intensity or peak shape as the solvent composition is changed from 100% hexane to 100% ethyl acetate. The observed change in intensity is cyclical in nature. This phenomena has been described previously. As the peak is sampled by the ion source, it is scanned and then the scan is reset. Because it is not continuous, one may or may not sample the actual peak top. From the data shown here it may be concluded that normal phase gradients may be utilized without concern for significant changes in spectral features or response.

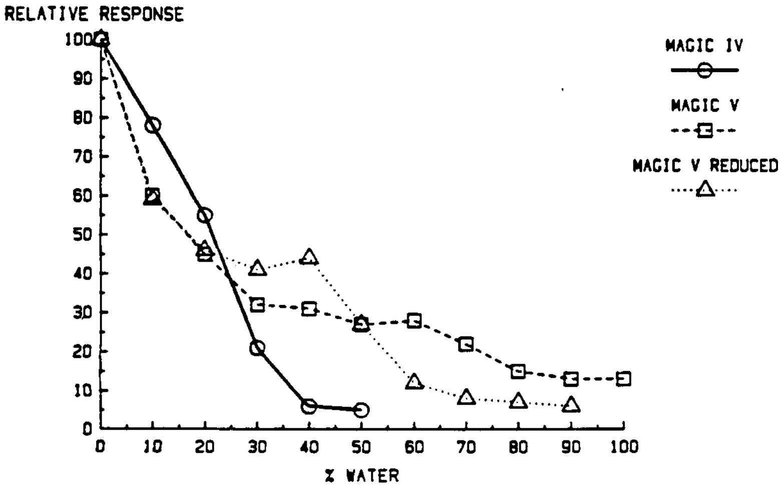

Figure 2: Mass Spectrometer Response vs. Solvent Composition

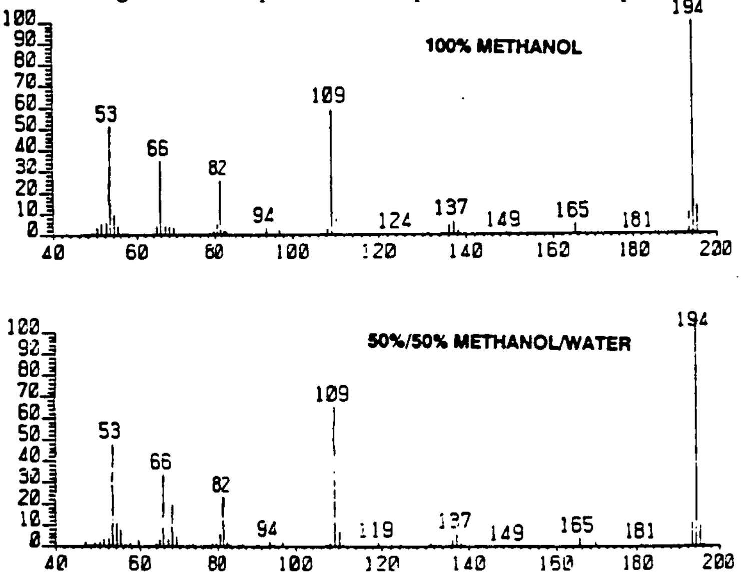

Figure 3: EI Mass Spectra of Caffeine at 100% MeOH and 50/50 MeOH/H_2O

A reversed-phase gradient involves the combination of water with some organic modifier, where the concentration of the organic modifier is gradually increased to bring all compounds in the separation off the column. Unfortunately, the presence of water is a more difficult situation with the MAGIC-LC/MS interface. From Figure 2, it can be seen that the response increases as the organic modifier concentration increases during a reversed-phase gradient. The increase in response from 50/50 methanol/water to 100% methanol will be approximately two and one-half times. These data are promising however, because no modifications were performed such as extraneous heating of interface parts or solvent.

