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THE DEVELOPMENT OF TECHNIQUES FOR COMPLEX MIXTURE ANALYSIS BY MEANS OF GAS CHROMATOGRAPHY/TRIPLE QUADRUPOLE MASS SPECTROMETRY

By

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ABSTRACT

THE DEVELOPMENT OF TECHNIQUES FOR COMPLEX MIXTURE ANALYSIS BY MEANS OF GAS CHROMATOGRAPHY/TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Adam Jeremiah Schubert

Current technology in several phases of organic mixture analysis have been integrated to demonstrate the analytical power of the combined technique of gas chromatography/triple quadrupole mass spectrometry (GC/TQMS). This research has included capillary gas chromatography, chemical ionization, triple quadrupole mass spectrometry, and the high speed instrument control and data systems which are required to make such a powerful instrument feasible.

The feasibility of GC/TQMS was enhanced by an increase in overall system speed and the development of programs to allow trace-level targeted component analyses on time variant samples introduced via the gas chromatograph. The performance of the instrument control system was achieved by dividing instrument control tasks among multiple processors rather than by increasing the power of the processor being used. This choice provides greater flexibility, modularity, and sufficient power to allow future incorporation of such features as intelligent dual-mode (pulse counting and analog) data acquisition and programmed ion optics.

The data acquisition software for the triple quadrupole mass spectrometer was converted from a single microprocessor to a four microprocessor configuration. To facilitate achievement of the goal of targeted component analysis, additional programs were developed to perform multiple reaction monitoring (MRM, analogous to the GC/MS technique of selected ion monitoring) GC/TQMS.

Methanol chemical ionization was investigated as a tool for the mass spectrometric determination of trace level polar components in petroleum products. Results from this study indicate that methanol enhances both the selectivity and sensitivity of the ionization when compared to the more conventional technique of methane chemical ionization. Studies on the effect of varying the pressure of the methanol reagent demonstrated a simple approach through which the analyst can adjust both the sensitivity and selectivity of the ionization.

Detection limits were determined for the determination of several thiophenes in a commercial jet aviation fuel by means of GC/TQMS. The combined use of capillary gas chromatography, methanol chemical ionization, and TQMS with specialized data acquisition programs, enabled the detection of these targeted components down to the low parts per million.

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Chapter 1.

Introduction

The research presented in this dissertation covers several aspects of the development of the multidimensional analytical technique of gas chromatography/triple quadrupole mass spectrometry (GC/TQMS). Emphasis has been placed on practical concerns relevant to developing a working tool for the analysis of trace components in complex organic mixtures.

Chapter Two opens with a discussion of what goes into the development of a practical analytical technique. The chapter continues with a discussion of how gas chromatographic and mass spectrometric techniques meet the needs for the analysis of complex organic mixtures and closes with the potential of GC/TQMS for addressing this type of problem.

Chapter Three begins the discussion of the actual development of the control and data systems for GC/TQMS. It covers the adaptation of TQMS instrument control and data acquisition software from a single microprocessor control system to a multiple microprocessor system. This adaptation provides analysts with greater speed, flexibility, and power to apply to solving a wide range of analytical problems in a reproducible manner.

Chapter Four relates the development of software to perform multiple reaction monitoring (MRM), the TQMS analog to the GC/MS technique of selected ion monitoring (SIM). By making highly efficient use of analytical "run time," MRM provides a sensitivity for trace analysis which is greatly enhanced relative to that which is available by full-scan TQMS.

Chapters Three and Four are supplemented by Appendices A and B. Appendix A presents a very detailed description of the software discussed in Chapter Three and Appendix B consists of annotated listings of several key portions of the software.

Chapter Five of this dissertation covers research which investigated the sensitivity and selectivity of methanol as a chemical ionization reagent for the analysis of trace polar compounds which may be present in fuels. Chapter Six applies this methanol chemical ionization work, in conjunction with the GC/TQMS instrument, and the control and data systems, to the analysis of trace level components in a complex organic mixture. Specifically, the chapter presents the use of capillary GC/TQMS with methanol chemical ionization for the determination of low parts per million levels of thiophenes in commercial jet aviation fuel.

Chapter Seven brings the dissertation into focus with a discussion of how the different elements of the GC/TQMS instrument individually, and in concert, contribute to the analysis of complex organic mixtures. The chapter closes with some conclusions and a brief discussion of possible future work.

Chapter 2.

The Analysis of Complex Organic Mixtures

Criteria for Complex Mixture Analysis

The identification and quantitation of trace species in complex mixtures is a classic problem confronting the analytical chemist. This dissertation covers the development of techniques for complex mixture analysis, thus, it is useful to begin with a discussion of criteria for the evaluation of this type of work. The necessary considerations for this type of analysis may be categorized as follows:

Qualitative and Quantitative Information - In order to select the compound(s) of interest from a complex sample matrix, sufficient qualitative information must be available to enable a positive identification. Quantitative accuracy requires an analytical signal that is sufficiently reproducible as to allow generation of stable working curves. Depending on the nature of the analysis, the working curve may be derived from internal standards, external standards, or standard addition.

Sensitivity and Detectability - The quantitative analysis of trace components necessitates both high sensitivity and low detection and quantitation limits. The limit of detection corresponds to the smallest quantity of analyte for which the analytical signal can be reliably distinguished from background (often defined as the amount of analyte giving a signal-to-noise ratio of 3.) For quantitative work, a limit of quantitation, the smallest concentration of analyte for which the working curve is valid, is often defined at a signal-to-noise ratio somewhat higher than the detection limit.

Sensitivity is defined as the derivative of signal with respect to analyte concentration (i.e. the slope of the working curve, if it happens to be linear.) The sensitivity of a technique must be sufficient that signals corresponding to the smallest concentration difference of interest can be distinguished. For example, it may be defined that the smallest difference in signals that can be reliably measured is three times the random noise. If it is then necessary to distinguish between analyte concentrations of 1.0 ppm and 1.1 ppm under conditions where the random noise is 10 units of signal, the sensitivity must then be at least 300 units/ppm ([3 x 10]/[1.1 - 1.0] = 300). However, measurement systems have a finite dynamic range, hence, excessive sensitivity can limit the range of analyte concentrations which may be determined.

Analysis Time - Three of the major factors influencing the choice of an analytical method are cost, throughput, and turnaround time. Complex mixture analyses are often required as responses to crisis situations. In such cases, the rapid availability of even preliminary results can be crucial. Two of the biggest cost factors in this type of analysis are the purchase and maintenance of the necessary instrumentation and the time of a skilled analyst. The high costs associated with instrumentation for complex mixture analyses are amortized across the number of samples which can be analyzed in the available instrument time. Usable instrument time in itself can often be the limiting resource. In such cases, decreased demands on instrument time effectively reduce the costs associated with the analysis of an individual sample while maximizing the availability of scarce instrumentation resources.

Another scarce resource is the time of skilled analysts who are frequently responsible for both instrument operation and data interpretation. Because of these factors, reducing the analysis time not only serves to reduce the overall time demands on the analyst, but also serves to maximize the portion of time available for interpreting the data, a task generally not amenable to automation.

For routine analyses, the predominant time constraint is the required sample throughput, defined as the number of samples which may be processed in a given time interval. Throughput for a given analysis is increased by reducing the analysis time of an individual sample or by increasing the

number of operations that may be either overlapped or performed in parallel for a number of different samples. The overall analysis time per sample is generally fixed by the choice of method. Where there are analysis steps that require little operator attention, or where more than one analyst/technician is available, several steps of the analysis may be overlapped -- for instance, an analyst may be interpreting the results of one sample, while an automated instrument is running the next one, and a technician is preparing additional samples. The throughput gains possible with overlapped tasks depends on how many analysis steps may be efficiently overlapped and the amount of time/attention required by the slowest step. Parallel operation refers to the case where a number of samples simultaneously, can be affected for a particular step in the analysis, such as a preliminary separation. This approach is a very effective way to increase sample throughput in cases where the parallel operation is the major time-consuming task in the analysis and the costs of the duplicate apparatus required for the parallel operations are sufficiently small.

Sample Preparation - The amount of sample preparation required for an analysis can have significant effects in terms of sample contamination, turnaround time, throughput, and analysis cost. Any manipulation of a sample provides an opportunity for causing undesired alterations in its composition. The magnitude of this problem is multiplied when analyzing for trace components. Sample treatments,

such as extractions and chromatography can also be very time-consuming to the extent of often being the time limiting factor in the analysis. The degree to which sample preparation activities for multiple samples can be either overlapped or performed in parallel can often be the limiting throughput factor when there is a high sample load. Finally, sample treatments consume analyst time, equipment, and supplies, all of which can add greatly to the cost of the analysis.

Cost - Before any analytical technique can be selected for solving a real problem, its costs, measured relative to alternate methods for obtaining the desired information for the required sample load, must be commensurate with the value of the information that it provides in the available time. Costs for a given technique can be attributed to the purchase cost of the instrumentation and related equipment, maintenance costs, the physical plant necessary to support the instrumentation, consumable supplies, analyst time for sample preparation, analysis, and interpretation, and training costs necessary to provide the skilled analysts and technicians necessary to support the technique.

For routine analyses, where there is a steady sample load, analysis costs are best measured relative to throughput. Within the bounds of available personnel, capital equipment and physical plant, increased throughput can allow the fixed costs associated with an analysis to be spread over a larger number of samples. The realization of

these throughput-related benefits, however, requires a sufficient sample load to justify the investment in additionally dedicated resources. That is, it does little good to set up a method for a fifty sample per hour throughput in a laboratory which only analyzes two hundred of these cases per month.

Repeatability and Reproducibility - Complex mixture analyses are often complicated by having to work with small amounts of sample, where replicate analyses are not possible. Additionally, the analyst must be able to make interpretations based on samples run over a period of time, and at different laboratories. These factors stress the need for repeatability and reproducibility in the analysis. These problems are generally approached by definition of standard method validation protocols and the analysis of standard reference materials both over time and in inter-laboratory comparisons. In the analysis of trace components in complex mixtures, however, time pressures and the need to do "one-of-a-kind" analyses makes it difficult to define standard protocols, and the manufacture and distribution of standards for trace components in complex mixtures is exceedingly difficult. As a result, the need for overall instrument reliability and good analytical practices is especially pronounced.

Approaches to Complex Mixture Analysis

In evaluating different methods of complex mixture analysis, consideration should be given to three distinct cases of the type of information desired. The first case occurs when the analytical goal is to quickly determine whether or not any items in a small list of targeted items are present and above some pre-determined threshold level. The second case to be considered occurs when detailed information on the identity and amount of a large number of mixture components is sought. A third case is where general information on the overall composition of the mixture is needed. Each of these cases place very different requirements on the analytical technique to be employed and, thus, all of the complex mixture analysis considerations discussed above must be weighed quite differently.

Screening Analysis - In the first case, which is a rapid screening analysis, the principal goals are speed and a yes/no type answer for each targeted item. For this type of analysis, conditions are adjusted to be selective for the targets, thus minimizing the amount of additional information required for positive identifications. Quantitative information is desired only to the extent of comparing it to a threshold value and the sensitivity and reproducibility need only be sufficient to make a reliable comparison to the action level. Cost considerations are exceedingly important due to the high sample load generally accompanying this type

of analysis. Further, high throughput is essential both as a means of spreading out the fixed analysis costs over a larger number of samples and to reduce the effective turnaround time.

Total Composition Analysis - The second case is that of a very detailed analysis. For this type of work, the goal is to obtain as much qualitative and quantitative information as is possible. Further, to enable the study of trace components, there are high demands on sensitivity and dynamic range. In order to reliably examine trace components, it is still quite important to minimize sample preparation. To obtain all of this information from the sample, a price is generally paid in terms of analysis time and cost.

Compound Type Analysis - The third case is a compound type analysis. In this approach the goal is to obtain general information on the overall composition of the mixture and the distribution of related components. Type analyses assume that a particular set of signals can be used to characterize the presence and abundance of specific compound types. This approach precludes the analysis of individual compounds which may be present as unknowns and impurities. In fact pre-separations are frequently employed to remove trace components which may interfere with the indicator signals. The great utility of this approach comes from the fact that specific, predetermined information of a statistical nature is being sought; This enables the use of highly

automated data treatments which reduce the data to a tabular or graphic format which can be easily interpreted. This approach is frequently used in the petroleum industry for the comparison of different samples of a particular type of fuel such as middle distillates, gas-oils, and low olefinic gasolines (1, 2, 3).

Data Organization - A further difference in these three analysis cases is the amount of information being generated. For the rapid screening only a small amount of information is generated -- a yes/no for each item in the target list. This simple information list allows for great flexibility in the manner in which the data are reported. In a detailed analysis, however, there can be an overwhelming amount of information generated. This data absolutely must be organized in some convenient a logical manner before any analyst can be expected to make a useful interpretation. Further, not all of the large quantities of data generated are relevant; much data must be weeded out and interpreted by the analyst before the desired information is obtained. Thus, as a very practical matter, the design of a technique for detailed analysis must consider approaches to reduce the data. The data obtained in a type analysis is readily summarized, but can only provide general information about the mixture as a whole.

Techniques for the Analysis of Complex Organic Mixtures

Many techniques are employed for the analysis of complex organic mixtures. These techniques have wide ranges in capability and convenience for different cases of analysis. These capabilities also vary widely among different instruments of the same type and are constantly evolving. In general, however, they all have some fundamental strengths and limitations which must be understood before different analysis methods can be properly considered.

Mass Spectrometry Applications - One of the first instrumental techniques to be employed for organic mixture analysis was mass spectrometry. Conventional mass spectrometry has been demonstrated to be extremely useful for the determination of individual components in simple mixtures. This approach may be used when the analyst has a fairly good idea of the components which are likely to be present, thus allowing the individual spectra to be deconvoluted by comparison with reference spectra. Conventional mass spectrometry has also been widely applied to the performance of type analyses on complex mixtures (4). A type analysis, however, does not yield information on individual components, but rather approximate totals for groups of structural isomers by means of monitoring a mass which is characteristic of that group. Additionally, in cases of

highly complex mixtures in which different compound types having isobaric characteristic peaks are present, the sample often requires a preliminary separation in order to simplify the identification process while still using a low resolution instrument (5). High resolution mass spectrometry can reduce this problem (6), but greater complexity in instrument operation tends to restrict usage, especially in routine cases. In general, type analyses are useful only when general information is required about the distribution of compound classes which make up the bulk of a sample of a type which is routinely analyzed.

Generally, mass spectrometry with a batch or probe inlet is best suited for the analysis of simple mixtures where interferences between the components are not disabling, or for type analyses of more complex mixtures when only general information on the distribution of major sample components is sought. Type analyses, however often require a pre-fractionation of the sample to minimize interferences and, by their algorithms, preclude the identification of any individual trace component.

Gas Chromatography Applications - Gas chromatography (GC) presents a complementary method for analyzing complex mixtures. Unlike mass spectrometry, where all of the sample components are analyzed simultaneously, GC disperses the individual components of the sample in time, allowing the detector to measure each individual component as it elutes from the column. The GC detector, however, does not

generally provide sufficient qualitative information to identify the compound. The only information available is the retention time and peak area for each resolved component. In general, the retention time for a given compound is a function of the column type, stationary phase, the column oven temperature program, the structure of the compound in question, and its boiling point. The peak area for a given component is dependent upon the functionality of the molecule, its elemental composition, and its concentration in the sample. Assuming that the resolution of the chromatogram is sufficient to prevent any significant overlapping of peaks, the retention time, measured relative to well-known reference components in the sample, can be extremely useful for routine analyses of similar mixtures where essentially the same peaks will be present from sample to sample. Retention times alone, while characteristic of specific compounds, are not sufficiently specific to enable the positive identification of an unknown peak in a mixture.

Overall, gas chromatography has the advantages of small sample requirements, ease of automation, quantitative results over a wide linear dynamic range, and, frequently, little or no sample pre-separation. However, GC analyses of complex mixtures suffer form a lack of qualitative information and long analysis times to attain high resolution.

Gas Chromatography/Mass Spectrometry Applications - The use of mass spectrometry alone for mixture analysis presents the problem of deconvoluting the spectra of each component and gas chromatography alone generally provides too little qualitative information for compound identification. The complementary data dimensions of these two techniques, however, allow them to be readily combined to form the extremely useful hybrid technique of gas chromatography/mass spectrometry (GC/MS). As a result, the combined analytical technique of GC/MS has been very highly developed for use in a wide range of applications (7). The combined GC/MS instrument can be viewed as either providing the GC with a highly selective detector in the case of selected ion monitoring or, as providing the mass spectrometer with an inlet which will isolate and provide, in sequence, each of the individual components in a mixture, as in the case of scanning GC/MS. In selected ion monitoring, the mass spectrometer acts essentially as a GC detector of very high sensitivity and, by selection of which mass to monitor, variable selectivity. In the case of scanning GC/MS, the GC inlet allows the mass spectrometer to obtain a spectrum of each of the components in the mixture with the "purity" of each spectrum being determined by the resolution of the GC and the scan frequency of the mass spectrometer. Use of both the chromatographic retention time and the qualitative

information in the mass spectrum of a given peak has been used in metabolic profiling: a technique demonstrated for its great power when applied to complex mixture analysis (8).

In any type of GC/MS analysis, however, the of GC analysis time needs to be traded off against the desired chromatographic resolution. Due to its thoroughness, scanning GC/MS analysis is an extremely powerful technique for the analysis of organic mixtures for which little prior information is available. Additionally, the systematic nature of this type of analysis renders it quite amenable to automation. Instruments for GC/MS are also widely available, thus easing the process of interlaboratory cooperation. As a consequence of the thoroughness of this type of analysis, however, there is the potential to generate tremendous quantities of data, of which only a fraction may actually be needed by the analyst.

Mass Spectrometry/Mass Spectrometry Applications - One method which has been developed to avoid the time/resolution tradeoff inherent in GC/MS, while still essentially allowing for the mass spectrometric identification of the individual components in a mixture is tandem mass spectrometry (MS/MS). One of the more popular and convenient implementations of MS/MS is the triple quadrupole mass spectrometer (TQMS) (9, 10, 11). In TQMS with a batch or probe inlet, the entire sample is available for analysis at all times. Thus, the components of interest may be examined without

having to wait for all of the other components to elute from the gas chromatograph. If the goal of the analysis is only the detection or quantitation of a few components, the great bulk of unneeded data frequently encountered in GC/MS is eliminated; indeed, it is never acquired. Additionally, TQMS allows the operator to change any of the system parameters at any time during the analysis. In order to take advantage of this feature, however, considerable operator attention is often required, whereas, in GC/MS, many instruments can run unattended. Problems with TQMS mixture analysis include the difficulty in getting a representative sample from a mixture of components with a wide range of volatilities (12), the necessity for maintaining constant sample composition for the duration of the analysis, and the high probability of having to deconvolute the spectra of the isomeric and isobaric peaks that are likely to be found in any mixture of reasonable complexity. Furthermore, in cases of non-routine samples for which little prior knowledge is available, the time advantage over GC/MS can easily be lost and the amount of data generated can be just as large and, potentially, even more difficult to interpret. Thus, TQMS has its greatest value in complex mixture analysis when only a few, targeted components need to be determined and differentiation of isomers is not required.

Gas Chromatography/Mass Spectrometry/Mass Spectrometry Applications - The addition of a GC inlet to the TQMS can frequently simplify the problems of performing TQMS on complex mixtures by dispersing the components in time and by allowing a more representative sampling of mixtures containing components with a wide range of volatilities. The time dispersion provided by GC can greatly facilitate interpretation of the data by allowing isomeric and isobaric components to be individually analyzed and by providing an additional dimension for interpreting experimental results. Additionally, in multiple reaction monitoring GC/TQMS experiments, several different components or component classes can be simultaneously and independently analyzed as they elute from the GC column. The power of having two stages of mass filtering allows for a wider range of control over the selectivity of detection than is possible with conventional GC/MS. The separation power of the first mass filter further serves to reduce demands on chromatographic resolution by allowing each component to be monitored in a different dimension. This has a net result of decreasing the analysis time. By providing the capability of increased selectivity at lower chromatographic resolution, GC/TQMS minimizes the two major problems of GC/MS, namely the long analysis times and large quantities of data which are generated.

Chapter 3.