Figure 3 is an example of the spectra obtained while changing the mobile phase composition. The spectra were obtained at 50/50 methanol/water and 100% methanol solvent compositions. Both spectra are nearly identical and also agree with the NIH spectrum. It is noteworthy that no solvent cluster ions are observed in the spectra obtained with the MAGIC-LC/MS interface.

External Variance. In conventional high performance liquid chromatography systems, external variance is caused by an increase in volume between the injector and the detector. External variance can also result

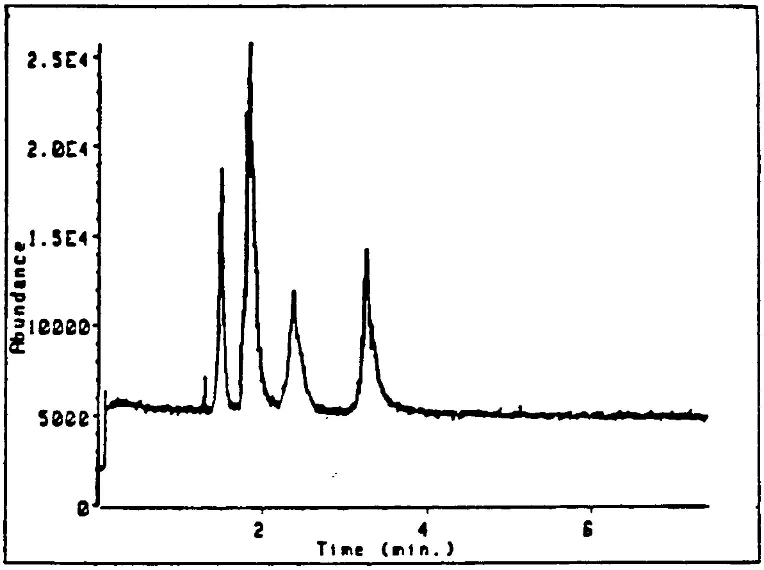

Figure 4: Total Ion Chromatogram Used for Measurement of Peak Variance

from an increase in the sample volume injected. The sources of external variance include large diameter or extra lengths of tubing, fittings which do not meet zero-dead volume requirements, and detector flow cells. In addition, the detector electronics and recording systems may contribute to the overall external variance.

In conventional liquid chromatography, liquid dispersion in the tubing is a consequence of a parabolic velocity profile across the tubing and turbulent rather than laminar flow. The MAGIC-LC/MS interface is an aerosol-based interface and the peak dispersion should be minimal as long as the flow is laminar and not turbulent. The velocity profile should largely be eliminated because less drag will occur against the gas phase and in partial vacuums than along a stainless steel surface of the tubing. In addition, dispersion will be reduced because the sample band will have reduced residence time as compared to a sample passing through the same length of tubing. It appears from the above discussion that the only point where variance may occur appreciably in the MAGIC-LC/MS interface is at the point where vaporization takes place.

The variance of a chromatographic band is measured by calculating the second statistical moment, M_2, of an eluting band. The total peak variance is a measure of the contribution of independent factors which can be treated as additives in their second moments or variances, σ^2.

$$\sigma^2_{ext} = \sigma^2_{col} + \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{conn} + \sigma^2_{oth}$$

These independent factors include contributions from the column variance, σ^2_{col}, the injector variance, σ^2_{inj}, the detector variance, σ^2_{det}, any variance that results from connections, σ^2_{conn}, and other factors, σ^2_{oth}. Extra-column effects lead to $\sigma^2_{tot} > \sigma^2_{col}$ and this additional band spreading causes the chromatographic resolution to fall short of the inherent column capability.

The method chosen here is that of the exponentially modified Gaussian (EMG) as the skewed peak model. This is a graphical method based on measurement of the retention time, t_R, peak width at 10% peak height, $w_{0.1}$, and the empirical asymmetry factor, B/A. The second statistical moment, M_2, which gives the total variance, r^2_{tot}, is given by the equation below.

$$M_2 = w_{0.1}{}^2/1.764(B/A)^2 - 11.15(B/A) + 28$$

This equation is accurate to within ±1.5% for $1.00 < B/A < 2.76$. All experimental values were within these limits.

Figure 4 shows the total-ion-chromatogram collected at 300 °C. Similar chromatograms were obtained for the other experiments. The retention times were obtained from the mass spectrometer data station. Table 1 contains the calculated values for σ^2_{ext}, the total variance; r,the correlation coefficient; and σ_{ext}, the external variance.

For conventional and fast liquid chromatography columns, the MAGIC-LC/MS interface system appears to be completely compatible at both 300 °C and 220 °C with the capillary tubing transfer line. The maximum allowable external variance for a conventional column is 15 μl. The values for MAGIC at 220 °C and 300 °C are 10.60 μl and 3.84 μl respectively, well below the limit. The MAGIC-LC/MS interface compares favorably to other LC/MS interfaces. The DLI interface has a reported variance of 10-40 μL. Most of this is attributable to the long length of tubing necessary to reach the ion source (at least 40 cm) utilized in most commercial DLI systems. The moving-belt interface has a reported value for fluorene at 1 mL/min of 15.8 μL. The reported variance for the moving-belt interface is compound dependent, however, and significant skewing is reported for polar compounds.

Table 1

Values calculated from linear regression analysis of variance and retention data.

Capillary Transfer Line

Temperature	$r^2_{ext}(sec^2)$	r	$r_{ext}(uL)$
300° C	0.59	.9789	3.84
220° C	4.49	.9077	10.60

Temperature Effects and Compound Response for the MAGIC-LC/MS Interface

The temperature of various components in many LC/MS interfaces has often played a crucial role. For instance, in thermospray LC/MS, a change in the vaporizer temperature of just 10 °C to 20 °C in either direction can modify signal levels by as much as an order of magnitude (1). Temperature control in the moving-belt interface is also crucial to vaporize the analyte off the belt itself; and in DLI, the ion source temperature can have profound effects on both spectra and intensity. Often, complicated means are necessary to control the temperature of LC/MS interface components.

Desolvation Chamber Temperature Studies. Figure 5 shows the results obtained as the desolvation chamber temperature was varied for three different solvent compositions. For a methanol mobile phase, the optimal performance corresponds to a temperature setting of between 27-32 °C measured at the wall. The center of the chamber had a temperature of between 18-26 °C.

As water is added to the mobile phase, the optimum desolvation chamber temperature increases. For a mobile phase consisting of 90%methanol/10%water, the most useful desolvation chamber temperature exists between 34 °C to 36°. An even higher water composition requires more desolvation chamber heating. At 50% methanol/50% water, the best temperature setting was approximately 39 °C.

An important observation of this experiment is that as the percentage of water is increased, the temperature of the desolvation chamber becomes more and more important. A variation in desolvation chamber temperature of 5 °C at 100% methanol causes only a 25% decrease in signal. At 50%methanol/50%water, however, the signal decreases by 65%. The implication of this observation appears