Adaptation of Triple Quadrupole Mass Spectrometry Programs for a Multiple Microprocessor Control System

The evolution of organic mass spectrometry from a specialized art employed in the slow scanning batch analysis of gases to a widely employed, high speed technique for mixture analysis and identification of trace quantity samples has been greatly assisted by the concurrent development of powerful, high speed data systems. Further, the recent evolution of high-powered, low cost microprocessors has, in recent years, forced an entire rethinking of data system design and functionality (13). This is especially true for multidimensional techniques such as MS/MS and GC/MS/MS where the data rates, quantities of data, and the number of instrumental parameters to control reach well beyond that which is feasible to perform manually (14).

Proper design of a data system requires that these demands be met in a user-friendly and economically feasible manner. Achieving these goals necessitates taking full advantage of the diversity of features now available in computer hardware and software. This entails assigning different data system tasks amongst various elements of hardware and software as needed to optimize cost and

performance. In other words, the use of several specialized, low-cost microprocessors can provide far greater instrument performance and flexibility than a similar cost system involving a single processor which must be chosen as a compromise of conflicting demands. Some of the advantages of such a distributed processing system are summarized in Table 3.1 (15).

The hardware (16) and systems software (17) for the triple quadrupole mass spectrometer control system used in this report have been described previously. Briefly, the system consists of four Intel 8088 processors. The first processor, the master, supports the user terminal and disk drives and directs the operation of the three slave processors. The first slave controls all of the ion path elements, the second slave performs peak-finding and controls the graphics display, and the third slave monitors the detector. The distribution of tasks among the four processors in the system is illustrated schematically in Figure 3.1.

This chapter covers the conversion of previously developed (18) single processor data acquisition routines for enhanced operation in a multiple processor environment. The result of this conversion has been to create a flexible and powerful TQMS control system.

As a first phase of the multi-micro conversion project, all of the user functions of the single microprocessor system were reproduced. Consistency with the old system was

Table 3.1 Advantages of Distributed Processing Systems

Faster Execution

* Parallel execution
* Less time spent in "overhead"
* Simpler addition of hardware controllers and processors

Independent Task Execution

* Non-interference of tasks
* Elimination of task interleaving problems
* Elimination of priority assignment problems
* Simpler task program modification

Modularity of Hardware and Software

* Consolidation of related tasks
* Simpler extension of instrument capability
* Simpler debugging and troubleshooting

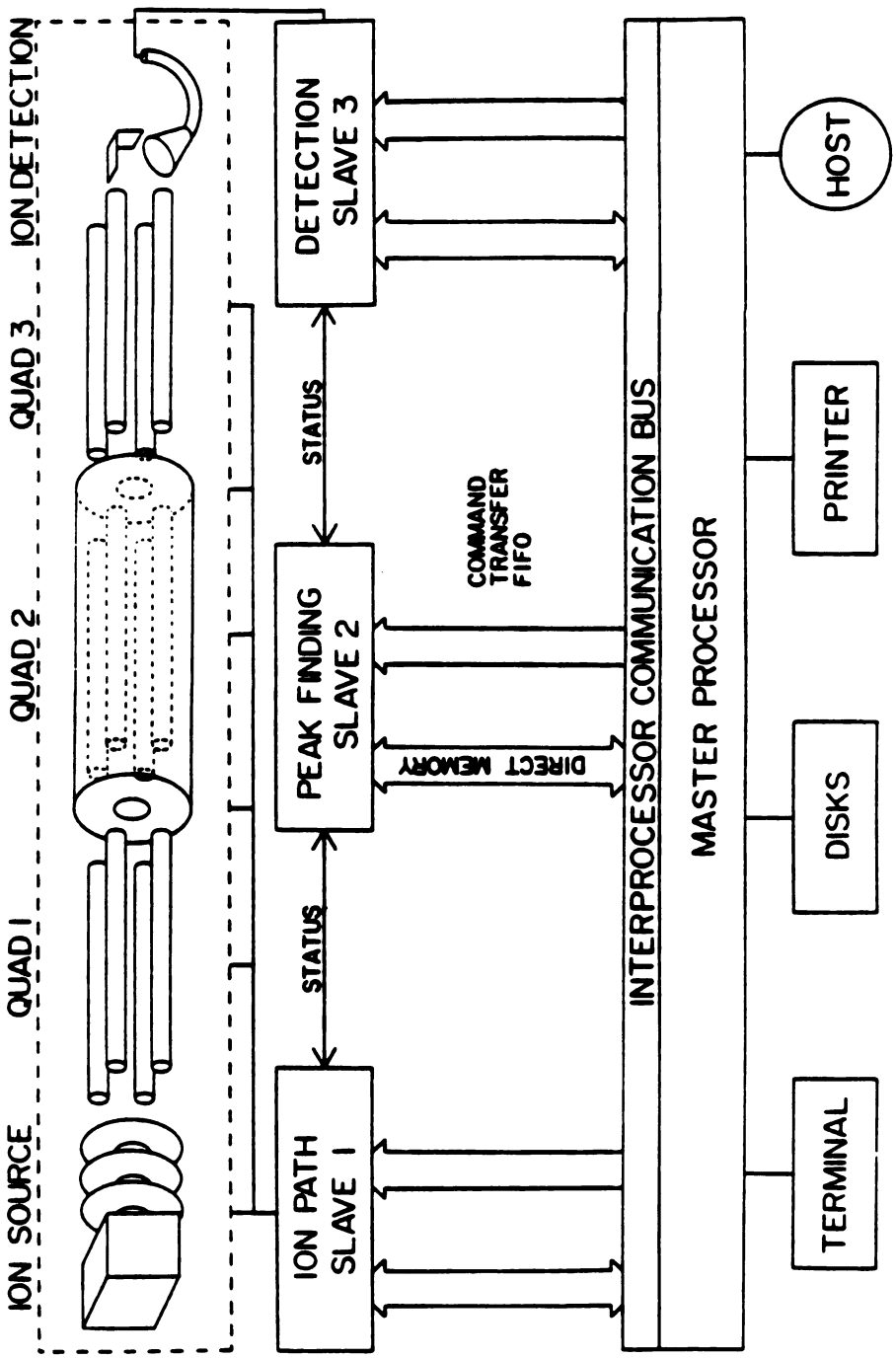


Figure 3.1 Distribution of Tasks in the Multiprocessor System

obtained for the most part. However, since the instrument was intended for a small group of sophisticated users, changes were made when needed to provide additional power or capability for future expansion.

Once the user interface was implemented, the next major step was to improve the speed of data acquisition by taking advantage of the distributed processing capabilities of the system. Additional objectives were the implementation of features such as real-time graphics, linked device sweeps, and dual-mode data acquisition; features which were not feasible in the single processor arrangement.

Two types of data acquisition routines are discussed: sweeps and scans. A third type of data acquisition, represented by selected ion monitoring and multiple reaction monitoring, will be discussed in the following chapter. Details of the data acquisition software operation are presented in Appendix A. Partial listings of the code discussed in the following sections are given in Appendix B.

Sweeps

Sweeps are used to acquire data while continuously sweeping the value of any ion path device. They save all of the raw intensity values without performing any peak-finding. This operating mode allows the operator to obtain intensity data as a function of mass or of the voltage on any of the ion path elements. The availability of this mode

provides a simple method for the operator to sytematically investigate the ion path tuning, use the analog data to verify the quality of the peak finding, and to examine the energetics of ion-molecule reactions under study.

A comparison of the software structuring of sweeps under the single and multiple processor configurations is illustrated in Figure 3.2. In this multiple processor scheme there is a fixed division of tasks among the three slave processors. The first slave controls the ion path configuration, the second slave monitors the ion current through control of the Data Acquisistion Board (Daq) (19), and the third slave receives the x-axis information from the first slave and the y-axis information from the second slave and stores them in a data buffer.

The first slave system has an 8 megahertz processor and is used to control the voltages on all of the ion path elements, the quadrupole mass selections, and the quadrupole RF/DC states. This slave calculates the value of each step in the sweep and passes this information to the reduction slave. A second function of this processor is to provide time synchronization through use of the AMU Timer (20) as well as synchronization with the detection slave. In addition, the ion path slave controls the sweep experiment by counting the number of steps remaining in the sweep and informing the other processors when the last step has been executed.

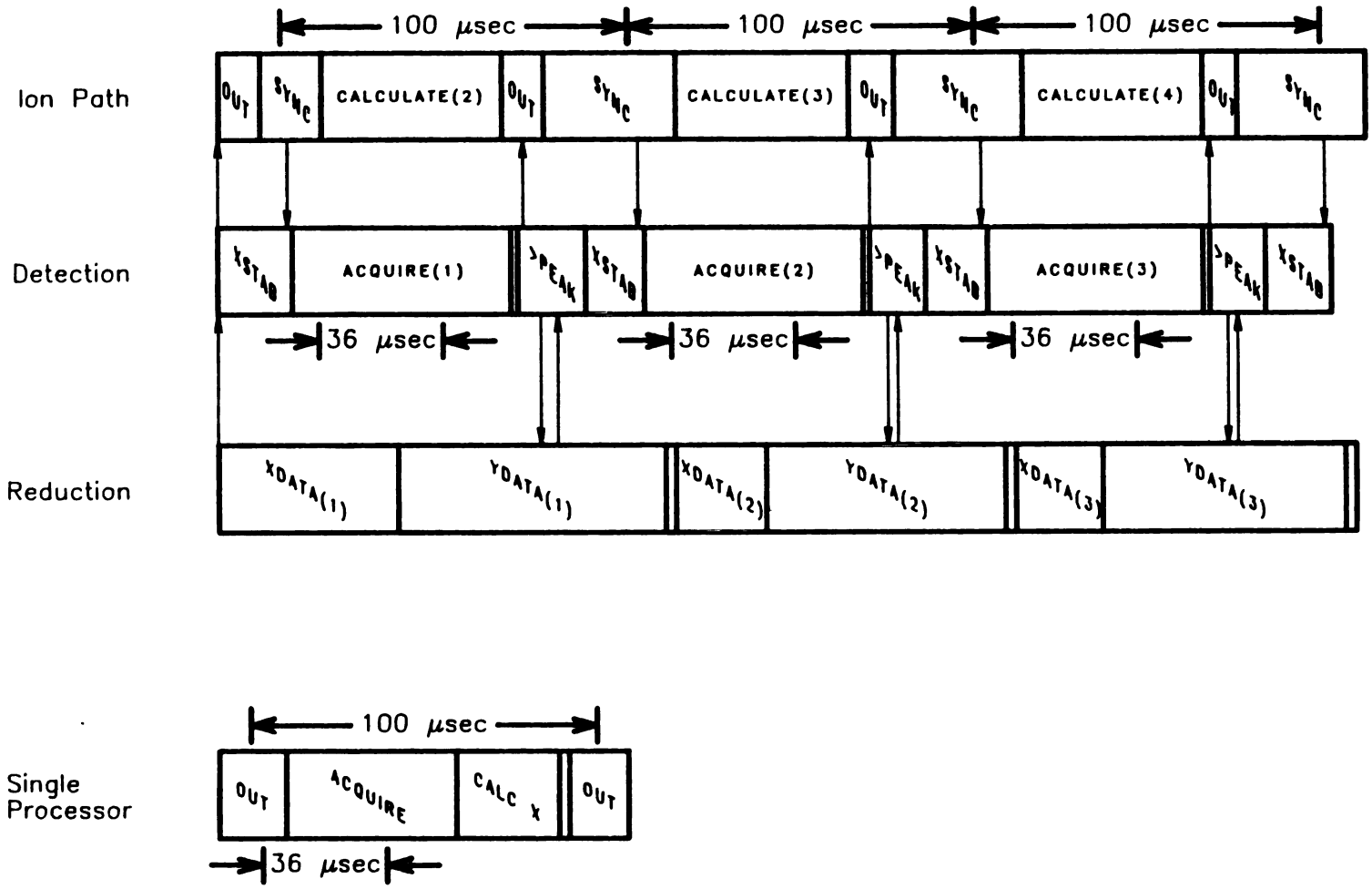


Figure 3.2 Timing Relationships for SWEEP's

The detection slave has a 5 megahertz processor and is used to interface with the Data Acquisition Board (Daq). In this capacity it provides the start signal to the Daq, monitiors its status byte for the completion flag, and readsback a 24-bit intensity value. This processor informs the ion path slave when acquistion of each point is completed and transmits a 32-bit ion intensity value (the 24-bit value from the Daq with an added high byte of zero) to the reduction slave when that slave is ready to accept it.

The third slave also has a 5 megahertz processor and is used to receive the current x-value from the ion path slave and the corresponding ion intensity from the detection slave. These values are then stored in a data buffer to be transmitted to the master processor at the end of the sweep. This processor obtains synchronization by waiting for the needed x and y values to be available from the other two slaves.

A more detailed discussion of the sweeps programs is provided in Appendix A. Overall, conversion of the sweeps code to a multi-processor system allows for greater modularity in the coding by allowing logically separate tasks to be performed on separate processors. This allows these tasks to be programmed as distinct modules as opposed to interleaving them, as would be necessary on a single processor system. Further, the partioning of data acquisition tasks allows the ion path processor nearly twice as

much time for calculations as would be available on a single processor system. This additional time is taken advantage of in more complex device contol, such as linked-device sweeps.

Scans

The second form of data acquisition is the scan. Five types of scans are defined -- single stage MS with the first quadrupole or the third quadrupole, the parent ion scan, the daughter ion scan, and the neutral loss scan. The principal difference between a sweep and a scan is that instead of storing raw intensities, a scan performs peak-finding and saves only the mass/intensity pair for each peak.

A schematic comparison of scanning with single and multiple processors is given in Figure 3.3. The task distribution for scans is generally the same as for sweeps with the exception that the reduction slave now performs on-the-fly peak finding instead of directly storing each data point into the buffer and the ion path slave sweeps the RF-power level on any RF-only quadrupole at one-half the magnitude of the scanning quadrupole. The timing diagrams in Figure 3.3 were developed for a neutral-loss scan. This is a "worst case" because the 32-bit calculation required to keep the quadrupole 3 mass tightly linked to the quadrupole 1 mass places the greatest load on the ion-path processor. The times indicated for ?PEAK also represent a worst case

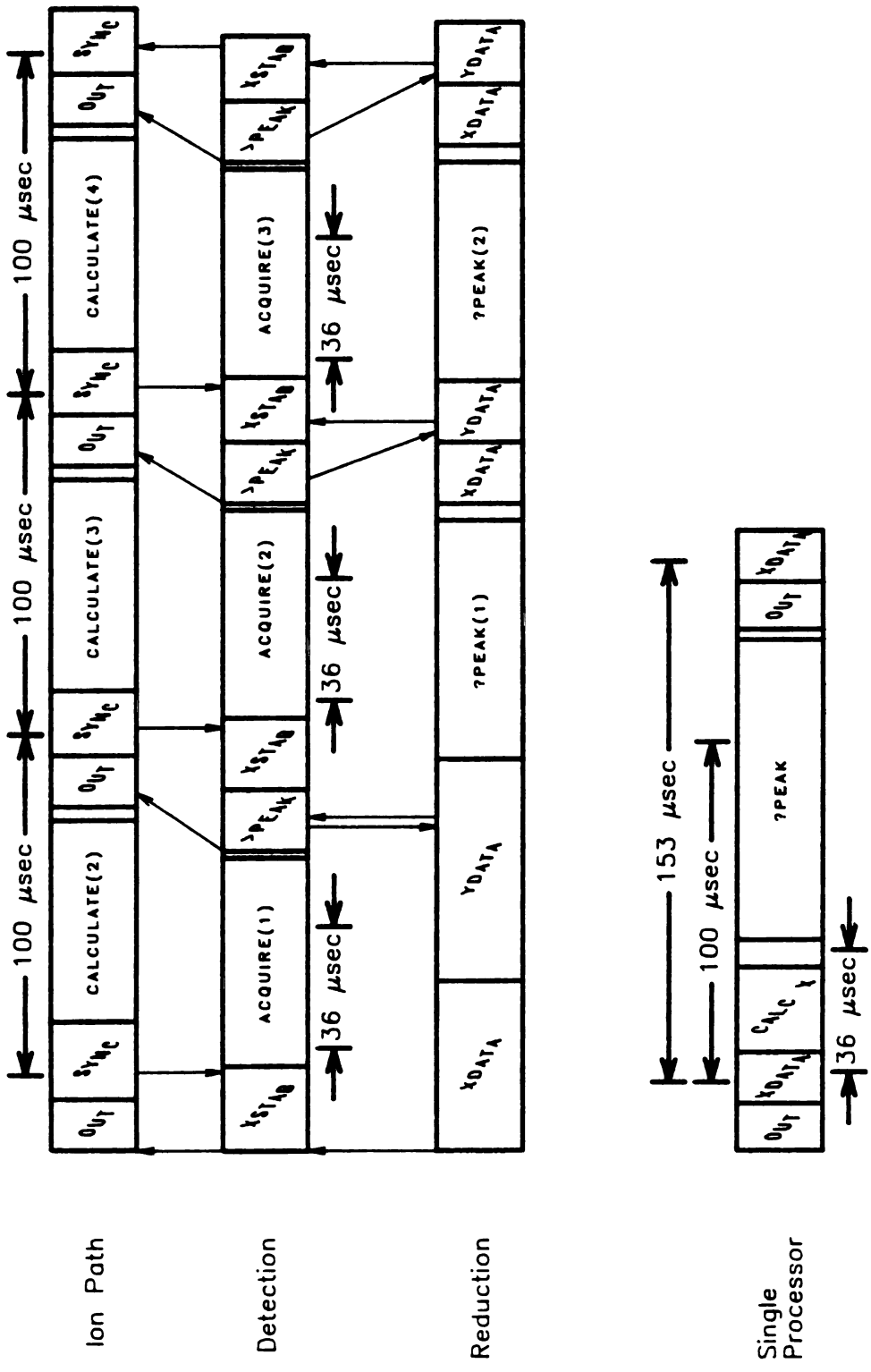


Figure 3.3 Timing Relationships for NSCAN's

condition (the intensity of the current data point is above threshold and the current peak exceeds the minimum peak width). This case generally occurs only for a small fraction of the actual steps. There are nine possible cases for the current peak-finding algorithm. For the multiple processor implementation, these cases range in time from 16 to 67 microseconds.

The schematic illustrates that the single processor system cannot realistically perform a neutral loss scan at 100 microseconds per step (1000 amu/sec at 10 steps per amu). Even evaluating a distribution of times required by the different cases of the peak-finding algorithm, the effective scan rate would vary considerably, depending on the number and size of peaks in the scan. In that mass step calculations are being performed during the data acquisition time of the single processor system, little, if any, gain in scan speed would be achieved by reducing the number of A-to-D conversions averaged for each step (the illustrated implementation uses 8 conversions per step).

In contrast to the single processor configuration, the multiple processor implementation of the neutral loss scan comes very close to the 100 microsecond per step goal, even assuming a constant worst case for the peak-finding algorithm. As illustrated in the schematic, this is achieved by performing separable tasks in parallel -- while the detection processor is monitoring the nth point, the ion path processor is calculating the masses for step n+1, and

the reduction slave is performing the peak-finding for step n-1. Even with the worst case of the peak-finding algorithm, the reduction slave has a cycle time of 110 microseconds, and it is reasonable to assume that the excess 10 microseconds for the worst case can be caught up with even a small abundance of the more favorable cases. The timing illustrated for the ion path processor allows over 60 microseconds for calculations, but even the calculation-intensive neutral loss scan requires only about five microseconds in the multiple-processor implementation. Thus, the multi-processor system can not only perform a high-speed neutral loss scan, but it also has the capability available to execute more sophisticated scanning algorithms.

Design Criteria for Future Systems

Study of the timing diagrams presented in this chapter reveal some interesting points on the relative merits of single processor and synchronous multiple processor systems. In the case of SWEEPs, the multiple processor system shows very little advantage over the single processor configuration, primarily due to the overhead imposed by the need for inter-processor synchronization. This comes despite the use of specialized inter-processor hardware designed to minimize software overhead. As a result, the only gains resulting from the multiple processor implementation of SWEEP are the increased amount of time available

for ion path calculations and improved modularity of the code. However, both implementations allow sufficient calculations time for most any reasonable scan algorithm.

The limited memory available with the 8088-based multiple processor system used restricts SWEEPs to narrow mass ranges (currently 100 amu at 10 steps/amu). Scans conserve memory by performing on-the-fly peak finding synchronized with each data point, allowing for wider scan ranges by throwing away potentially useful peak shape information. The use of synchronized, on-the-fly peak-finding, however, requires multiple processors for repeatable scan rates. Additionally, even with the multiple processor system, the sophistication of the peak-finding algorithm is compromised by the need to operate within scan rate limitiations (1000 amu/sec at 10 steps/amu). The increasing use of 2000 amu quadrupoles and scan rates of over 2000 amu/sec in commercial instruments further limits the utility of a 1000-point data buffer and underscores the need for fundamental changes to accommodate higher scan rates.