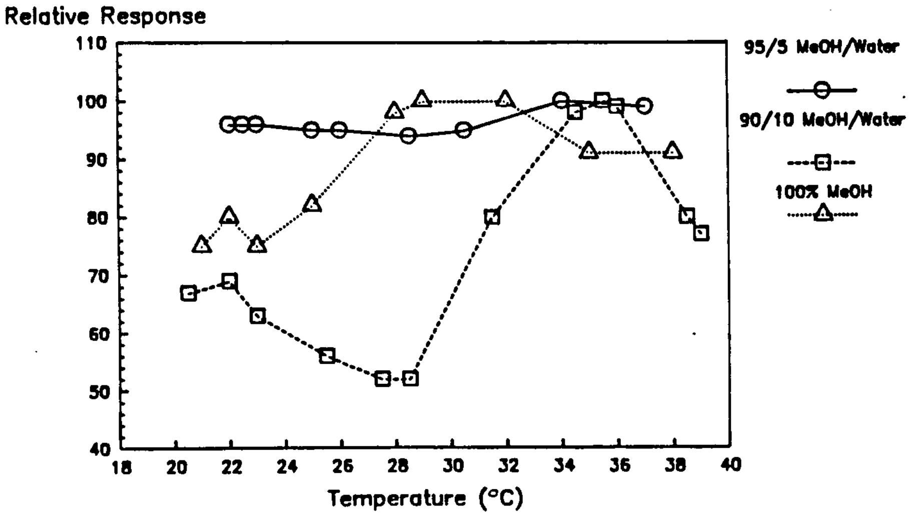

Figure 5: Influence of Desolvation Temperature on Response

to be that some of the problems of reversed-phase gradients can be solved with a variably thermostated desolvation chamber.

Ion Source Temperature Effects. The proposed mechanism for ionization with the MAGIC-LC/MS interface involves vaporization of the analyte particles off the walls of the ion source. It is therefore apparent that the ion source temperature is expected to play an important role in the signal production.

The ion source temperature of the mass spectrometer was varied while all other parameters were held fixed. An easily volatilized compound, methyl stearate, was tested along with some less volatile fatty acids. The results of the test appear in Figure 6. Finally, a thermally labile and involatile compound, glucose, was studied. It may be observed that the more volatile compound is best run at a lower ion source temperatures than the less volatile compound. However, temperatures between 180-280 °C, give good results without affecting the signal by more than 20% for both volatile and involatile compounds. The response of the labile sugar, glucose, peaks at a somewhat lower temperature than the fatty acid response due to the decomposition which occurs at high temperatures producing fragments which appear below the solvent cutoff and are thus not recorded.

Thermally Labile Compounds

Aldicarb. Many compounds are precluded from gas chromatography/mass spectrometry analysis not by their volatility, but by their thermal lability. In other words, these compounds decompose rapidly when exposed to thermal energy. Such a group of compounds is the carbamate pesticides which find much current use due to their effectiveness and low mammalian toxicity. Liquid chromatography is highly suited for thermally labile compounds and its combination with mass spectrometry should give sensitive, specific detection.

Many LC/MS interfaces, unfortunately, utilize heat in the process of transforming the mobile phase and analyte into vapor and analyte gas molecules. Thermal degradation of compounds has been reported with moving-belt LC/MS, as well as, thermospray LC/MS.

In order to test the MAGIC-LC/MS interface capabilities with thermally labile compounds, a sample of aldicarb, was obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository (MS-8)(Research Triangle Park, NC). Aldicarb is a carbamate pesticide sold under the name of Temik. It rapidly degrades in

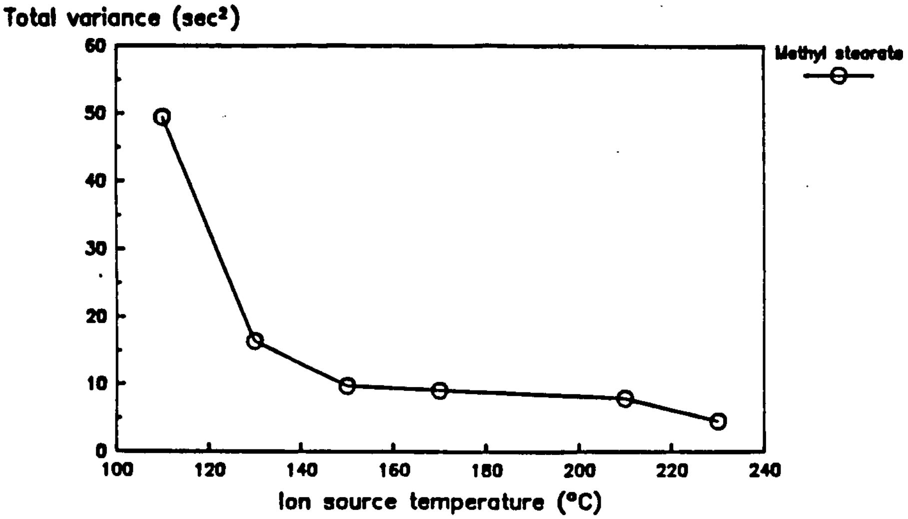

Figure 6: Influence of Ion Source Temperature on Response for Methyl Stearate

the injection port or on the column during gas chromatographic methods. The degradation product of aldicarb is aldicarb nitrile. Aldicarb would produce a molecular ion at m/z 190, whereas the molecular ion for aldicarb nitrile would appear at m/z 115.

At an ion source temperature of 82 °C, enough of the aldicarb molecule remains intact to give a molecular ion at m/z 190. As the ion source temperature is increased to 250 °C, degradation of the compounds clearly occurs. The spectrum obtained at 250 °C resembles the NIH spectrum reported for aldicarb.

Note that it was possible to obtain the intact molecular ion by lowering the ion source temperature. This illustrates that alternate ionization sources which do not require molecules in the gas phase can be utilized with this interface to obtain intact molecules of thermally labile compounds. The degradation of the compound is not significant in the interface itself, but occurs in the ion source.

Fast Atom Bombardment Ionization

The MAGIC-LC/MS interface has been used in an on-line mode to generate fast atom bombardment (FAB) spectra from a range of solvent types. By a relatively simple modification of the instrument, the fast atom beam is directed on the surface of a probe in the ion source of the mass spectrometer. Matrix material (*e.g.* glycerol) is combined with the analyte, either by post column addition or by placing the matrix directly on the probe. The particle beam then interacts with the analyte suspended in the matrix surface.

Classical FAB spectra are generated in this mode. These compare exactly with probe FAB spectra generated on the quadrupole, and also with spectra generated from a probe using a magnetic sector instrument. The important conclusions which may be drawn from this preliminary study are the following: (1) FAB spectra may be generated with a particle beam interface without any changes from its normal mode of operation for EI and CI spectral generation, with only the addition of a suitable collection surface in the mass spectrometer (2) The FAB mass spectra generated are independent of solvent type (*e.g.* hexanes, acetonitrile, MeOH, H_2O and gradients of these show no differences). This is because the solvent is removed very efficiently in the interface (3) Solvent flow rates which may be used are typical for EI/CI particle beam mass spectrometry, and are in the range 0.3-1.0 mL/min. (4) Matrices used for normal FAB probe work are also suitable for on-line particle beam use (5) Signal responses for the same mass of analyte generated directly from the probe and obtained with the particle beam interface are generally comparable.

Selected Applications of MAGIC-LC/MS Interfacing

The optimum columns for use with MAGIC-LC/MS interfacing are: (1) small bore columns with an internal diameter of 2.1 mm i.d., packed with 5 μm particles. Typically, flow rates range from 0.2 mL/min to 0.6 mL/min. (2) 4.6 x 60 mm columns, packed with 3 μm particles. Although these columns are usually employed at high flow rates (3-6 mL/min) they also work well at MAGIC's flow rates of 0.2-1.2 mL/min because their small particle size provides fast analysis time and little dilution. They are also ideal for high sample throughput.

Fatty Acids. A number of fatty acids were measured. All spectra showed the molecular ion peak as well as characteristic fragment ions. All peaks contained a base peak at m/z 129. This is not the normal base peak of a free fatty acid. Normally, the base peak is at m/z 74, the McLafferty rearrangement. Due to an impurity, however, the scan began at m/z 80. The base peak at m/z 129 is the fragment $[HOCO(CH_2)_6{}^+]$. Other prominent peaks represented include fragments formed from losses of $[CH_2]$.

Phenyl Urea Herbicides. Diuron, neburon and metoxuron show significant degradation in gas chromatography/mass spectrometry analyses. Electron impact spectra collected with the MAGIC-LC/MS interface were observed to contain the molecular ion as well as an abundance of structurally significant fragment peaks. The base peaks in all four spectra represent the cleavage of the nitrogen-carbonyl bond which is secondary to the phenyl ring. The appearance of isotope peaks which are indicative of chlorine presence are easily identified in the spectra.