With the rapidly decreasing cost of computer memory, a first step in reducing these problems and simplifying the computer system would be to increase the size of the data buffer so that SWEEPs may be performed on a 2000 amu mass window, and peak-finding can either be postponed until after data acquisition is complete or performed asynchronously from the data acquisition. For SWEEPs employing a constant

step size, this temporary data buffer need only contain four bytes for each data point, plus a four byte header region containing the starting x value and the step size. For a 2000 amu scan at 10 steps/amu, this corresponds to 80,000 bytes. Many 16-bit processors, such as the 8088 used here, employ memory segmentation schemes that make it very awkward to address a buffer of larger than 65,536 (64K) bytes.

The two most viable approaches to this problem are to use a processor that can directly address an 80,000 byte data buffer (such as the Motorola 68000 family), or to devise a scheme to perform peak finding on-the-fly, but asynchronously from the SWEEP. The first solution gives a surface impression of simplicity in that it can be readily implemented with a single processor. Such an approach, however, would come at the expense of the many modularity advantages of multiple processor systems, as described earlier in this chapter. More importantly, this would result in considerable dead time between scans to allow for post scan peak-finding, thus negating many of the advantages of a high scan speed.

The second approach, performing peak-finding asynchronously from data acquisition, can remove the requirement that the 80,000 bytes of data be resident in memory simultaneously while also eliminating the inter-scan dead time problem. This solution requires the introduction of a second processor to perform most of the peak-finding operations during scan time. To avoid the timing

restrictions observed with synchronized on-the-fly peak-finding, mass and intensity data must be made available to the peak-finding processor in a manner other than the point by point mechanism employed in this work. Two approaches to this problem are a dual port first-in-first-out (FIFO) buffer or providing a segment of dual port memory for use as a data buffer.

The use of a FIFO avoids the need for an 80,000 byte buffer, thereby opening the design to processors with 64 Kbyte memory segmentation. A FIFO data transfer path will effectively decouple peak-finding from data acquisition so long as the FIFO is sufficiently deep that it never fills. FIFO memory chips are available in a variety of widths and depths and designed for combination to form most any desired configuration. However, the FIFO configuration and support circuitry would need to be custom designed for the given hardware configuration. Additional complications are that the software to reliably read and write the FIFO's can be just as involved as the interprocessor synchronization used in the current system. Further, the use of FIFO's make it awkward to employ peak-finding algorithms that require non-sequential access to the raw data (such as correlation functions and centroiding).

The problems associated with FIFO memories suggest that shared memory be considered as an alternative. Commercial microprocessor buses, such as the Multibus and VMEbus, readily provide for shared memory, which can be implemented

using most commercial memory boards (the custom inter-processor bus used in this work allows the master processor to access all of address space of the slave processors, but the slaves are unable to address anything other than 64 Kbytes of local address space). Data transfer through use of shared memory can be implemented with little software overhead. This type of data transfer can also be implemented using the shared memory space as a large wrap-around buffer, so that processors with 64 Kbyte memory segmentation may still be employed. If this wrap-around buffer is sufficiently large, data in the buffer can be resident long enough to allow the reduction processor to employ non-sequential peak-finding algorithms.

Recommendations

This work has provided considerable experience in the design of multiple processor control systems. Combined with the evolution in quadrupole mass spectrometry and microprocessor hardware which has occurred since this multi-microprocessor system was designed, a number of recommendations can be made towards the design of faster configurations with greater power and flexibility. The rich array of commercial, board-level hardware now available should be incorporated wherever practical. This will result in reduced development time and generally improved reliability and documentation. Large RAM buffers in regions of shared

memory are now economically viable and can be implemented with standard commercial memory boards. Their use will greatly simplify interprocessor communications and allow peak-finding to be decoupled from instrument control and data acquisition. Such an approach will provide more reproducible scan rates and allow the use of sophisticated peak-finding algorithms. A large wrap-around buffer in shared memory should be used for raw data, with additional buffers available for peak data. These buffers are configured for ready access by additional processors, allowing peak data from one scan to be transparently written to disk or displayed while the next scan is being acquired.

A single high-speed processor can perform both the detection and ion path control functions with somewhat reduced modularity if analog-only detection is to be used. An additional processor creates a more modular system by isolating the instrument control and data acquisition functions and provides sufficient power to perform dual-mode detection. The instrument control and data acquisition processors may be synchronized through status bytes located in shared memory. Both the detection and ion path slaves can write directly to the raw data buffer. Finally, a master processor is needed to control the slave processors and provide interactive foreground functionality during data acquisition.

Conclusions

Data acquisition code for triple quadrupole mass spectrometry has been converted to take advantage of the enhanced power of a multiple processor control system. This conversion has allowed for higher speed operation while maintaining modular software. Combined with the modularity of the hardware, this provides a highly flexible system which can be readily tailored to perform demanding and specialized experiments, including such possibilities as intelligent dual-mode data acquisition and sophisticated linked-scanning algorithms. Based on the experience gained in developing this prototype system, recommendations have been made for the design of a faster, more powerful system.

Chapter 4.

Development of Multiple Reaction Monitoring Programs for Triple Quadrupole Mass Spectrometry

A traditional approach for improving the detection limits and reproducibility of GC/MS is selected ion monitoring (SIM). As opposed to full scan GC/MS, where the mass filter is continuously and repetitively swept through a range of masses bracketing all ions of interest in the sample, SIM involves repetitively cycling the mass analyzer through a small number of pre-determined m/z values. This improves the signal to noise ratio of the analysis by allowing the instrument to spend the bulk of its time monitoring ions of specific interest while not wasting time gathering data in irrelevant regions of the spectrum. As a result, detection limits can be improved by as much as two to three orders of magnitude over those attainable in full scan mode. This approach is especially valuable in conjunction with capillary gas chromatography where sample concentrations in the ion source may vary significantly over the time required to obtain a full mass spectrum. Clearly, this enhanced sensitivity comes at the expense of information content -- the analyst no longer has full mass spectra available to aid in evaluation of unexpected mixture

components. However, for targeted analyses, involving mixtures of a type which has been sufficiently characterized, this is frequently not a limiting factor.

The analog to SIM in tandem mass spectrometry is to cycle through a short list of parent ion/daughter ion pairs, or reactions. This technique is referred to as multiple reaction monitoring (MRM). Through judicious choice of reactions, MRM provides much greater selectivity than is attainable with SIM. Cooks describes MRM as employing a fixed parent ion and cycling only through the daughter ions (21). The use of tandem quadrupoles, however, allows for rapid cycling of both the parent ion and daughter ion masses; in fact, their hardware and control software are identical. To take full advantage of this feature of quadrupole MS/MS, the software designed here allows cycling of both the parent ions and daughter ions in pre-determined reactions. This design is illustrated schematically in Figure 4.1.

The design of the selected ion monitoring/multiple reaction monitoring software attempted to incorporate several criteria. These may be summarized as follows --

1. Provide a user interface which is generally similar to that designed for sweeps and scans.

2. Define four types of selected ion monitoring experiments --

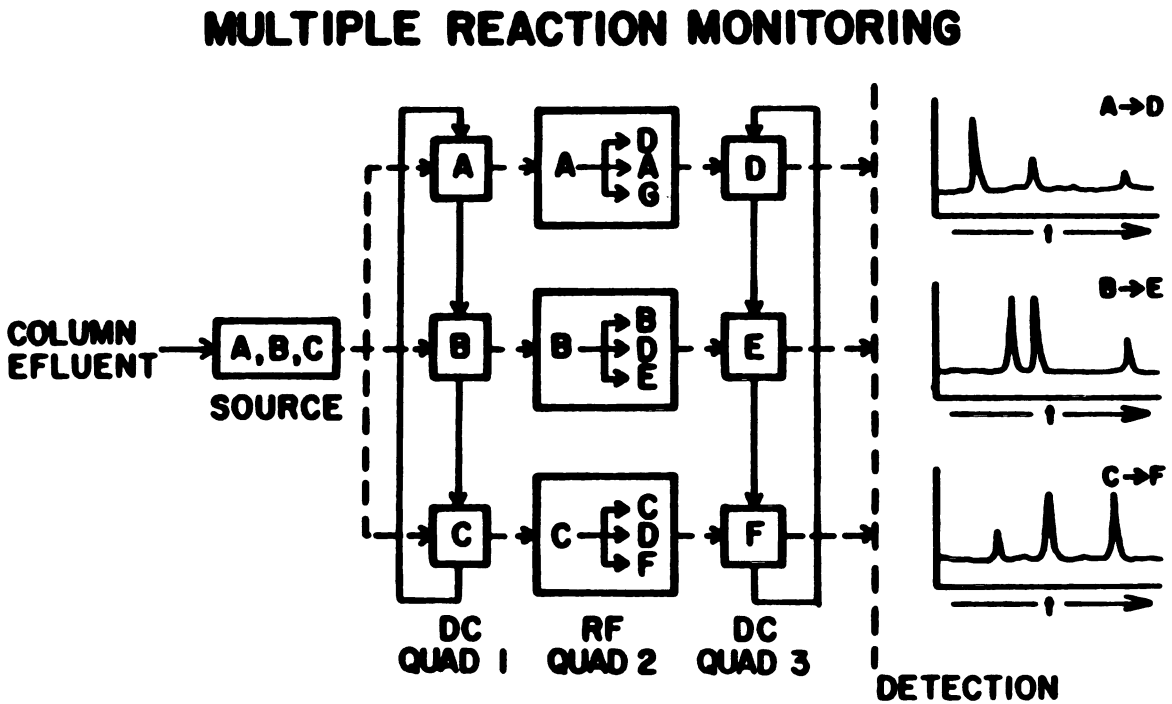


Figure 4.1 The Multiple Reaction Monitoring Experiment

a. setting quadrupole 1 while quadrupoles 2 and 3 are in RF-only mode (1SIM),

b. setting quadrupole 3 while quadrupoles 1 and 2 are in RF-only mode (3SIM),

c. keeping quadrupole 3 at a fixed mass while setting quadrupole 1 (PSIM),

d. and keeping quadrupole 1 at a fixed mass while setting quadrupole 3 (DSIM).

These SIM modes would be analogous to the scanning modes 1SCAN, 3SCAN, PSCAN, and DSCAN.

3. Use a single set of utilities for the four SIM modes and multiple reaction monitoring (MRM).

4. Allow for the simple creation and storage of several sets of ions and reactions (elements), with the capability of specifying a different parameter set for each element in a set.

5. Provide a mechanism to allow the user to select from several possible cycle frequencies.

6. Allow the user to set a duration for data acquisition while retaining a capability to force a clean termination of the run at any earlier time.

7. Use real-time graphics to provide the user with immediate feedback from the current run.

8. Provide a mechanism to scale the real-time display for effective representation of data with a potentially wide dynamic range.

9. Define a set of post-run utilities to generate summary reports, provide a range of graphics capabilities from quick and simple to publication quality, allow for archival data storage and generate dumps of the raw data.

Consistent with the overall system design, that of separate control and data systems (22), the experimental set up and execution are performed by the control system, while most of the post-run utilities exist as tasks on the data system. Simple graphics and data listing capabilities are provided on the control system, however, to allow for more rapid feedback to the experimenter and to provide some redundancy.

Experimental set up consists of two phases -- definition of the elements to be monitored and definition of the various run-time parameters. These steps need to be executed once before the first run is executed. For subsequent runs, however, they only need to be repeated when

the experimenter wishes to change the elements or parameters.

Elements are defined through the use of one of two screen editors -- RED, the Reaction EDitor, and SIMED, the Selected Ion Monitoring EDitor. These two editors are very similar. SIMED allows for the definition of up to eight ions to be monitored and a parameter set to use with each of the ions. Physically, the editor presents the user with a table of ions and parameter sets which the user may edit by using the cursor control keys to position the cursor over the entry to be edited and then typing over the current entry. During run-time the system cycles through the list of ions from the beginning through the last non-zero ion entry. The choice of which quadrupole to use for selecting the various ions is made by choice of the appropriate run command (1SIM, 3SIM, PSIM, or DSIM). Any RF-only quadrupoles are set to one-half of the value of the current ion selection. The fixed mass quadrupole in PSIM and DSIM is kept at the "current" mass for the corresponding quadrupole using the parameter set which is active when the run is initiated. All of the run commands use the ion/parameter set table which is active when the run is started. In addition, up to 32 ion/parameter set tables may be stored on disk for future use. Storage and retrieval of these tables is performed by the commands n SIMSAVE and n SIMGET, respectively, where n is the table number, ranging from 0 to 31.

Reactions for a multiple reaction monitoring run are defined using the reaction editor, RED. This editor works in essentially the same manner as SIMED. The principal difference is that RED provides for definition of both the parent and daughter ions for each reaction. In addition, it allows the user to specify an RF-value to use for the second quadrupole. This value will default to one-half the lesser of the parent and daughter ion masses. Similar to SIMED, the user may specify a parameter set to be used with each reaction. A maximum of sixteen reaction tables may be saved on the disk. There is a pair of commands, n RSAVE and n RGET, to store and retrieve the nth table, respectively.

The second step in setting up a SIM/MRM run is the SSET command. This command prompts the user for inputs. The parameters to be specified are cycle period, scaling for the real-time display, and the maximum duration of the run. Six possible cycle periods are allowed -- 0.1, 0.2, 0.4, 1.0, 2.0, and 4.0 sec/cycle. The display scaling allows the user to specify the vertical range to be used in the real-time display. The default value is 1,048,575 which is the full scale response of the detection electronics. This value is used for all of the elements to be displayed. It is important to note that this value has no effect on the actual data acquisition; it only scales the real-time display. The third item requested is the run duration. In addition to automatically terminating the run after the given time elapses, this value is also used, along with the

cycle period, at the start of a run to verify that sufficient disk space is available to store the entire run. For each of these parameters, the user is given the choice of either entering a new value or just entering a carriage return to keep the present value. All values entered in SIMED, RED, and SSET are maintained by the computer until they are explicitly changed by the user.

An actual run is initiated when the user enters one of the five command words -- 1SIM, 3SIM, PSIM, DSIM, or MRM. The computer then initializes everything and comes back with a prompt for the user to strike any key to begin data acquisition. This provides the experimenter with an opportunity to let the GC oven equilibrate and to make the injection. When the injection is made, a key may be struck and the instrument will go immediately into data acquisition mode. During data acquisition the monitor will have a real-time display of element intensities versus time for each element being analyzed, up to a maximum of five. If more than five elements are being monitored, the system uses the first five entries in the table. This display is erased and restarted after every 1000 data points, thus the time window depends upon the cycle period. The user may allow the experiment to continue until the specified time has elapsed or may stop the analysis at any time by striking the "Q/q" key.

Each run is stored as a scan at the end of the most recent experiment in the current data file. This allows the

user to access any SIM/MRM run in the same manner as used for scans and sweeps. As with any other data, Data LISTings may be obtained through use of the **DLIST** command. For SIM/MRM data this produces a raw data dump of the intensity for each element at each time sampled. Because of the sheer volume of data potentially present in a run, DLIST does not perform any normalization or peak-finding. The control system can also generate simple graphics through use of the **DISP** command. DISP works somewhat differently, however for SIM/MRM data than for scans and sweeps. Instead of using system defaults or the optional **DSET** (Display SET) command there is the **CSET** (Chromatogram SET) command. Generally, this command must be executed before every DISP of SIM/MRM data. CSET prompts for user input or a carriage return to retain the current values. It allows the user to specify which elements to display, to a maximum of five, and what time interval to use (the user may enter zero for the end time to default to the end of the run). Additionally, there is a command **NEXT** which allows the user to display a set of elements as a series of displays containing successive time window of constant width, without having to return to the CSET command.

As is the case with sweeps and scans, archival storage of data and generation of high quality plots is performed on the PDP-11/23 data system. Data may be uploaded to the data system by means of a 16-bit parallel link. Unlike scans and sweeps, however SIM/MRM data cannot be conveniently

incorporated into the MSU-LLNL Multi-Dimensional Database structure (23). This is because the data points cannot be represented as an ordered, x-y pair, as required by the multi-dimensional database. Instead, they must be represented as ordered triples, composed of quadrupole 1 mass, quadrupole 3 mass, and intensity. Thus, a new set of utilities had to be written to process these data on the PDP-11/23 data system.

Data from the control system are uploaded to the data system as "experiments". Each experiment consists of some header information, user-supplied comments, and headers and data for one or more "scans", which are presumably related. Each scan corresponds to an MRM/SIM run and consists of a header record, the full set of parameter values for each element monitored, and one data record for each cycle (regardless of how many elements were actually monitored). The control system uploads one experiment at a time up the 16-bit parallel link to the data system. The receiving task on the data system, UPLOAD (24), creates a specially formatted file. The data system command GCTRAN is used to take the file created by UPLOAD, and separate it into one file for each of the scans in the experiment. Each one of the scan files contains the scan header, all of the comments for the experiment, the scan parameter sets, and all of the scan data. In addition, the user is prompted for any additional comments to go into each individual scan file as it is being generated.

A second data system command, MRM, may then be used for each of the scans to be processed. The current functions of the MRM routine are to generate a run listing and to generate a special file compatible with MULPLT, a locally developed graphics package (25). In addition to the MULPLT data file, MRM generates a command file which may be executed by the user to automatically generate default reaction/ion chromatograms for each element which was monitored for the entire run. Each of these chromatograms will be separately normalized and will indicate the maximum for each element. The run listing generated by MRM consists of the date and time for the run, the parameter values used for each element, and the maximum intensity and corresponding time for each of the elements. Any data files transferred to the PDP-11/23 data system may be archived by use of the general file utilities included in the RSX-11 operating system (26).

Summary

A flexible and easily learned set of programs has been created for use with a multiple microprocessor triple quadrupole mass spectrometry control and data system. The structure and user interface designed into these programs is consistent with the existing programs for sweep and scanning data acquisition which have been described in chapter 3. Additionally, the use of multiple processor allows

incorporation of parameter switching for each ion or reaction being monitored; this gives the user a powerful tool for optimized data acquisition for each element while maintaining a sampling rate which is high enough for accurate monitoring of capillary GC peaks.

Chapter 5.

The Relative Efficiency of Methanol Chemical Ionization for Selected Hydrocarbons and Heterospecies Present in Fuels

Introduction

A key element in any mass spectrometric analysis is the ionization process employed. Over the years, mass spectrometrists have developed a large number of ionization techniques, each having advantages for one or another of the wide array of applications in which mass spectrometry is employed. One of the ways various ionization techniques may be classified is by the degree to which they fragment the sample molecule; another is by their selectivity towards different compounds and classes. A third classification is the technique's efficiency; what fraction of the sample molecules can be converted into characteristic ions?

Energetic ionization techniques, of which 70 eV electron impact is the most common example, frequently result in extensive fragmentation of the sample molecule. High energy methods such as EI are often used to obtain universal ionization since they are capable of putting sufficient energy into most organic molecules to remove an electron. Putting so much energy into the sample molecule,

however, often comes at the price of creating virtually nothing but fragment ions and thus losing one of the most valuable single pieces of information on the sample molecule -- the molecular weight.

The fragmentation patterns found in the EI spectrum are highly characteristic of individual compounds and compound types. As a result, they can be used by a trained mass spectrometrist to gain valuable insight into the identity of an unknown molecule. One limitation of such a sample spectrum is that it often consists of a large number of relatively minor peaks. It is very rare that any of these peaks, taken individually, are selective for the individual compound. As a result, they are of limited utility for quantitation from complex samples by selected ion monitoring (SIM.) Thus, ionization techniques which cause large amounts of fragmentation are often inadequate when attempting to quantitate trace components in complex mixtures.

In cases where the electron impact spectrum does not yield molecular weight information or creates excessive fragmentation of trace species, chemical ionization (CI) techniques are often employed (27). Essentially, CI employs an indirect approach to ionization. A relatively closed ion volume is flooded with a reagent gas or gas mixture to a pressure typically on the order of 1 torr (measured inside of the source). This creates a large excess of reagent compared to sample partial pressures which are generally on the order of a few millitorr. A high

energy (ca. 300 eV) electron beam is passed through the ion volume. Due to the large excess present, the electron beam essentially ionizes only the reagent gas. The ions thus formed can then react with other reagent molecules present in the source to form what amounts to a steady-state reagent plasma.