CI spectra showed the $(M+H)^+$ ion as the base peak. The identification of a chlorine containing compound was again evident with the $(M+H+2)^+$ isotope peaks. This class of compounds is one of the most successful yet found to display the capabilities of MAGIC with low nanogram detection limits comparable to thermospray. The spectra are also much more informative than those of thermospray due to the presence of significant fragment ions.

Antioxidants. Antioxidants are commonly used as additives in plastics to enhance their lifetime and physical properties. A mixture of such compounds was readily measured using MAGIC-LC/MS. Elution was in order of molecular weight and all compounds analyzed displayed a molecular ion. BHT showed a base peak at m/z 205, an obvious loss of 15 mass units, the CH_3 moiety. The second eluting compound 2-hydroxy-4(octyloxyl)-benzophenone showed a base peak at 213 Da. This fragment is left after the loss of the alkane C_8H_{17}. The 4-4'-methylene-bis-(2-6-di-t-butylphenol) also gave a base peak which results from the loss of a CH_3 group. The final compound octadecyl-3-5-di-t-butyl-4 hydroxycinnamate gave a base peak from a much smaller fragment, m/z 73. Detection limits were in the hundreds of nanograms for this type of compound on the MAGIC interface.

Appendix VIII Compounds. Appendix VIII is a list of chemical constituents which have been shown in scientific studies to have toxic, carcinogenic, mutagenic or teratogenic effects on humans and other life forms.

Appendix VIII compounds are found in Part 261: Identification and Listing of Hazardous Wastes of the Federal Registry. The compounds examined included some benzidene compounds which cannot be analyzed

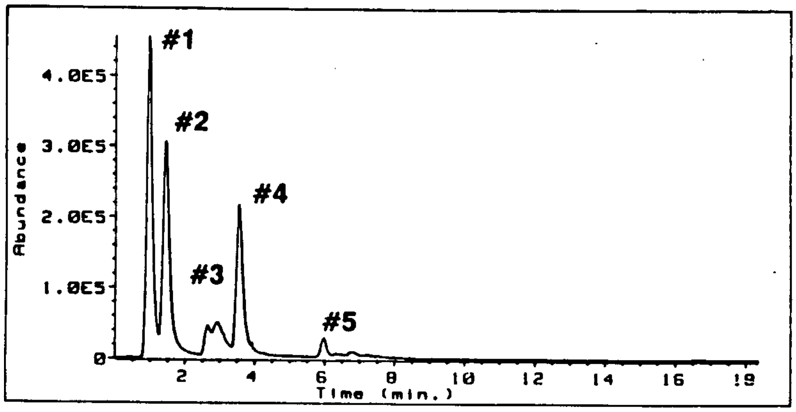

Figure 7: Total Ion Chromatogram for Selected EPA Appendix VIII Compounds. Solvent System pH5 Buffer

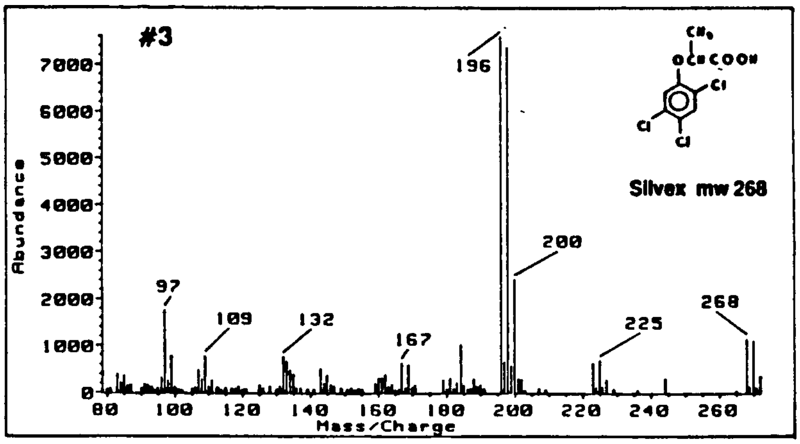

Figure 8: EI Spectrum for Silvex. (Peak #3 of TIC from Fig.7)

by gas chromatography, silvex which is a chlorphenoxy acid herbicide and reserpine, an indole alkaloid tranquilizer which is difficult to characterize by mass spectrometry.