Sample molecules present in the source may be ionized through ion-molecule interactions in the reagent plasma. Depending on the choice of reagent gasses, the temperature, the pressure, and the nature of the sample, these interactions may include such processes as proton transfer, charge exchange, and hydride abstraction (28). The energy available for ionization and fragmentation by any of these processes depends primarily on the thermodynamics of the particular reaction for the involved species.

The choice of reagents, pressure, and temperature allows the analyst to use ionization conditions which will have increased selectivity for the species of interest and, if desired, yield little or no fragmentation. As a result, molecular weight information, which may not have been available in the EI spectrum, can be obtained much more readily. Further, by having fewer, more characteristic peaks in the spectrum, CI conditions can often be tailored to be highly selective and sensitive for trace compounds, even in complex mixtures.

The primary ionization mechanism involved with many of the more common chemical ionization reagents (such as

methane, iso-butane, and ammonia) is proton transfer. In these cases, the energy transfer upon ionization of the sample is due to the difference in the proton affinities of the reagent ion, a protonated Bronsted base (primarily CH_5^+, $C_4H_{11}^+$, and NH_4^+ for methane, iso-butane, and ammonia, respectively) and the sample molecule. When this difference in proton affinities is such that proton transfer from the reagent ion to the sample molecule is exothermic, chemical ionization occurs and the MH^+ ion is formed. The appearance of this ion at $(M+1)^+$ in the mass spectrum (M being the molecular weight of the sample) can then be used to obtain the weight of the sample molecule.

The difference in proton affinity between the reagent ion and the sample molecule determines the amount of excess energy deposited into the sample molecule upon ionization. This energy is primarily released by collisional deactivation and/or fragmentation of the collisional complex of the sample molecule and reagent ion. The extent to which either of these processes is observed in the spectrum is dictated by their relative rates.

Thus, if the analyst seeks to minimize fragmentation and concentrate the ion current into fewer, more abundant peaks, ionization conditions should be selected so as to minimize the amount of excess energy which must be released by the sample ion. This may be done by choosing ionization conditions which yield reagent ions with the highest proton affinity which will still allow efficient transfer of a

proton to the sample components of interest. This approach may be particularly valuable in cases where it is desirable to discriminate against low proton affinity components in the sample.

One area where the above approach may be particularly useful is the quantitation of trace hetero-atom containing species present in fuels. The presence of such compounds in fuels, even at the parts-per-million or below levels, can have a profound impact on the storage stability and other properties of the fuel. Detection of such components in fuels using conventional ionization techniques is exceedingly difficult due to the vast differences in concentrations between the hydrocarbon and heteroatom species. Even the very low abundance fragmentation products of the hydrocarbons, which appear at virtually every mass in the spectrum, dominate over the base peaks in the spectra of individual trace components. Further, the fuels matrix is sufficiently complex that even capillary GC/MS may be unable to resolve trace components from the background.

The heterospecies, with lone pairs of electrons on the sulfur, oxygen, and nitrogen atoms, are characterized by high proton affinities. As a result, they can be successfully ionized using "soft" proton transfer chemical ionization conditions. This makes it possible to enhance sensitivity for trace species by concentrating the ion current from a particular compound into a small number of peaks, generally dominated by the MH^+ ion. In contrast, the

hydrocarbons, especially the saturates, are characterized by low proton affinities. Because of this, the "soft" proton transfer reagents, which work very well at ionizing the heterospecies tend to strongly discriminate against the hydrocarbons, which make up the bulk of the fuel matrix.

One "soft" proton transfer reagent which has been successfully employed in this laboratory is methanol (29). The first reported use of methanol chemical ionization, by Stan (30), was for the study of fragmentation patterns in the mass spectra of organo-phosphorus pesticides. Its use in that project was essentially abandoned due to the fact that it provided too little fragmentation for their studies. It was this lack of fragmentation which was successfully employed by Bauer (31) in developing screening methods for organo-phosphorus pesticides by MS/MS.

Methanol, under CI conditions in the ion source of a mass spectrometer, primarily tends to form a series of proton bound cluster ions of the form $(CH_3OH)_nH^+$. Under conditions employed in this work, the predominant values of n ranged from one to three. In addition, there is a second series of ions formed corresponding to the general formula $[(CH_3OH)_nH^+ - H_2O]$.

This current study was undertaken to investigate the utility of methanol chemical ionization for the analysis of trace heterospecies in fuels.

Experimental Conditions

This study employed an ELTQ-400-3 Triple Quadrupole Mass Spectrometer and Control System manufactured by Extranuclear Laboratories Inc., Pittsburgh, PA. Chemical ionization (CI) was employed using an Extranuclear Simulscan™ ionizer maintained at 200 C. All of the work reported in this chapter used the instrument as a single stage mass spectrometer with the first quadrupole performing the mass filtering and the second and third quadrupoles operating in RF-only mode, tuned to transmit all masses. No collision gas was present in the second quadrupole.

Chemical ionization was performed with methane (ultra-high purity) and methanol (anhydrous reagent grade). Methanol for CI was introduced from a 500 ml expansion volume maintained at ambient temperature. Pressure of the CI reagent gas was monitored by means of Bayard-Alpert type ionization gauge in the source vacuum chamber. Comparison of methane chemical ionization spectra obtained on this instrument with those obtained on instruments with direct readings of the actual ion source pressure indicates that the pressure reading obtained on this gauge is approximately four orders of magnitude lower than the pressure inside of the ion source. Thus, the pressure values, on the order of 10^{-4} torr, being reported here, roughly correspond to source

pressures of about 1 torr. Electron energy for the chemical ionization was maintained at 300 eV.

Sample introduction was achieved with a Hitatchi 633-30 Gas Chromatograph (Hitatchi Ltd., Tokyo, Japan) equipped with a split/splitless injector maintained at 200 C. Separations were achieved using a 30 meter narrow-bore (0.25 mm ID) DB-1 chemically bonded-phase fused silica capillary column with a 1 micron coating (J & W Scientific, Rancho Cordova, CA.) The column was interfaced to the ion source by passing it through a transfer line at 250 C to a direct inlet. Helium carrier gas was used at a flow of 1 ml/min (a flow velocity of 40 cm/sec at 200 C.) The column was held isothermal at 200 C. Sample injections of 2 microliters were used, split 25:1.

The test blend used in this study was prepared in reagent grade n-Heptane purchased from Mallinckrodt. Blend components were obtained either from Chemservice Inc. (West Chester, PA) or Aldrich Chemical (Milwaukee, WI.) All chemicals were used without further purification.

Data interpretation was performed on a PDP-11/23 mini-computer running the RSX-11M operating system after performing a 16-bit parallel data transfer from the instrument control microcomputer system. Full scan data were interpreted with the aid of a multi-dimensional database package (32). Selected ion monitoring (SIM) data were obtained at a rate of 5 samples per second for each ion; each intensity an average of 128 ADC readings. These

SIM data were interpreted with the aid of another locally developed software package (see Chapter 4).

A 10 ml blend of seven components in n-heptane was prepared for this study and its composition is summarized in Table 5.1. The seven compounds used, two hydrocarbons and five heterospecies, were chosen to represent a wide range of functionality and have boiling points in the range of a middle distillate fuel. The structures, molecular weights, and boiling points of these compounds are given in Figure 5.1.

Comparison of Spectra under Methane and Methanol CI

The first step in this study was to compare the full spectra of each of the components obtained using methane and methanol chemical ionization. The CI with methanol was performed at three different pressures. The comparison of methanol CI spectra at taken at different pressures can be used to sort out the effects of the change in reagent ion composition as a function of pressure. The full scan spectra were taken by repetitive scanning GC/MS of a 2 microliter injection from the test blend, split 25:1.

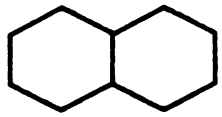
Spectra were taken over the mass range of 42-180 amu at a nominal scan rate of 250 amu/sec. The individual spectra presented here were those scans, selected from the data set, which exhibited the maximum net ion counts for the $(M+1)^+$

Table 5.1 Composition of Test Blend

Compound	Total Mass/g	ug/2 ul split 25:1	mole %
n-Heptane	6.22	49.7	91.0
Decalin	0.0769	0.615	0.816
1-Octanethiol	0.156	1.24	1.56
n-Butylbenzene	0.342	2.73	3.74
1-Phenylpyrrole	0.0292	0.570	0.299
Benzothiophene	0.0868	0.574	0.948
p-Methoxyphenol	0.0712	0.694	0.840
2-Methylindole	0.0718	0.234	0.802
Total	7.051 g in 10.00 ml		

n–Heptane

MW=100, BP=98.4C

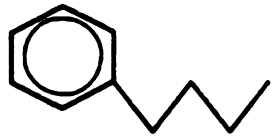


Decalin

MW=138, BP=190C

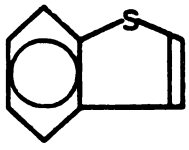
1–Octanethiol

MW=146, BP=198C



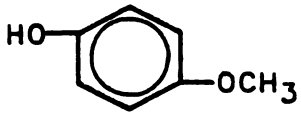
n–Butylbenzene

MW=134, BP=183C



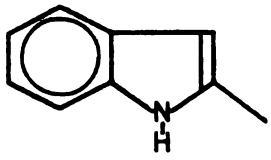
Benzothiophene

MW=134, BP=221C



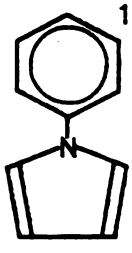
p–Methoxyphenol

MW=124, BP=243C



2–Methylindole

MW=131, BP=273



1–Phenylpyrrole

MW=143

BP=234C

Figure 5.1 Compounds for Methanol CI Study

peak of the corresponding mixture component. Background subtraction was used to remove the CI reagent peaks.

Spectra Obtained Using Methane Chemical Ionization

The first set of spectra, shown in Figures 5.2a and 5.2b, were taken using methane chemical ionization at a total pressure of 2.0 X 10^{-4} torr measured in the source vacuum chamber. This pressure reading includes a base pressure of approximately 2 X 10^{-5} torr, primarily due to a flow of approximately 1 ml/min (at atmospheric pressure) of helium from the gas chromatograph.

Under these conditions, the spectra of all eight compounds show significant amounts of molecular ion formation in addition to the expected MH^+ ion. Additionally, the hydrocarbons (n-heptane, n-butylbenzene, and decalin) and 1-octanethiol show extensive fragmentation, with little difference from their reference EI spectra. These observations, taken with the high concentration of helium in the ion source suggests that charge transfer from He^+ (ionization potential 24.587 eV (33)) is competing with proton transfer from CH_5^+.

Charge transfer ionization would transfer an amount of energy approximately equal to the ionization potential of the reactant ion to the reactant molecule. In the case of He^+, this would amount to 24.587eV and would be expected to generate spectra similar to that observed in EI with an

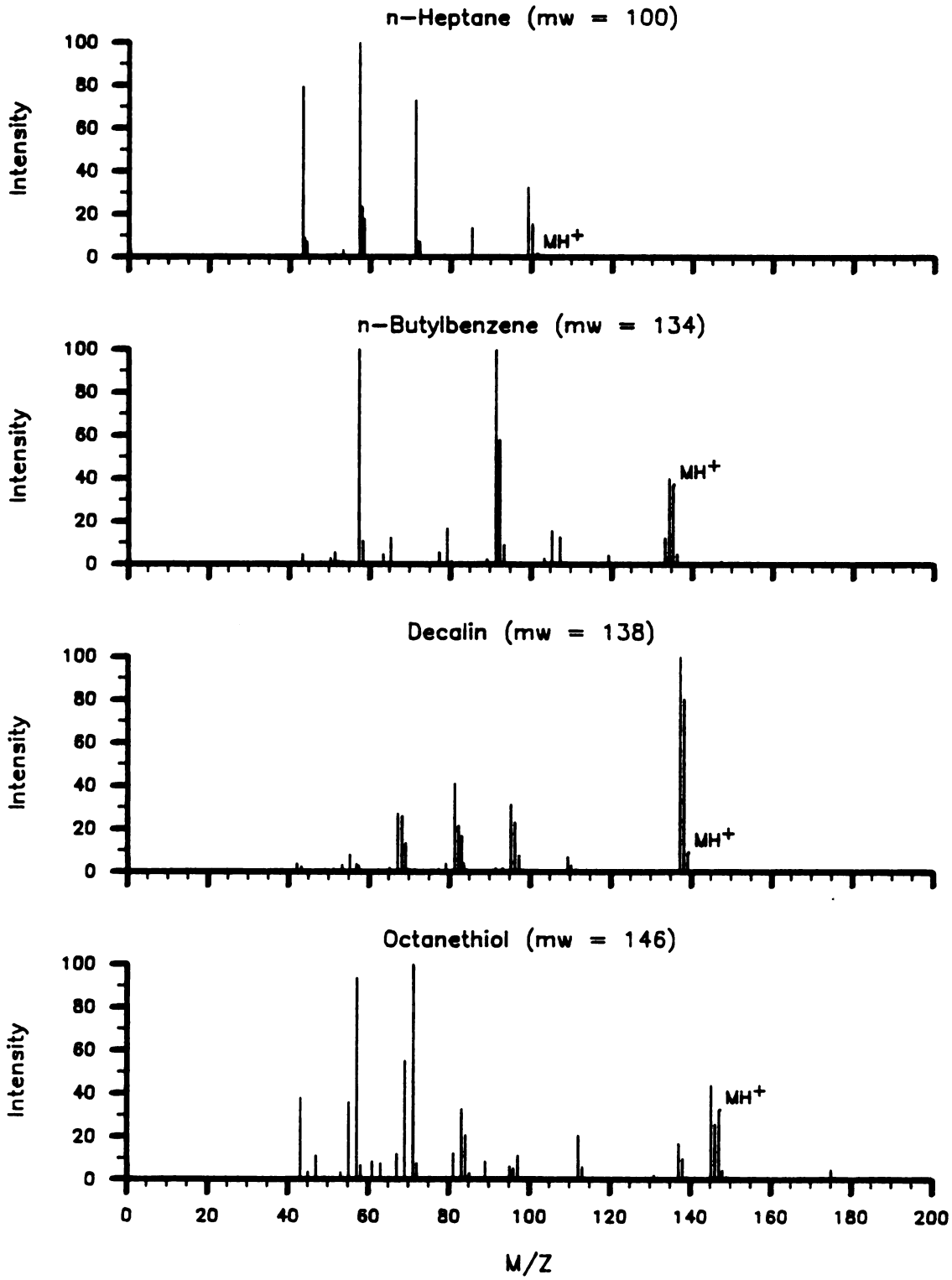


Figure 5.2a Methane CI 2.0 X 10^{-4} torr

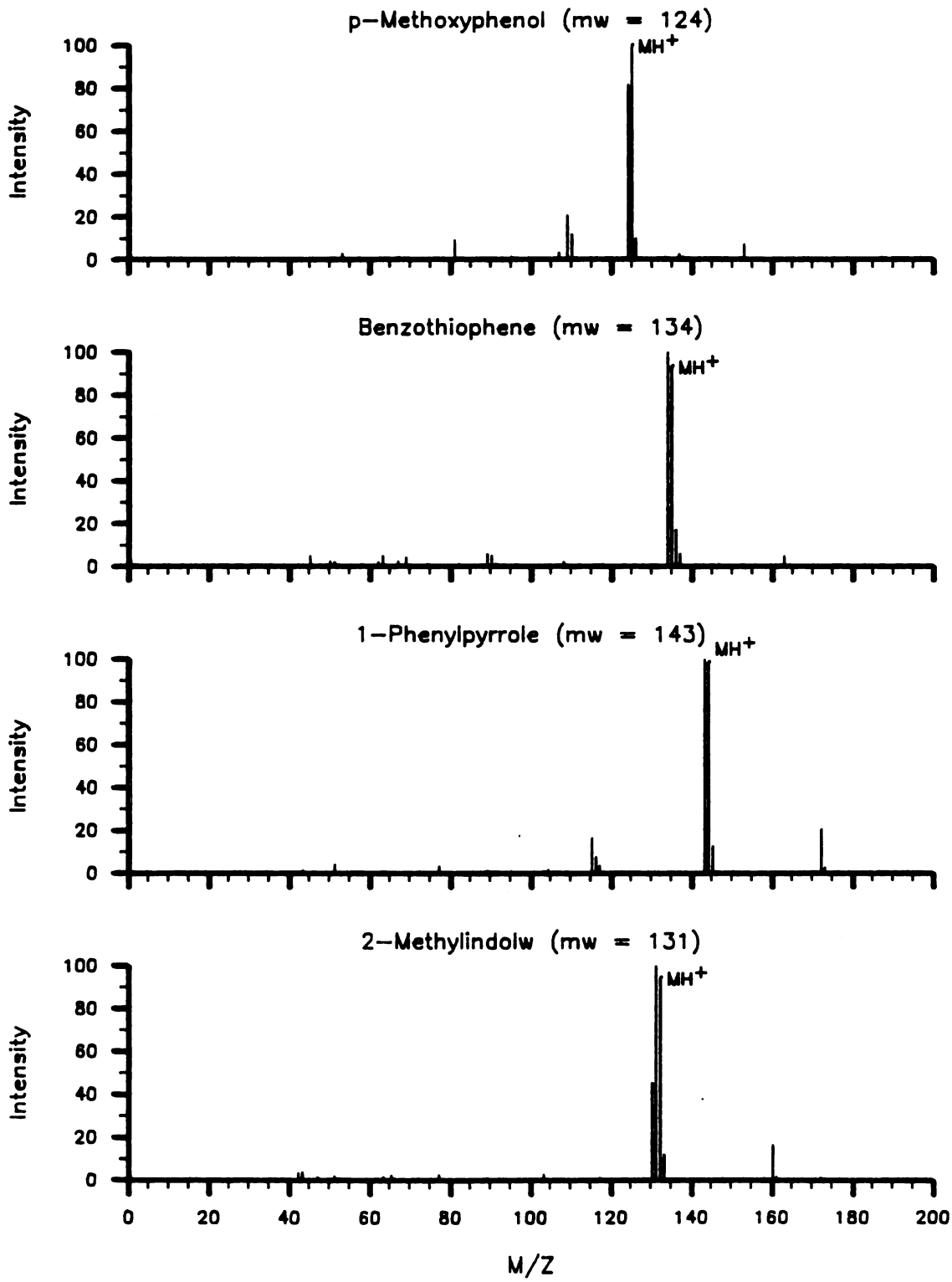


Figure 5.2b Methane CI 2.0×10^{-4} torr

equivalent electron energy. Such spectra would be expected to have enhanced molecular ion abundances relative to 70eV EI spectra with somewhat reduced fragmentation (34). This is very similar to what was observed in these methane CI spectra. An alternate explanation for the appearance of the M^+ peaks would be that the peak-findeer in the data system was splitting up the MH^+ peaks. This explanation, however, fails to account for the extensive fragmentation observed in several of the spectra.

The n-heptane exhibits the typical alkane peaks at 43^+, 57^+ (base peak), and 71^+, with smaller amounts of $(M-1)^+$ and M^+. The small amount of $(M+1)^+$ can be mostly attributed to the ^{13}C isotope. N-Butylbenzene does show a significant $(M+1)^+$ peak, but this is still smaller than the M^+ peak. The base peak in the spectrum is due to the butyl ion (57^+) with other major peaks at 91^+ (tropyllium) and 92^+. Decalin shows a large $(M+1)^+$ peak, but again it is smaller than the M^+ peak. There is, however, considerably reduced fragmentation, presumably due to stabilization from the fused ring structure. The spectrum of 1-octanethiol is dominated by the hydrocarbon chain, showing the characteristic alkene pattern of peaks at 43^+, 55^+, 57^+, 69^+, and 71^+ (base peak). This suggests the elimination of H_2S as a first step in the fragmentation process. That possibility is also supported by the presence of a significant peak (ca. 25%) at 113^+, corresponding to $[(M+1) - H_2S]^+$. This domination by the hydrocarbon peaks is similar to that observed in the EI

spectra of the straight-chain primary thiols (35). There are also significant peaks at $(M-1)^+$, M^+, $(M+1)^+$, and a $(M+29)^+$ adduct peak at 175^+.

The other four compounds studied, p-Methoxyphenol, Benzothiophene, 1-Phenylpyrrole, and 2-Methylindole, as shown in Figure 5.2b, all exhibit higher relative abundances of $(M+1)^+$. The presence, in all four cases, of a large M^+ ion again suggests that helium charge transfer is competing with proton transfer. Each of the four also shows an adduct peak at $(M+29)^+$. Further, the 2-Methylindole shows a peak of 45% relative abundance at $(M-1)^+$, possibly due to loss of the nitrogen-bound proton. The fragmentation of these heterospecies is much less than that observed for the hydrocarbons. Much of this effect can be attributed to the charge stabilization afforded by the unsaturated ring structures. However, the ion current is still being divided between at least two major peaks in each spectrum.