The total-ion-chromatogram for the separation of Appendix VIII compounds appears in Figure 7. The elution order was benzidine, dimethoxybenzidine, silvex, dichlorobenzidine and reserpine. Spectra for silvex and reserpine are shown in Figures 8 and 9. The base peak for all benzidine related compounds was the molecular ion. A molecular ion for silvex is present along with isotope peaks indicative of three chlorine atoms. The base peak for silvex at m/z 196 is the [$Cl_3C_6H_2OH^+$] fragment. Reserpine displays a considerable molecular ion at m/z 608 and a base peak at m/z 195, the [$^+OC(C_6H_2)(OCH_3)_3$] fragment ion.

Summary

Scope of the MAGIC-LC/MS interface. As yet, the full range of compound types accessible with the MAGIC-LC/MS interface has not been fully investigated. In general terms, all compounds which are known to be capable of generating EI and CI spectra and which have been tried with the interface have generated

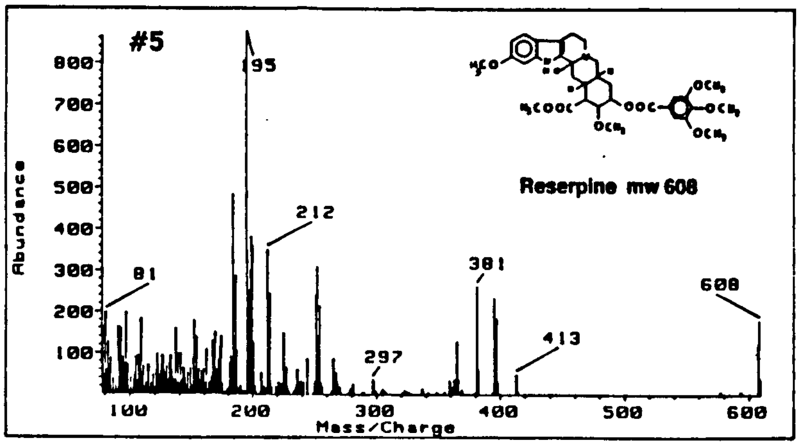

Figure 9: EI Spectrum for Reserpine. (Peak #5 from Fig.7)

good quality spectra. These include carbamate and triazine pesticides, phenyl urea herbicides, polynuclear aromatic hydrocarbons, plant alkaloids, antioxidants and EPA Appendix 8 compounds. Compounds known *not* to generate EI spectra, such as simple sugars and certain azo dyes, predictably do not generate spectra with the current system. The primary mode of ion formation appears to be through a flash vaporization step in the ion source, followed by EI or CI ionization, as selected. Any limitation on compound type accessible with MAGIC-LC/MS will be influenced by: (1) whether the molecule is functionally capable of generating an EI spectrum (2) whether the compound is sufficiently volatile to be capable of forming an adequate vapor pressure in the ion source. This latter property will ultimately represent the interface limitation for generation of EI and CI spectra, rather than the molecular weight of the species. Nevertheless, it is possible to generate good searchable EI spectra with strong molecular ions even for quite involatile species. An example is provided by reserpine, with a molecular weight of 608 daltons, which generates a searchable EI spectrum with a strong molecular ion at m/z 608. Very recently, good CI spectra have been generated of quite high molecular weight species. An excellent CI spectrum of Gramicidin-S (mw 1154) has recently been obtained.

Additionally, the recent work with FAB generation of spectra using on-line MAGIC-LC/MS has been extended to include environmentally important classes of compounds, such as azo dyes. The potential for FAB is to extend the MAGIC-LC/MS mass range initially to values in the 1,000-2,000 Da range, and ultimately well above this value.