Spectra Obtained Using Methanol Chemical Ionization

These methane CI spectra provide a basis for comparison with spectra obtained from the same compounds, under similar instrument conditions, and using methanol at three different pressures as the reagent gas. The three spectra in Figure 5.3 illustrate the ion composition of the reagent gas plasma at three different source chamber pressures, namely 6.5×10^{-5}, 2.0×10^{-4}, and 4.5×10^{-4} torr. The first pressure

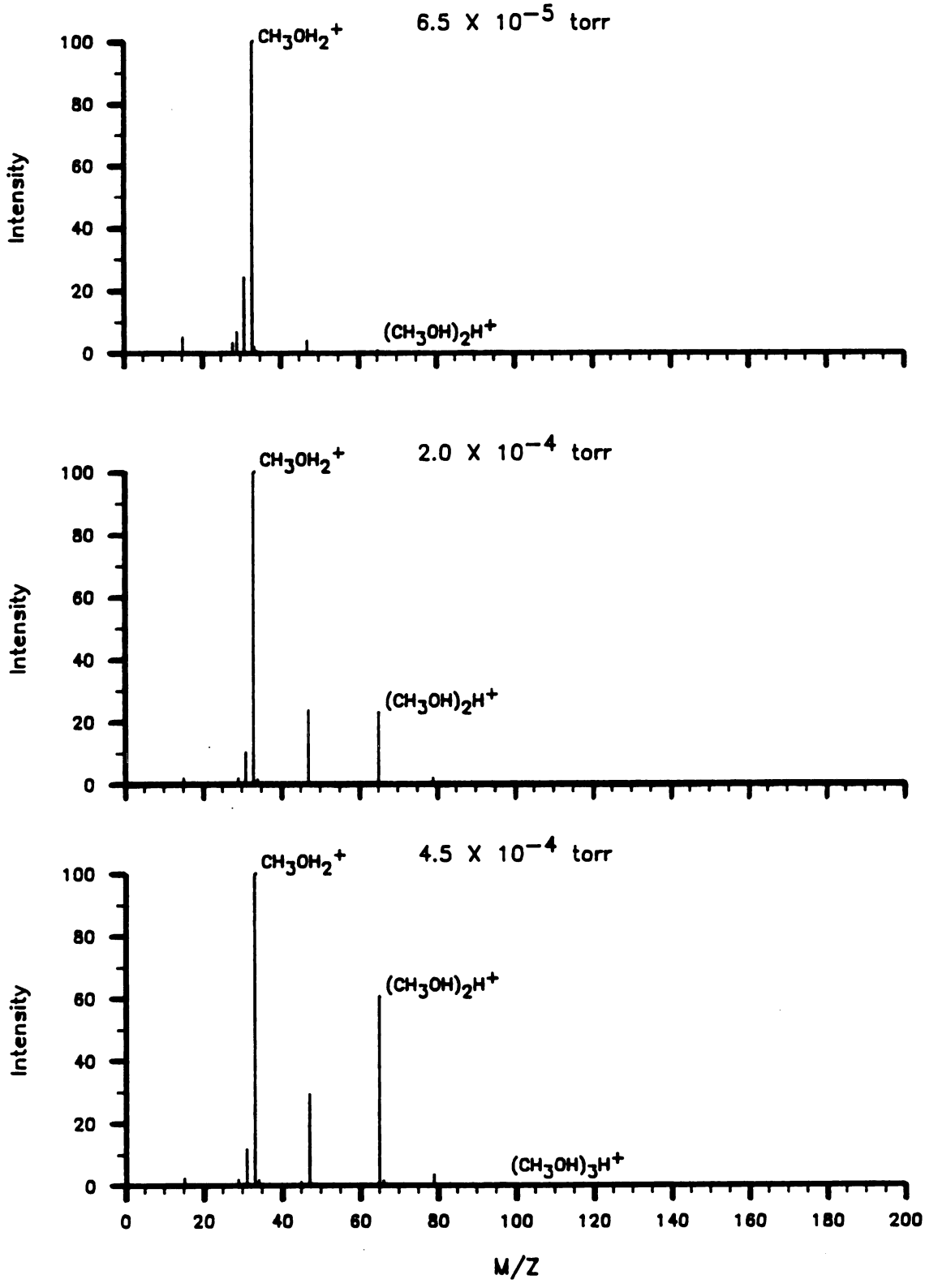


Figure 5.3 Methanol Reagent Ions

was chosen to maximize the absolute intensity of the 33$^+$ ($CH_3OH_2^+$) peak while minimizing the contribution of 65$^+$ $[(CH_3OH)_2H]^+$. There is a small peak (ca. 3% relative abundance) at mass 47$^+$, representing loss of water from 65$^+$. The third pressure, 4.5 X 10^{-4} torr, was chosen to maximize the contribution due to the 65$^+$ ion. Under these conditions, 65$^+$ has a relative abundance of about 60% and 47$^+$ has a peak of roughly 30%. A small peak (ca. 3%) at 79$^+$ represents loss of water from the methanol cluster ion at 97$^+$, $[(CH_3OH)_3H]^+$. The second pressure, 2.0 X 10^{-4} torr, was chosen as an intermediate between the first and the third. At this pressure, the peaks at 47$^+$ and 65$^+$ are found to be of roughly equal relative abundance, 25%.

These spectra, taken at a source temperature of 200 C and with a helium flow of 1 ml/min from the GC column show significantly less clustering of the methanol than in the work of Bauer (36). In that study, which employed a source temperature of 100 C and no helium flow, the methanol pressure was adjusted so as to maximize the absolute intensities of the 65$^+$ and 97$^+$ ions. This resulted in a spectrum with the base peak at 65$^+$ and the 33$^+$ and 97$^+$ ions having approximately equal relative abundances at about 80%. The source conditions in the present study were dictated by the need to optimize within conditions appropriate for GC/MS. The reduced abundances of the higher methanol clusters at elevated temperatures is reasonable

considering the weakness of the hydrogen bonds which bind the clusters.

Figures 5.4a and 5.4b present the spectra of the blend components obtained with methanol CI at 6.5 X 10^{-5} torr (the lowest pressure). Generally reduced fragmentation, relative to that observed with methane CI, is observed, even with this relatively low methanol pressure. As was the case in the methane CI spectra, the extent of fragmentation and the presence of M^+ ions in several of the spectra suggests that helium charge transfer ionization is again competing with proton transfer, this time from the methanol reagent ions. This is consistent with the high helium concentration resulting from the use of a low methanol pressure.

The appearance of the n-heptane spectrum is virtually unchanged from that observed with methane. The additional peak at 65^+ can be attributed to a residual reagent gas peak which was incompletely removed in the background subtraction. The n-butyl benzene shows greatly reduced fragmentation. The butyl peak at 57^+, which was the base peak with methane, has been virtually eliminated, and $(M+1)^+$, at 135^+ has taken its place. The relative abundances of the 91^+ and 92^+ ions have also been reduced by about 30-40%. The decalin spectrum shows a greatly reduced M^+, in favor of $(M+1)^+$, but the large number of fragment ions are essentially unchanged. 1-Octanethiol shows a greatly enhanced $(M+1)^+$ ion, which is now the base peak, but the fragment ions, other than their reduced relative

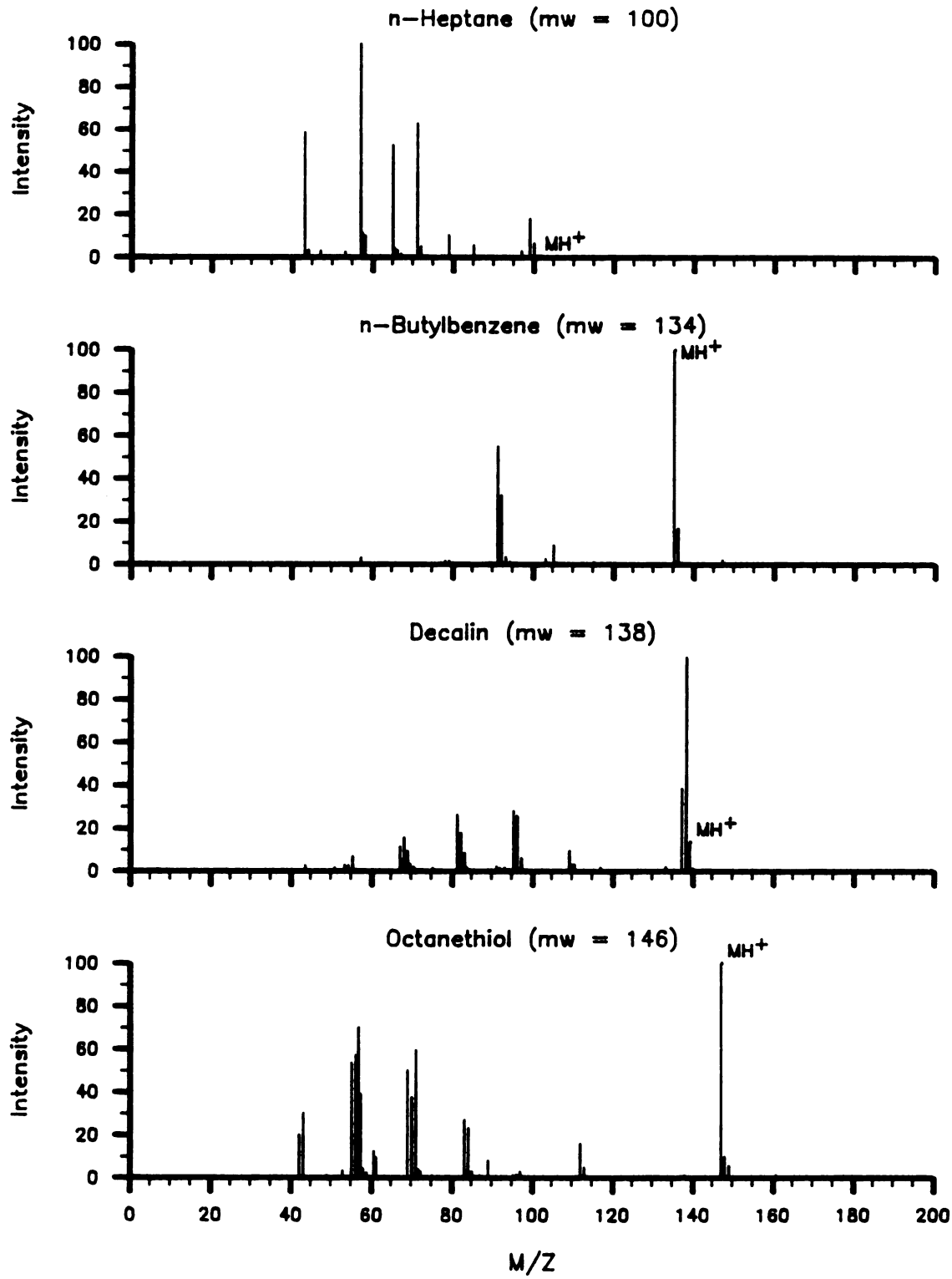


Figure 5.4a Methanol CI 6.5×10^{-5} torr

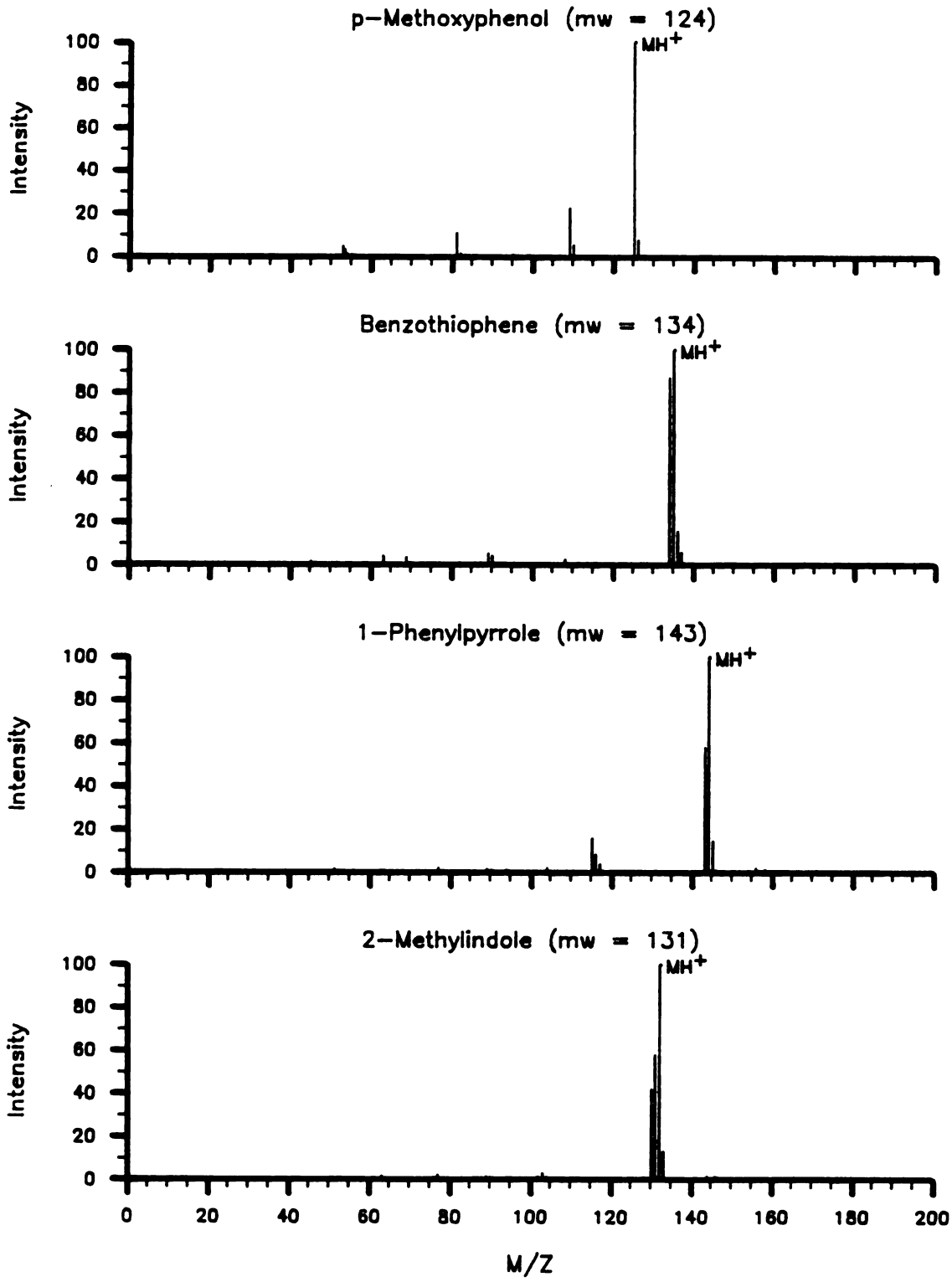


Figure 5.4b Methanol CI 6.5×10^{-5} torr

abundance, remain the same. The spectra of the four heterospecies in Figure 5.4b are very similar to those obtained with methane except that the $(M+1)^+$ ions are enhanced relative to M^+. For p-Methoxyphenol, the M^+ ion at 124^+ is completely eliminated.

These differences from the methane CI spectra observed for the polar compounds and n-butylbenzene occur despite the high concentration of helium in the ion source. This suggests that the efficiency of proton transfer from the methanol reagent ions to these compounds is higher, relative to helium charge exchange, than is the case for the non-polars.

Increasing the methanol pressure to 2.0×10^{-4} torr, as illustrated in Figures 5.5a and 5.5b, generally results in further reduced fragmentation. The exceptions are the n-heptane and the decalin. The spectrum of n-heptane is essentially unchanged once more, and the decalin actually shows increased fragmentation. All of the heterospecies, including 1-octanethiol, as well as the n-butylbenzene, show increased relative abundances for $(M+1)^+$. The M^+ peaks are now eliminated from the spectra of benzothiophene, 1-phenyl-pyrrole, and 2-methylindole.

The third set of methanol CI spectra, taken at a pressure of 4.5×10^{-4} torr, are presented in Figures 5.6a and 5.6b. In this set, the fragmentation of decalin has undergone further increase. Additionally, n-butylbenzene is

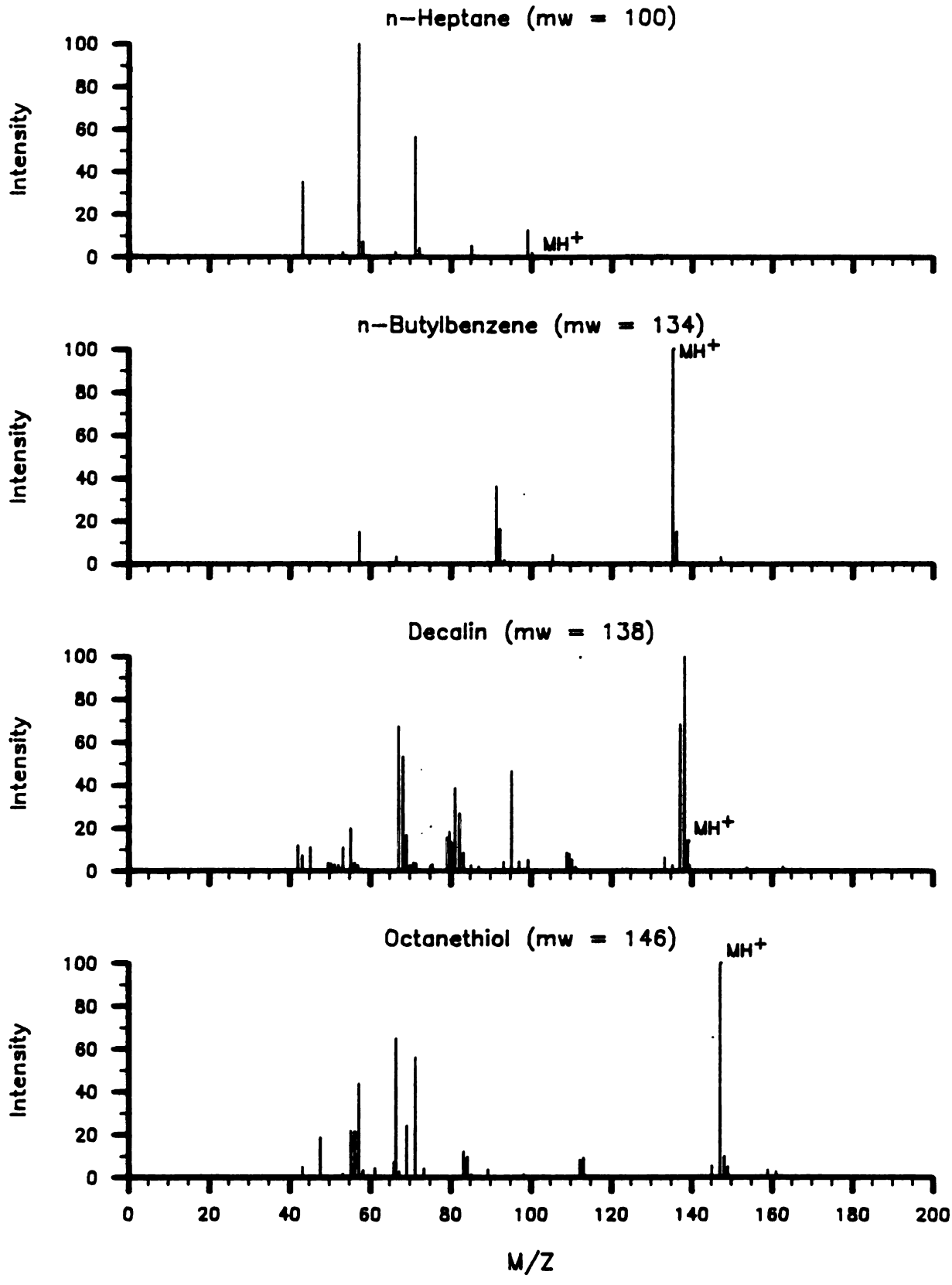


Figure 5.5a Methanol CI 2.0 X 10^{-4} torr

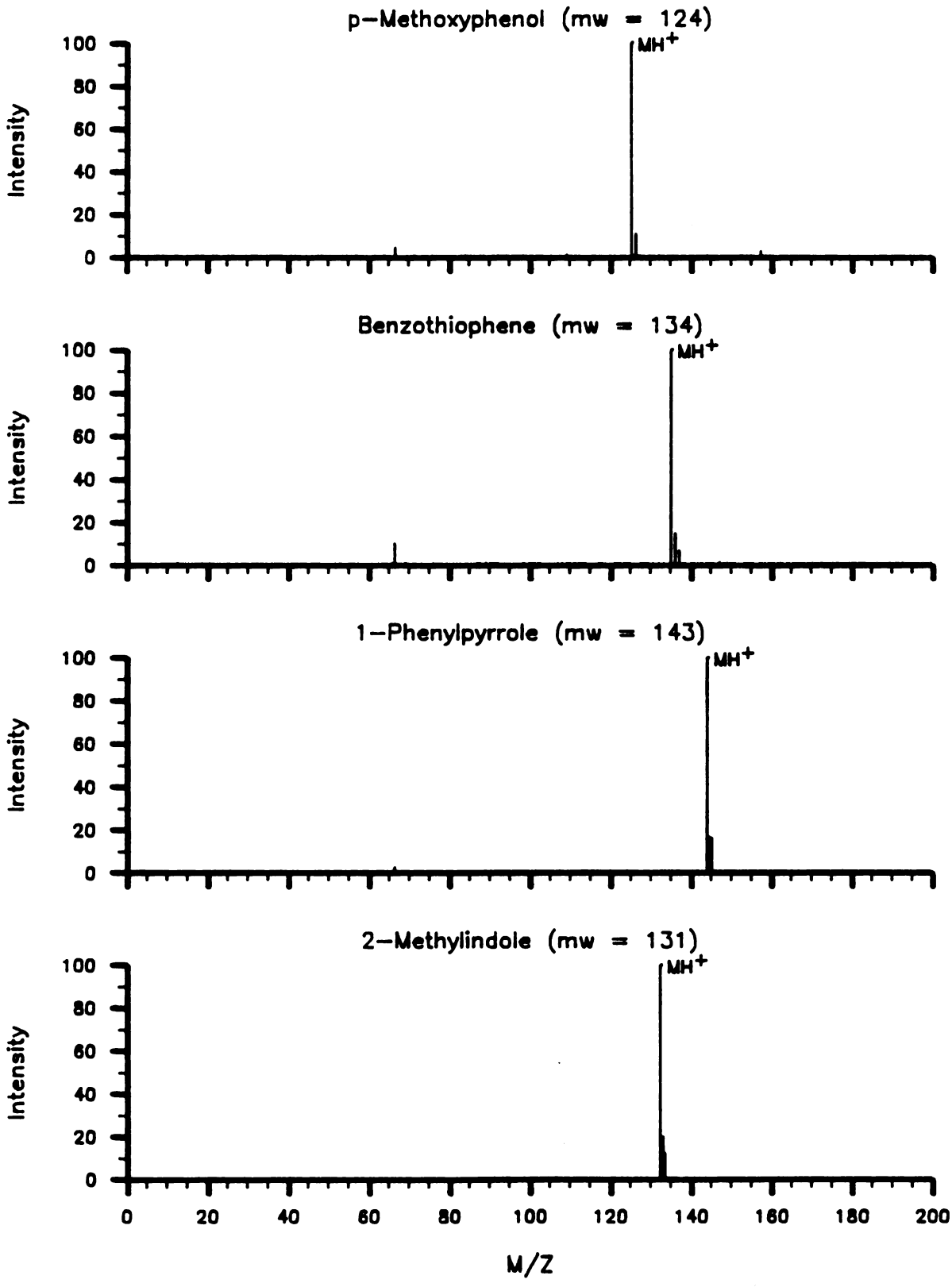


Figure 5.5b Methanol CI 2.0×10^{-4} torr

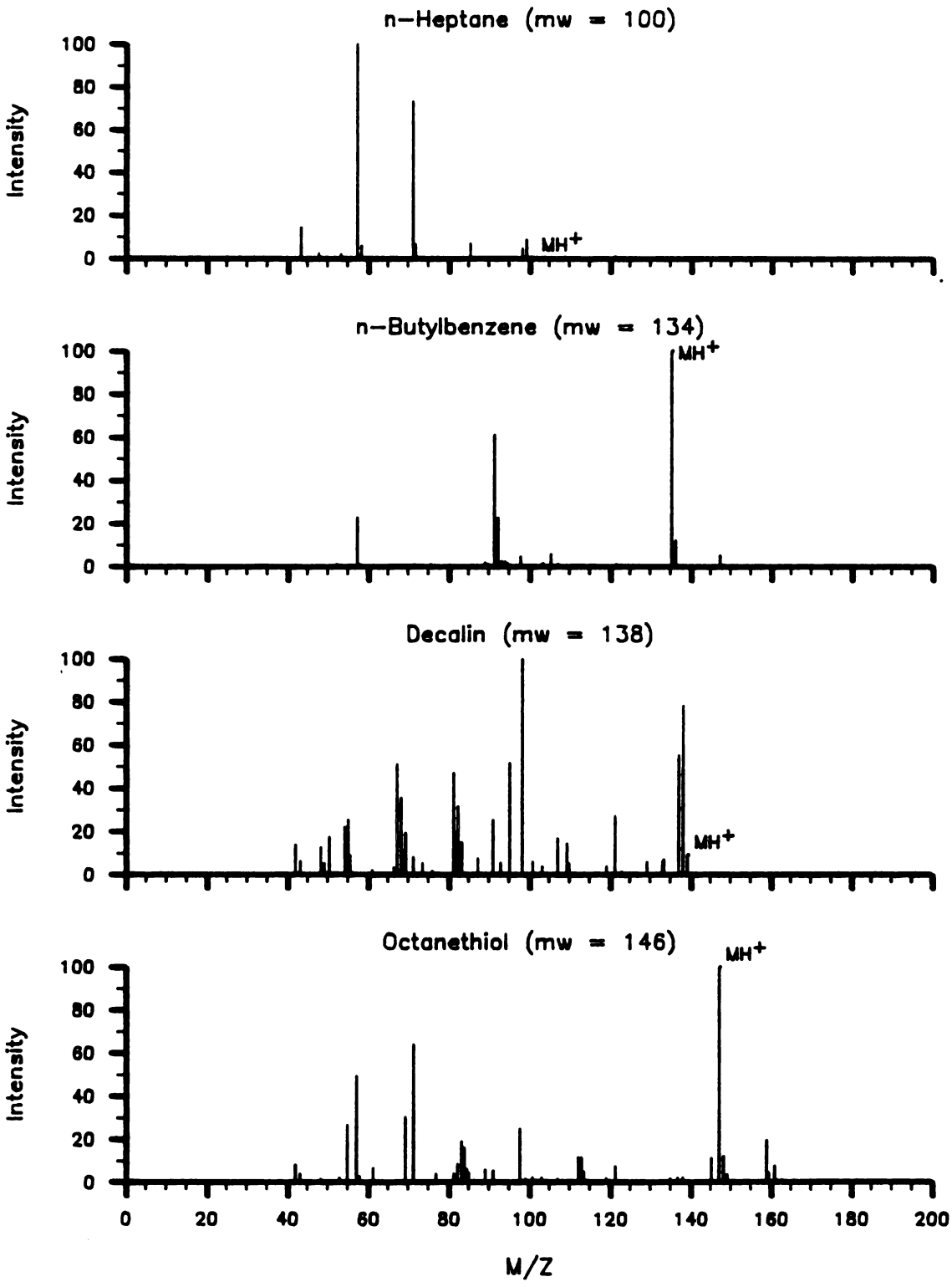


Figure 5.6a Methanol CI 4.5×10^{-4} torr

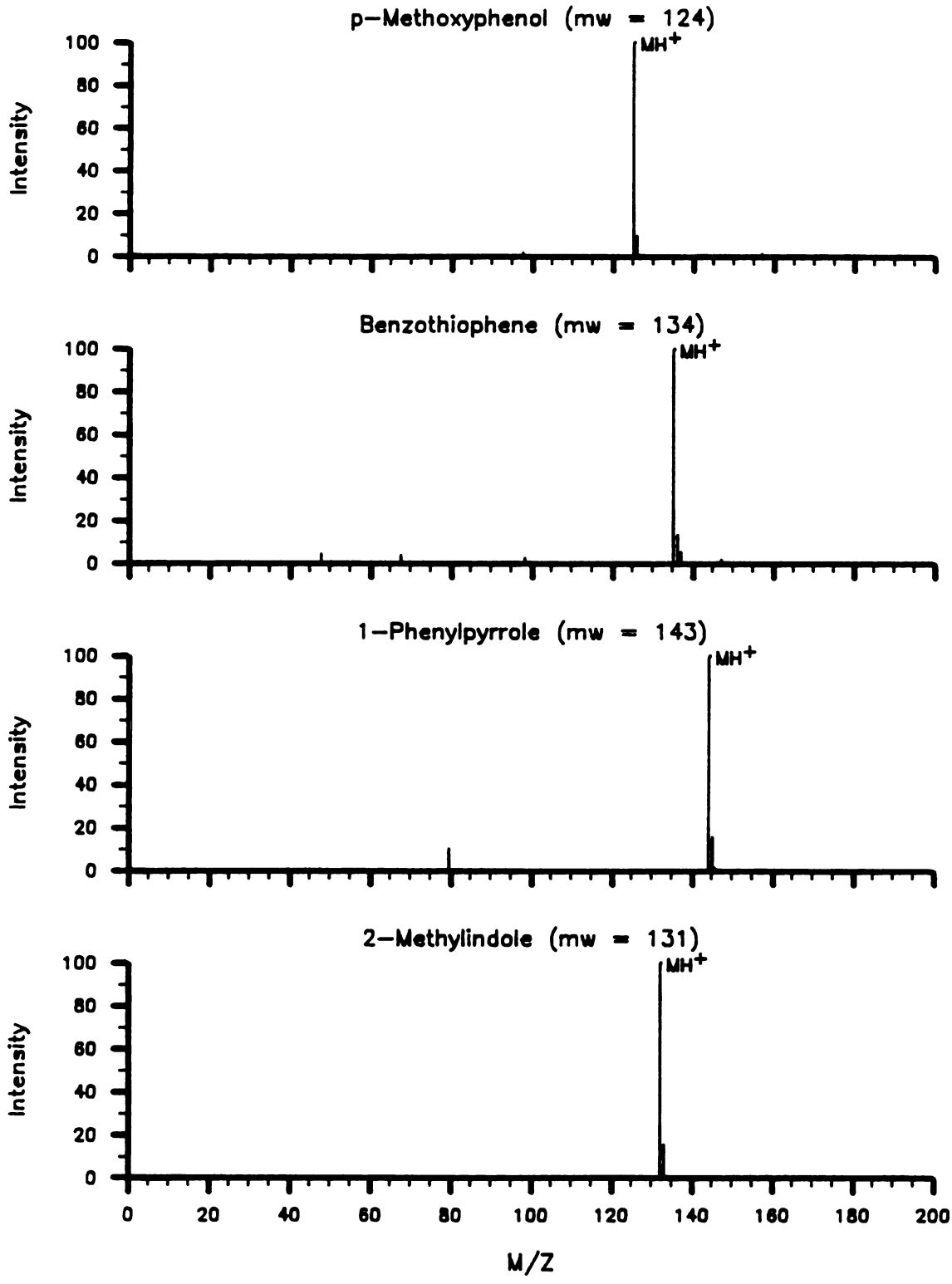


Figure 5.6b Methanol CI 4.5×10^{-4} torr

now showing increased fragmentation. The normalized spectra of all the other compounds remain essentially unchanged.

In summary, increasing the methanol pressure results in greatly reduced fragmentation and elimination of the M^+ peaks. This may be attributed to an effective dilution of helium in the ionization region and the fact that higher methanol pressures increase the probability that any helium ions formed would collide with, and ionize, methanol molecules rather than sample molecules. The fact that this is not observed for 1-octanethiol, heptane, and decalin suggests that these compounds are not very sensitive to methanol CI, possibly due to low proton affinities, and, instead, the bulk of the ionization of these molecules is the result of helium charge transfer.

Discussion of Full Scan Chemical Ionization Spectra

Comparison of the spectra taken under methane CI with those obtained using methanol CI show significant differences in behavior for the different compound classes studied. The observed differences all appear quite reasonable considering the diversity of the eight compounds under study.

The spectra of n-heptane and decalin are the least affected by the choice of ionization conditions. Changes in the spectral patterns observed for these two compounds may be explained by poor proton transfer efficiency from the

methanol reagents ions to these saturated hydrocarbons. As a result, charge exchange ionization from helium (present in the ionization region from the gas chromatograph) appears to be the dominant ionization mechanism. This effect is particularly noticeably at the highest methanol pressure (i.e. the lowest helium concentration) where the spectra of the polar compounds show virtually no fragmentation and the non-polar compounds undergo extensive fragmentation.

The third hydrocarbon studied, n-butylbenzene, shows a significant change under all of the methanol CI conditions studied. At all three methanol pressures, the n-butylbenzene shows enhanced abundance of the MH^+ ions and reduced abundances for M^+, $(M-H)^+$, and fragmentation products. This difference in behavior from that observed for n-heptane and decalin can probably be attributed to the stabilizing affect of the aromatic ring. There appear to be no significant differences in the spectral patterns obtained using the three different methanol pressures.

Perhaps the most interesting compound studied is 1-octanethiol. It's spectra contain both a fragmentation pattern typical of an alkene and an enhanced abundance of the MH^+ ion, as would be expected for a hetero-atom containing species. Switching from methane to methanol CI retains the alkene fragmentation pattern, but at reduced relative abundance, while the increase in the abundance of the MH^+ ion is such that it becomes the base peak. The contributions of M^+ and $(M-H)^+$ are both greatly reduced.

Again, there are no major differences in the spectral patterns obtained under the three different methanol pressures.

The remaining four compounds in this study -- p-methoxyphenol, benzothiophene, 1-phenylpyrrole, and 2-methylindole -- all show very similar trends. Generally, they all exhibit minimal fragmentation under any of the four ionization conditions studied. The one major trend is a reduction in the relative abundance of the M^+ ion from being approximately equal to the MH^+ intensity under methane CI, to a reduced abundance in the low- and medium-methanol CI spectra, and finally being eliminated in the high-pressure methanol spectra. This may be explained as a reduction in the occurrence of charge exchange ionization as the concentration of helium in the ion source is effectively reduced. In all four cases, the high-pressure methanol spectra are virtually fragment free and show only the MH^+ ion and the related isotope peaks.

In general, methanol CI is observed to result in reduced fragmentation relative to the spectra obtained using methane CI. This effect holds true for n-butylbenzene and all of the heterospecies studied. Due to the apparent occurrence of helium charge exchange ionization in this work, the spectral patterns for the saturated hydrocarbons appear basically unaffected by the choice of ionization conditions, though their sensitivities appear to be reduced under methanol CI conditions.

The reduced sensitivity of methanol chemical ionization for non-polar compounds can be used to discriminate against the hydrocarbon matrix in a fuel sample, thereby facilitating the study of heterospecies. As a result, the costly and time-consuming preliminary separations required by less selective techniques can, potentially, be avoided. Further, the reduced fragmentation observed for the heterospecies can be used to simplify the spectra of fuels, yielding a single characteristic ion for each heterocompound of interest.

Relative Sensitivities of Methane and Methanol CI

After examination of the normalized spectra of the blend components under different ionization conditions, the next area for examination was the relative sensitivities of the different ionization techniques. As discussed earlier, an experimental goal was to find ionization conditions which maximize sensitivity for heterospecies which may be present at trace levels in fuels, while minimizing contributions to the ion current due to the hydrocarbon species which make up the bulk of the fuel matrix; in other words, to develop a technique that is selective for the heterospecies.

To obtain this information, selected ion monitoring (SIM) was employed. Peak areas were determined for the $(M+1)^+$ ions from each of the components in the previously described test blend. The choice of ions was made on the basis that both methane and methanol CI were observed to

produce ions at $(M+1)^+$ for all of the compounds in the test blend. Conditions for the GC were identical to those previously described for the full scan experiments. Six ions were simultaneously monitored, representing the $(M+1)^+$ ions for each of the mixture components (n-butylbenzene and benzothiophene are isobaric with molecular weights of 134, but are readily distinguished on the basis of their retention times.) Triplicate runs were made at each of the four ionization conditions described above. Observed retention times are reported in Table 5.2.

To compare the relative sensitivities of the trace heterospecies under these ionizing conditions, n-butylbenzene was chosen as a reference compound. This choice was made because n-butylbenzene is expected to be present in middle distillate fuels at the high parts per million level, it was observed to yield large relative abundances of $(M+1)^+$ with all of the ionization conditions employed in this study, and it produced more reproducible spectra than could be obtained for the alkanes (which show very poor sensitivity with methanol CI). Thus, measurement of the ionization efficiency for each of the heterospecies, relative to that observed for n-butylbenzene, will be an indicator of the selectivity of a given ionization technique for heterospecies in a hydrocarbon matrix. Results obtained using this approach are reported in Tables 5.3 - 5.6. They are presented in terms of the ratio of the peak area of each component to that obtained for n-butylbenzene for the

Table 5.2 Retention Times of Blend Components

Compound	Retention Time (sec)
n-Butylbenzene	98.2 ± 0.3
1-Octanethiol	105.4 ± 0.3
Decalin	110.6 ± 0.3
p-Methoxyphenol	113.5 ± 0.2
Benzothiophene	123.2 ± 0.3
1-Phenylpyrrole	135.6 ± 0.2
2-Methylindole	161.5 ± 0.3

Table 5.3 Sensitivities for Several Heterospecies Relative to n-Butylbenzene with Methane CI at 2.0×10^{-4} torr

Compound	ug/inj	Area Ratio	Corrected Ratio	Sensitivity (area/pg)
n-Butylbenzene	2.74	1.00	1	0.3 ± 0.2
1-Octanethiol	0.16	0.12±0.01	2.1 ± 0.1	0.6 ± 0.4
p-Methoxyphenol	0.071	0.6 ± 0.1	23 ± 4	7 ± 1
Benzothiophene	0.087	2.1 ± 0.6	70 ± 20	20 ± 5
1-Phenylpyrrole	0.029	1.4 ± 0.4	130 ± 40	40 ± 10
2-Methylindole	0.072	3 ± 1	100 ± 40	30 ± 10

Table 5.4 Sensitivities for Several Heterospecies Relative to n-Butylbenzene with Methanol CI at 6.5 X 10^{-5} torr

Compound	ug/inj	Area Ratio	Corrected Ratio	Sensitivity (area/pg)
n-Butylbenzene	2.7	1.00	1.00	0.9 ± 0.3
1-Octanethiol	0.16	0.19±0.01	3.3 ± 0.2	2.9 ± 0.2
p-Methoxyphenol	0.071	0.18±0.01	6.8 ± 0.4	5.9 ± 0.4
Benzothiophene	0.087	0.45±0.03	14.3 ± 0.1	12.5 ± 0.1
1-Phenylpyrrole	0.029	0.38±0.03	36 ± 3	31 ± 3
2-Methylindole	0.072	0.52±0.04	20 ± 2	17 ± 2

Table 5.5 Sensitivities for Several Heterospecies Relative to n-Butylbenzene with Methanol CI at 2.0 X 10^{-4} torr

Compound	ug/inj	Area Ratio	Corrected Ratio	Sensitivity (area/pg)
n-Butylbenzene	2.74	1.00	1.00	1.3 ± 0.3
1-Octanethiol	0.16	0.29±0.01	5.1 ± 0.2	6.5 ± 0.3
p-Methoxyphenol	0.071	0.77±0.07	30 ± 3	39 ± 4
Benzothiophene	0.087	0.85±0.05	27 ± 2	35 ± 3
1-Phenylpyrrole	0.029	1.8 ± 0.1	70 ± 10	90 ± 10
2-Methylindole	0.072	2.8 ± 0.1	105 ± 4	135 ± 5

Table 5.6 Sensitivities for Several Heterospecies Relative to n-Butylbenzene with Methanol CI at 4.5 X 10^{-4} torr

Compound	ug/inj	Area Ratio	Corrected Ratio	Sensitivity (area/pg)
n-Butylbenzene	2.74	1.00	1.00	0.263±0.006
1-Octanethiol	0.16	0.208±0.007	3.7 ± 0.1	0.97 ± 0.03
p-Methoxyphenol	0.071	1.73±0.07	67 ± 3	17.6 ± 0.8
Benzothiophene	0.087	1.29±0.03	40.7 ± 0.9	10.7 ± 0.2
1-Phenylpyrrole	0.029	5.3 ± 0.2	500 ± 20	130 ± 5
2-Methylindole	0.072	9.6 ± 0.5	370 ± 20	97 ± 5

amounts indicated and also the sensitivity in peak area per picogram of component. This approach minimizes uncertainties in the data resulting from fluctuations in instrument sensitivity due to factors other than the ionization conditions and serves to emphasize how the use of selective ionization conditions can enhance sensitivity for trace components in complex mixtures. The mass corrected ratios are a measure of the selectivity of each set of ionization conditions because they directly compare the signal obtained for each analyte to that obtained from an equal weight of a typical interference, with all other analysis conditions being held constant.

Discussion of Selected Ion Monitoring Data

The area ratio approach to sensitivity comparison becomes somewhat distorted due to the major variations in the fragmentation pattern of n-butylbenzene across the four different ionization conditions employed. It is, nonetheless, a valuable approach considering that quantitative studies are generally done on the basis of peak areas obtained by selected ion monitoring of one, or perhaps two, ions for each compound of interest. As a result, however, these data are best evaluated by comparing the trends across ionization conditions for each of the heterospecies in the blend. This is done graphically in Figure 5.7.

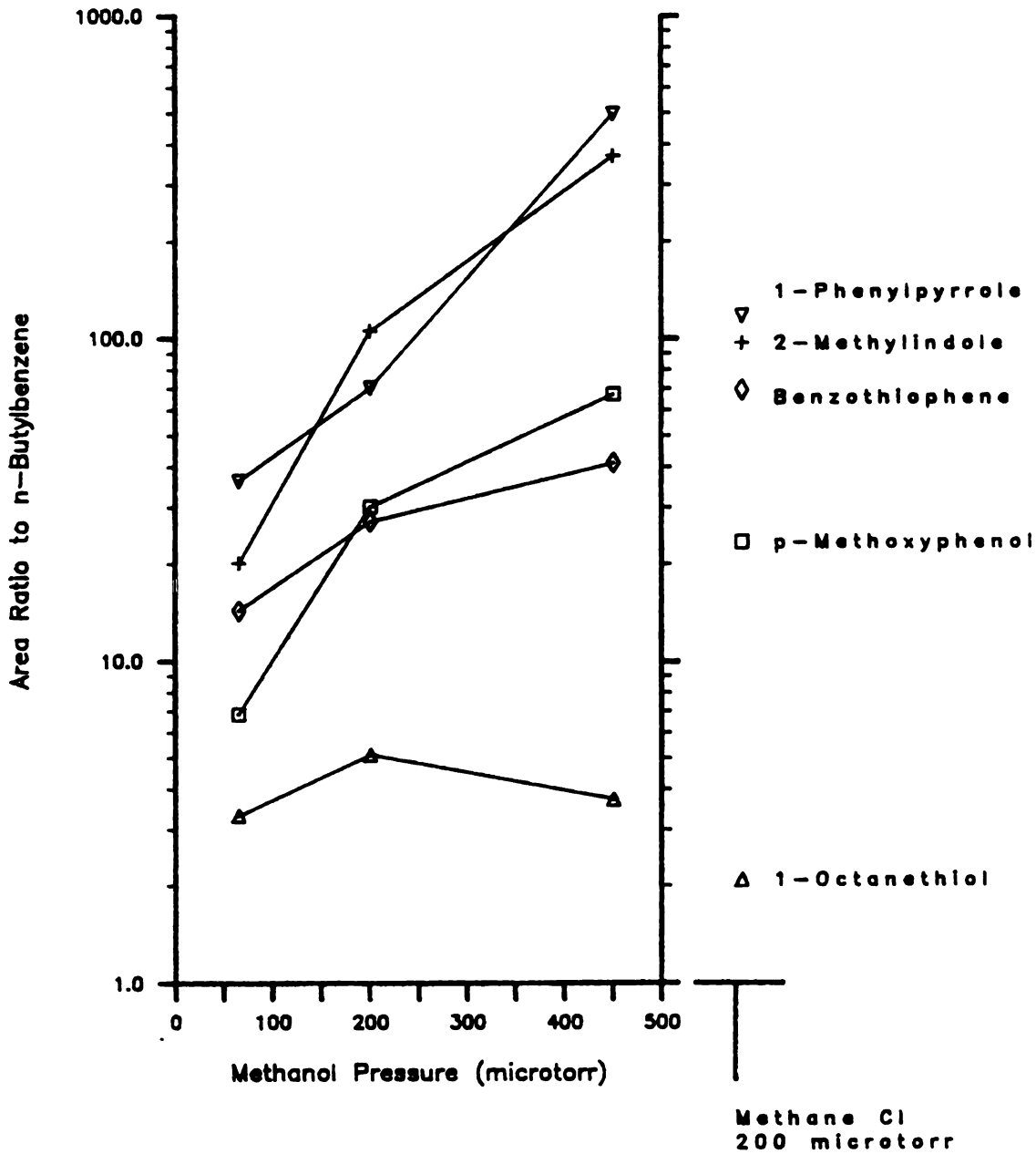


Figure 5.7 Selectivity Factor vs. Ionization Mode

Observing the trends for the five heterospecies studied shows that 1-Octanethiol is the only one to increase in selectivity factor going from methane to low pressure methanol CI. The selectivity factors of the other four heterospecies are seen to actually fall by factors of roughly three to five under that change of conditions. It may be recalled that under these two ionization conditions, the spectrum of 1-octanethiol was observed to be dominated by the alkyl portion of the molecule, showing an alkane fragmentation pattern, while the other compounds were showing very little fragmentation. The fact that the selectivity factors of the heterospecies actually declined under this change in ionization conditions can be attributed primarily in the dramatic increase in the relative abundance of the $(M+1)^+$ ion for n-butylbenzene from about 10% of total ionization with methane to almost 50% under low pressure methanol CI.

Further examination reveals that the selectivity factors of the five heterospecies studied show a general increase with increasing methanol pressure. These increases are quite dramatic for the p-methoxyphenol, 1-phenylpyrrole, and 2-methylindole for which the selectivity factors increase approximately 10, 14 and 18-fold, respectively, in going from the low to the high methanol pressure.

In contrast, benzothiophene shows a much less pronounced increase in relative sensitivity with increased methanol pressure, going from 14.3 at the low pressure to

40.7 at the high pressure - an overall improvement of less than threefold. It can be further noted that even at high methanol pressure, relative sensitivity is lower than that which was obtained using methane, the only one of the five heterospecies studied to behave in this manner.

1-Octanethiol also shows a trend different from the other heterospecies studied. Its selectivity factor is very low and exhibits relatively little change, with corrected area ratios of 3.3 at low pressure and 3.7 at high methanol pressure. The selectivity factor of 1-octanethiol is actually greatest at the middle methanol pressure, with a value of 5.1. As Figure 5.7 shows, 1-octanethiol exhibits the lowest relative response of all the heterospecies studied at under all four ionization conditions employed. The anomalous behavior of 1-octanethiol, however does not seem unreasonable in light of the full mass spectra, examined earlier, which were dominated by the alkyl portion of the molecule.

The selectivity of an ionization technique, however, is of little use if that technique is not sufficiently sensitive to produce a reliable signal at the concentration range of interest. Tables 5.3 - 5.6 also present the sensitivity of each of the four ionization conditions studied for each of the five heterospecies in the test blend. These numbers were obtained by calculating the product of the sensitivity of n-butylbenzene and the corrected area ratio for each of the heterospecies. To

better illustrate the trends across the different ionization conditions, these values are plotted in Figure 5.8.

Four of the five heterospecies studied show reduced sensitivity in going from methane to low pressure methanol as the chemical ionization reagent. This can be attributed to fragmentation resulting from helium charge exchange ionization. 1-Octanethiol, which shows a significantly increased relative abundance of the MH^+ ion under low pressure methanol CI conditions, is the one exception. It shows nearly a five-fold gain in sensitivity in going from methane to low pressure methanol CI.

Increasing the methanol pressure from 6.5 X 10^{-5} to 2.0 X 10^{-4} torr, as measured in the source vacuum chamber, results in major increases in sensitivity for all five heterospecies. This can be attributed to a general increase in the overall ionization efficiency, illustrated by a nearly 50% increase in the sensitivity for n-butylbenzene. Recall too, that all five of the compounds exhibit increased selectivity in going to the medium pressure methanol. The medium pressure methanol is the most sensitive ionization condition for all but one of the compounds studied, the one exception being 1-phenylpyrrole.

With the exception of 1-phenylpyrrole, all of the heterospecies showed a major loss in sensitivity as the methanol pressure was increased to the highest value used. This can be primarily attributed to reduced ion extraction efficiency resulting from the high source pressures

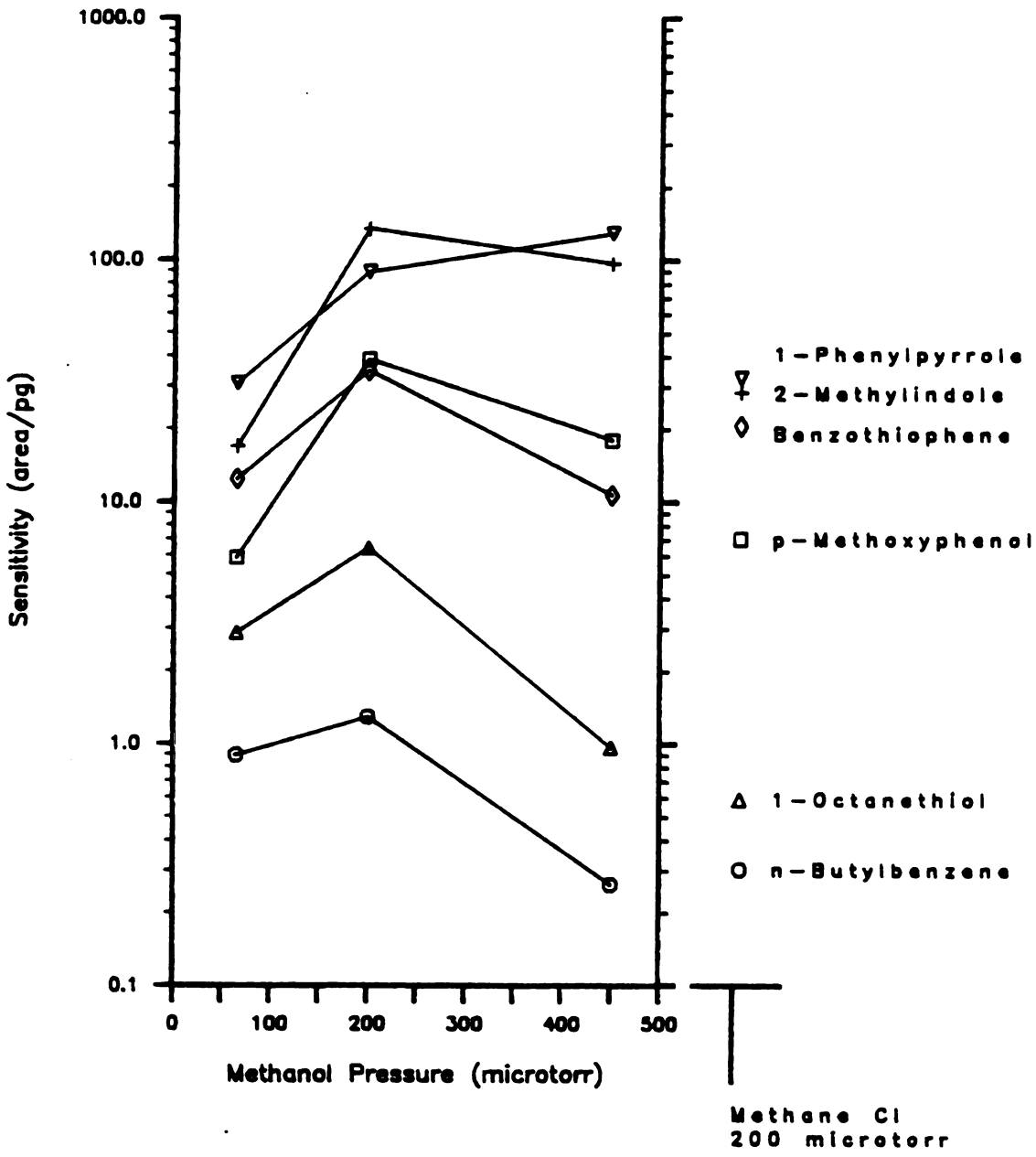


Figure 5.8 Sensitivity vs. Ionization Mode

Summary and Conclusions

This study examined the methanol chemical ionization mass spectra of compounds representing a wide range of functionality and representative of species which may be present in a middle distillate fuel. These spectra exhibited reduced fragmentation when compared to spectra of the same compounds obtained with the more conventional technique of methane chemical ionization. The degree of fragmentation using methanol CI was observed to generally decrease with increased methanol pressures, where proton-bound clusters of methanol are present. The optimum methanol pressure varied for the different compounds studied, with higher optimum pressures observed for the more polar compounds. In general, the overall optimum pressure was found to occur at levels at, or somewhat higher, than those generally employed in this laboratory for methane chemical ionization.

Additional studies examined the selectivity and sensitivity of methane and methanol chemical ionization for the MH^+ ions of the same compounds studied above. Under these conditions, the selectivity factors of all five heterospecies, measured relative to that of the representative hydrocarbon, n-butylbenzene, increase with increasing pressure of the methanol CI reagent. For all but 1-octanethiol, the highest relative selectivity under methanol CI conditions, is measured at the highest methanol pressure

studied. The selectivity factor for all of the heterospecies studied, except for benzothiophene, is higher at the maximum methanol pressure than that observed using methane CI. All five of the heterospecies studied exhibit higher sensitivity at the middle methanol pressure than with methane as the CI reagent.

The ionization process employed is a crucial factor in determining the overall selectivity and sensitivity of techniques such as GC/MS, MS/MS, and GC/MS/MS. The power of multi-dimensional methods for trace analysis depends upon each independent dimension contributing some selectivity for the species of interest. Small selectivity gains can often be made without requiring severe sensitivity tradeoffs. The combination of even small enhancements, however, allows the analyst to tailor a highly selective technique while retaining good sensitivity.

This study has quantitated the selectivity versus sensitivity tradeoff resulting from the use of methanol chemical ionization for the analysis of five diverse heterospecies which may be present in a middle distillate fuel. The optimum methanol pressure to employ in such an analysis in a fuel is largely dependent upon which additional analytical dimensions are also available. In low resolution GC/MS, for instance, background from unresolved mixture components is often a limiting factor. In such a case, the use of high-pressure methanol, with its very high selectivity, is indicated, especially for compounds such as

2-methylindole and 1-phenylpyrrole. The analyst must realize, however, that this increase in selectivity is coming at a stiff price in terms of sensitivity. The use of additional selectivity elements such as high-resolution chromatography, high-resolution mass spectrometry, or tandem mass spectrometry serves to lower the selectivity demand placed on the ionization process. As a result, the analyst could then use the medium pressure methanol CI conditions which, though less selective, are much more sensitive. For a targeted analysis, the methanol pressure can be fine-tuned along with other instrument elements employed to allow the maximum overall selectivity and sensitivity. Such an optimization of the selectivity can greatly reduce interferences from the hydrocarbons which compose the fuels matrix, thereby allowing the heteroatom species to be analyzed without the need for time-consuming and costly preliminary separations.

Thus, methanol chemical ionization provides a sensitive and selective technique for the analysis of selected trace heterospecies present in a low polarity matrix, such as a fuel.

Chapter 6

The Use of Multiple Reaction Monitoring Gas Chromatography/Triple Quadrupole Mass Spectrometry for the Detection of Trace Components in Jet Fuels

A number of studies have shown that the storage stability and thermal stability of jet aviation fuels can be greatly affected by low levels of heteroatom-containing species (36, 37, 38, 39, 40). The storage stability of a fuel is determined by the extent of polymerization and sediment formation upon long term exposure to temperatures in the range of 50-125 C. Thermal stability is the degree of deposit formation resulting from short term exposure to temperatures in the 200-400 C range such as in the hot section of a jet engine. These problems have considerable economic and safety implications in the aviation industry and are expected to become increasingly serious in the future as lower quality crude oils and non-petroleum sources (e.g., shale oil and coal liquefaction products) enter the hydrocarbon pool used in the refining of these fuels.

Jet fuels present a complex hydrocarbon matrix containing large numbers of isomeric and isobaric species spanning a wide range of concentrations. As a result, at unit mass resolution, it is virtually impossible to obtain

unique peaks in the electron impact spectrum; one generally finds a peak at virtually every mass. The use of full-scanning GC/MS greatly simplifies the mass spectra and thus their interpretation, however, even with high resolution capillary chromatography, the time resolution of individual components in the bulk of a fuel sample is incomplete. This is especially true when relatively large sample injections are required to enable detection of low-level components. Further, the peaks which are not resolved are likely to represent isomeric and isobaric compounds whose spectra are especially difficult to deconvolute, or trace components which are lost in competition with the matrix. The use of chemical ionization can serve to reduce much of the general background and increase sensitivity by concentrating the ion current into fewer peaks. This sensitivity, however, is obtained at the cost of the structural information necessary to distinguish closely related compounds present in the sample and likely to be co-eluting.

Triple quadrupole mass spectrometry (TQMS), coupled with capillary gas chromatography, provides a powerful tool for addressing these characteristic problems of complex mixture analysis. Selective reactions (parent ion/daughter ion pairs) may be multiplexed in time over the course of a chromatographic run to provide for the simultaneous and independent analysis of co-eluting peaks in different data dimensions. Selective reactions also have the advantage of reducing the requirements for chromatographic resolution,

effectively allowing for larger sample injections and more rapid temperature programming. Additionally, the structural information available from the CAD spectra in TQMS allows chemical ionization to be used while retaining a mechanism for obtaining structural information.

The limited selectivity of mass spectrometry for trace quantities of heteroatom-containing species in complex hydrocarbon mixtures has, in the past, required a preseparation of the aromatic and polar species from the aliphatic constituents which make up the bulk of fuels. In addition to being a time-consuming process, preseparation provides many opportunities for altering the distribution of trace components in a sample.

In this study, the power of capillary GC/TQMS has enabled the detailed analysis of selected trace heteroatom-containing species in jet fuels without any sample pretreatment. The thiophenes were selected as the targeted compound class for this research because they have been previously reported in jet fuels (41) and there is evidence of their participation in the thermal degradation of fuels (42, 43).

Background

Batch MS/MS has been previously reported for the analysis of thiophene in gasoline (44) and middle distillate fuels (45) and for the determination of organosulfur compounds in crude oil fractions (46). In the first study,

McLafferty and Bockhoff claim a detection limit of < 25 ppm using 70 eV EI on a MIKES instrument. The authors also suggest that GC/MS/MS could be a valuable technique for the analysis of high complexity mixtures. Myerholtz, in the second study, reports the use of a single neutral loss scan for the rapid screening of several thiophenes in middle distillate fuels using TQMS and 20 eV EI. This study did not report any quantitative information or attempt to identify individual thiophene components. The third study, by Hunt and Shabanowitz, uses methane and NO CI to obtain qualitative profiles of the 370 to 535 °F cut from different crude samples. This work was primarily concerned with the dibenzothiophenes and the di- and tri-thienyls prevalent in that particular crude fraction and reported very different behavior from the alkylthiophenes which are more likely to be present in refined middle-distillate fuels.

Experimental Conditions

This study employed an ELTQ-400-3 Triple Quadrupole Mass Spectrometer and Control System manufactured by Extranuclear Laboratories Inc., Pittsburgh, PA. Both electron impact (EI) and chemical ionization (CI) were employed using an Extranuclear Simulscan™ ionizer maintained at 200 C.

Electron impact spectra were obtained at both high (70 eV) and low (20 eV) energies. Chemical ionization was

performed with methane (ultra-high purity) and methanol (anhydrous reagent grade). Methanol for CI was introduced to the ion source by leaking it through a needle valve from a 500 ml expansion volume maintained at ambient temperature. Collisionally activated dissociation (CAD) spectra were obtained using argon (99.9 %) at laboratory pressures ranging from 1-4 x 10^{-5} torr in the analyzer chamber.

For pure components, liquid samples were introduced by leaking them through a needle valve and solids were introduced by means of a solids probe. Fuel samples were introduced by means of a Hitatchi 633-30 Gas Chromatograph (Hitatchi Ltd., Tokyo, Japan) equipped with a split/-splitless injector maintained at 200 C and a 30 meter narrow-bore DB-1 chemically bonded phase fused silica capillary column with a 1 micron coating (J & W Scientific, Rancho Cordova, CA.) The column was interfaced to the ion source by passing it through a transfer line at 250 C to a direct inlet. Helium carrier gas was used at a flow of 1 ml/min (a flow velocity of 40 cm/sec at 200 C.) The column was temperature programmed from 50 to 250 C at 6 C/min.

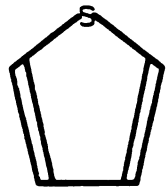
Pure components used in this study were obtained from either Chemservice Inc. (West Chester, PA) or Aldrich Chemical (Milwaukee, WI) and were used without further purification. Fuel samples were obtained from Dr. Gary Seng at NASA-Lewis Research Center and were analyzed without any pretreatment.

Data interpretation was performed on a PDP-11/23 minicomputer running the RSX-11M operating system after performing a 16-bit parallel upload from the instrument control microcomputer system. Full scan data were interpreted with the aid of a multi-dimensional database package (47). Selected ion monitoring (SIM) and multiple reaction monitoring (MRM) data were interpreted with the aid of another locally developed software package (see Chapter 4.)

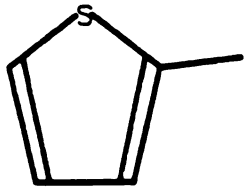
Preliminary MS and MS/MS Studies

The first stage in this work was a MS and MS/MS characterization of several thiophenes under different ionization conditions. Structures for the thiophenes used in this study are given in Figure 6.1. These particular thiophenes were chosen because they represent the type of functionality expected for thiophenes in a refined fuel and they cover the molecular weight and boiling point ranges typical of the middle distillate fuels.

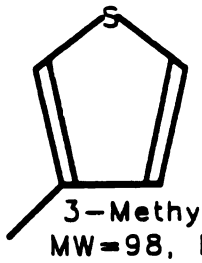
The EI spectra of the thiophenes were obtained at electron energies of 70 and 20 electron volts. CAD spectra of the principal ions in the EI spectra were also obtained at both electron energies. These data are summarized in Table 6.1. Considerable fragmentation was observed at both



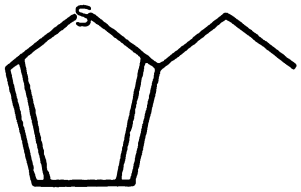
Thiophene
MW=84, BP=84C



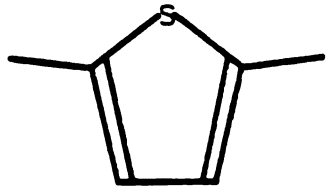
2-Methylthiophene
MW=98, BP=113



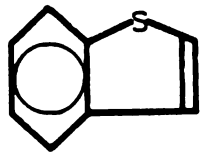
3-Methylthiophene
MW=98, BP=114C



2-Ethylthiophene
MW=112, BP=133C



2,5 Dimethylthiophene
MW=112, BP=134C



Benzothiophene
MW=134, BP=221C

Figure 6.1 Structures of Pure Thiophenes Studied

electron energies for each of the thiophenes investigated with the exception of benzothiophene which, at 70 eV, produced only the molecular ion and which was not ionized at 20 eV.

The mass spectral behavior of the thiophenes studied fell into two groups. The first group consists of thiophene itself and the alkyl substituted thiophenes. Members of this group tended to undergo neutral losses of 45 (corresponding to CHS) and also formed 45^+ both in the source and under CAD with argon at $P_{lab} = 2 \times 10^{-5}$ torr and collision energies on the order of 20 eV. The formation of 45^+ is generally considered characteristic of aliphatic organosulfur compounds (48). The neutral loss of 45 from the thiophenes is quite reasonable in that it results in the formation of $C_3H_2-R^+$, a substituted aromatic ring where R is the ring substituent of the parent thiophene. The 3-methylthiophene was found to give essentially the same pattern as other members of this group despite the difference in position of the ring substituent, even at the low electron energy. The one disubstituted thiophene examined, 2,5-dimethylthiophene, gave the same peaks as were characteristic of other thiophenes studied, plus additional peaks at 77^+, 78^+, and 65^+. These peaks probably correspond to ring rearrangement incorporating a methyl group to form benzene and phenyl ions. These ions exhibit substantially reduced abundance under in the 20 eV spectra. The principal reaction of the substituted thiophenes is the cleavage of

Table 6.1 Spectra of Pure Thiophenes Ionized by Electron Impact

Thiophene	
70 eV EI	84 (100), 58 (70), 45 (40), 39 (35)
20 eV EI	84 (95), 58 (100), 45 (60), 39 (60)
70 eV CAD(84^+)	84 (100), 58 (50), 45 (60)
20 eV CAD(84^+)	84 (100), 58 (60), 45 (60), 39 (15)
2-Methylthiophene	
70 eV EI	98 (60), 97 (100), 45 (18), 39 (10)
20 eV EI	98 (65), 97 (100)
70 eV CAD(97^+)	97 (100), 53 (25), 45 (10)
20 eV CAD(97^+)	97 (100), 53 (60), 45 (25)
3-Methylthiophene	
70 eV EI	98 (59), 97 (100), 53 (10), 45 (21)
20 eV EI	98 (75), 97 (100), 45 (5)
70 eV CAD(97^+)	97 (100), 53 (50), 45 (35)
20 eV CAD(97^+)	97 (100), 53 (35), 45 (15)
2,5-Dimethylthiophene	
70 eV EI	112 (92), 111 (100), 97 (75), 77 (22), 59 (27), 45 (18)
20 eV EI	112 (90), 111 (100), 97 (60), 77 (10), 59 (10)
70 eV CAD(111^+)	111 (100), 97 (15), 78 (30), 77 (50), 67 (60), 65 (15), 45 (30), 41 (10)
20 eV CAD(111^+)	111 (100), 97 (20), 78 (15), 77 (25), 67 (25), 65 (10), 45 (15), 41 (10)
2-Ethylthiophene	
70 eV EI	112 (36), 97 (100), 45 (19)
20 eV EI	112 (60), 97 (100)
70 eV CAD(112^+)	112 (25), 97 (100)
20 eV CAD(112^+)	112 (15), 97 (100), 53 (5)
70 eV CAD(97^+)	97 (100), 53 (90), 45 (25)
20 eV CAD(97^+)	97 (85), 69 (10), 53 (100), 45 (40)
Benzothiophene	
70 eV EI	134 (100), 90 (10), 89 (15)
70 eV CAD(134^+)	134 (100)

the substituent group gamma to the sulfur. Proposed mechanisms for this group are given in Figure 6.2. This observed behavior is consistent with previous studies of the EI spectra of the alkylthiophenes (49).

The second type of thiophene studied was benzothiophene. As would be expected, its molecular ion is highly stabilized by the fused aromatic ring and gives much less fragmentation in the primary spectrum, even at 70 eV. This compound also undergoes a neutral loss of 45^{+} in its CAD spectrum, although less readily than the alkyl thiophenes.

In that the experimental goal was to maximize sensitivity by multiple reaction monitoring, an ionization method was sought which would reduce fragmentation from that observed with electron impact, even at low energy. As illustrated in Chapter Five, ionization techniques which minimize fragmentation while maintaining the overall ionization efficiency can improve sensitivity by concentrating the ion current into essentially one ion for each compound under study. Preferably, this ion would also carry molecular weight information. Since the actual analysis would use multiple reaction monitoring (MRM) of the targeted compounds, the loss of structural information in the primary spectrum was not a problem. The combined information available with the retention time from a capillary gas chromatograph and the choice of reactions to monitor was expected to provide sufficient selectivity to identify the

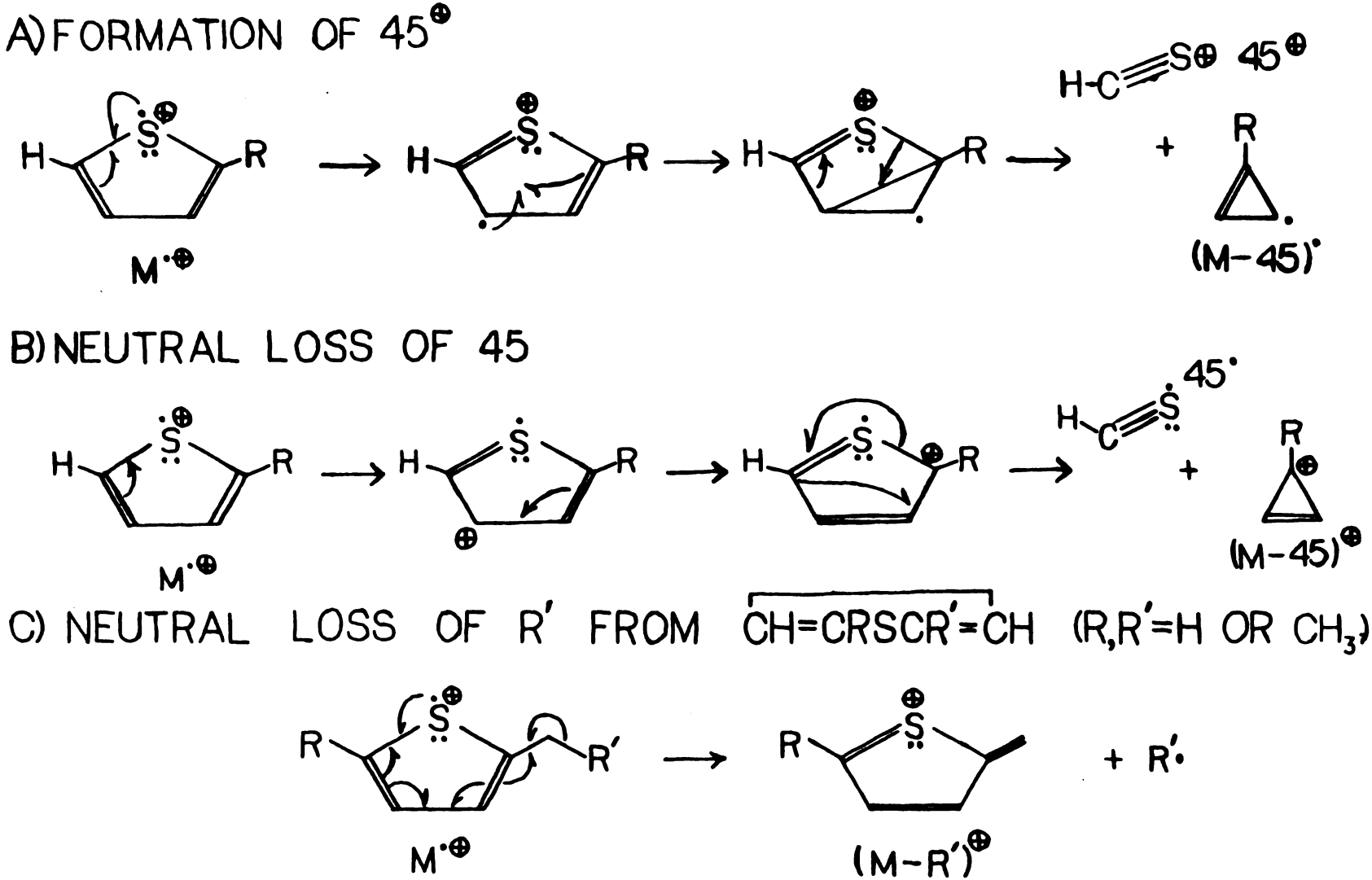


Figure 6.2 Proposed Fragmentation Paths for Substituted Thiophenes

targeted components. Consideration of these criteria suggested the use of chemical ionization (CI).

The most widely used reagent gas for chemical ionization is methane. Methane CI is expected to ionize virtually all organic compounds, yielding a base peak at $(M+H)^+$, where M is the molecular weight of the compound under study, but with much less fragmentation than in EI (50). The primary and CAD mass spectra of the thiophenes were obtained with methane CI at 200 C and helium present from the GC column (column flow ca. 1 ml/min.) These primary spectra, summarized in Table 6.2, showed extensive fragmentation of the alkyl thiophenes, forming significant (20 - 100% of the base peak) abundances of 97^+ and as much as 90% relative abundance of 111^+ in the case of 2,5-dimethylthiophene. Thiophene and benzothiophene showed very little fragmentation. The CAD spectra of the $(M+H)^+$ ions of thiophene and the alkyl thiophenes all showed neutral losses of 44 mass units (corresponding to elimination of CS.) In addition, the alkyl thiophenes all showed a neutral loss of 34 mass units (corresponding to H_2S.) The principal CAD product of the $(M+H)^+$ ion of 2-ethylthiophene was loss of 28 mass units (elimination of ethylene, C_2H_4.) The major CAD product of 97^+ from the alkyl thiophenes was loss of 44 mass units to yield the aromatic species 53^+, which was also observed in the EI spectra. CAD of the $(M+H)^+$ ion from benzothiophene resulted in the neutral loss

Table 6.2. Spectra of Pure Thiophenes using Methane CI

Thiophene

CI	113 (5), 87 (5), 86 (7), 85 (100), 84 (14)
CAD(85^+)	85 (100), 45 (28), 42 (32)

2-Methylthiophene

CI	127 (4), 101 (3), 100 (8), 99 (100), 98 (14), 97 (12)
CAD(99^+)	99 (100), 65 (20), 55 (37)

3-Methylthiophene

CI	127 (5), 101 (3), 100 (8), 99 (100), 98 (9), 97 (13)
CAD(99^+)	99 (100), 65 (10), 55 (45)

2-Ethylthiophene

CI	141 (10), 114 (8), 113 (100), 112 (22), 111 (5), 97 (20), 85 (31)
CAD(113^+)	113 (100), 98 (5), 85 (65), 79 (7)

1,2-Dimethylthiophene

CI	141 (1), 114 (9), 113 (100), 112 (26), 111 (19)
CAD(113^+)	113 (100), 98 (8), 79 (32), 77 (12), 69 (19), 45 (8)

Benzothiophene

CI	135 (100), 134 (25)
CAD(135^+)	135 (100), 91 (37)

of 44 mass units, presumably to form the highly stable tropyllium ion at 91^+.

Overall, the methane CI generally produced a smaller number of peaks in the primary mass spectra of the thiophenes being studied. However, using source conditions simulating those present in a GC/MS/MS analysis, a few fragment ions still accounted for a large fraction of the ion current. Additionally, since methane is capable of ionizing virtually all organic compounds (51), it provides little if any gain in selectivity over EI when dealing with a mixture as complex as a fuel where there are large numbers of isomeric and isobaric compounds that are not completely resolved, even with high resolution chromatography. This is especially true when analyzing for trace components which suffer from interferences from even very low abundance reactions of the major mixture components.

Methanol has been previously reported as providing extremely little fragmentation when employed as a CI reagent gas (52, 53). Under GC/MS/MS conditions in the source (200 C and ca. 1 ml/min of helium flow) optimum ionization of the thiophenes occurs when the methanol pressure is adjusted to provide approximately equal abundances of the ions at masses 33^+ ($CH_3OH_2^+$) and 65^+ (corresponding to $(CH_3OH)_2H^+$, the methanol dimer cluster ion.) Other significant reagent ions present under these conditions are 97^+ (the methanol trimer cluster ion $(CH_3OH)_3H^+$,) 79^+ (loss of water from 97^+) and 47^+ (loss of water from 65^+.)

Using the source conditions described above, methanol CI gave virtually no fragmentation in the primary mass spectrum of any of the thiophenes studied. All of the primary spectra consisted only of the $(M+H)^+$ ion from the sample and reagent ions. The CAD spectra of $(M+H)^+$ from each of the thiophenes gave the same product ions as were the case for methane CI. Methanol CI spectra of the thiophenes, and the corresponding CAD spectra, are listed in Table 6.3.

An additional, very significant, advantage of methanol chemical ionization is its high selectivity. As reported in the previous chapter, methanol CI has been found to discriminate against the hydrocarbons, and gives essentially no ionization of the saturates which make up the bulk of the matrix in a fuel sample. As a result, matrix interferences are greatly reduced, thus lowering the requirements for chromatographic resolution and enabling shortened analysis times.

Determination of Detection Limits

On the basis of studies of the MS/MS characteristics of the thiophenes under methanol CI conditions described above, reactions may be chosen for the detection of these compounds by means of multiple reaction monitoring. This choice of reactions is summarized in Table 6.4. The loss of 44 u (CS) was chosen for the detection of thiophene, the methylthiophenes, and benzothiophene. The $113^+ \rightarrow 79^+$ reaction

Table 6.3 Spectra of Pure Thiophenes using Methanol CI

Thiophene

CI	87 (6), 86 (9), 85 (100), 84 (32), 58 (4), 45 (4), 39 (2)
CAD(85^+)	85 (100), 59 (2), 45 (49), 41 (82), 39 (19)

2-Methylthiophene

CI	101 (6), 100 (8), 99 (100), 85 (2)
CAD(99^+)	99 (100), 97 (2), 84 (6), 65 (22), 55 (78), 45 (7), 39 (1)

3-Methylthiophene

CI	101 (5), 100 (9), 99 (100), 85 (1)
CAD(99^+)	99 (100), 84 (8), 65 (12), 55 (65)

2-Ethylthiophene

CI	114 (8), 113 (100), 111 (2), 97 (10), 85 (8)
CAD(113^+)	113 (100), 98 (6), 85 (86), 79 (8)

1,2-Dimethylthiophene

CI	114 (10), 113 (100)
CAD(113^+)	113 (100), 98 (8), 79 (35), 77 (11), 69 (25), 45 (5)

Benzothiophene

CI	137 (5), 136 (12), 135 (100)
CAD(135^+)	135 (100), 91 (53)

Table 6.4 Reactions for Monitoring Thiophenes in Fuels

Compound	Parent Ion	Daughter Ion
Thiophene	85^+	41^+
2-Methylthiophene	99^+	55^+
3-Methylthiophene	99^+	55^+
2-Ethylthiophene	113^+	85^+
2,5-Dimethylthiophene	113^+	79^+
Benzothiophene	135^+	91^+

(loss of 34 u, H_2S) was used for detection of 2,5-dimethylthiophene since it was found to be much more sensitive. Similarly, for 2-ethylthiophene, the $113^+ \rightarrow 85^+$ reaction (loss of 28 mass units, cleavage of the ethyl side chain) was also employed for reasons of sensitivity.

Potential interferences for thiophene and the alkyl thiophenes are the cycloparaffins and the olefins which are isobaric. Monitoring the reaction corresponding to the neutral loss of 34 mass units (H_2S) from 2,5-dimethylthiophene should discriminate against both of these interfering compound types. The reactions chosen for thiophene and the methylthiophenes (loss of 44 u) and for 2-ethylthiophene (loss of 28 mass units) are not as selective as the loss of 34 reaction. However, these reactions are much more sensitive and, since the targeted compounds have boiling points at the low end of the middle distillate boiling range, the overall analysis will be sufficiently selective to attain unambiguous identifications. Benzothiophene is isobaric with the C_4-substituted benzenes and both compound classes readily undergo neutral losses of 44 mass units (corresponding to CS for benzothiophene and C_3H_8 for the substituted benzenes) to form the highly stabilized ion of mass 91. Benzothiophene does have a somewhat higher boiling point, however, so it can be resolved chromatographically. (It is interesting to note that benzothiophene cannot be resolved from the C_4-substituted benzenes by batch

MS/MS at unit mass resolution, in that they are isobaric and both undergo the loss of a 44 u neutral.)

Using these reactions, detection limits for thiophenes in jet fuels were determined by preparing a series of blends of Jet A (the standard commercial jet fuel) with known amounts of thiophene, 2-methylthiophene, 3-methylthiophene, 2-ethylthiophene, 2,5-dimethylthiophene, and benzothiophene. Triplicate analyses were performed on each blend by methanol chemical ionization GC/MS/MS using multiple reaction monitoring. The detection limit is defined as the lowest analyzed concentration where the average ratio of the integrated peak area to the background area was greater than 2:1. The detection limits so determined are reported in Table 6.5. Retention times reported are the average of all gc/ms runs. Typical single reaction chromatograms used to determine these detection limits are given in Figures 6.3a through 6.3d.

Table 6.5 Detection Limits for Thiophenes in Jet Fuels

Compound	Retention Time (sec)	Detection Limit (ng)	Signal to Background
Thiophene	87.7	2.5	2.2
2-Methylthiophene	111.3	2.2	6.9
3-Methylthiophene	113.7	2.1	5.0
2-Ethylthiophene	147.6	1.7	4.5
2,5-Dimethylthiophene	148.8	1.5	3.5
Benzothiophene	352.1	7.6	4.3

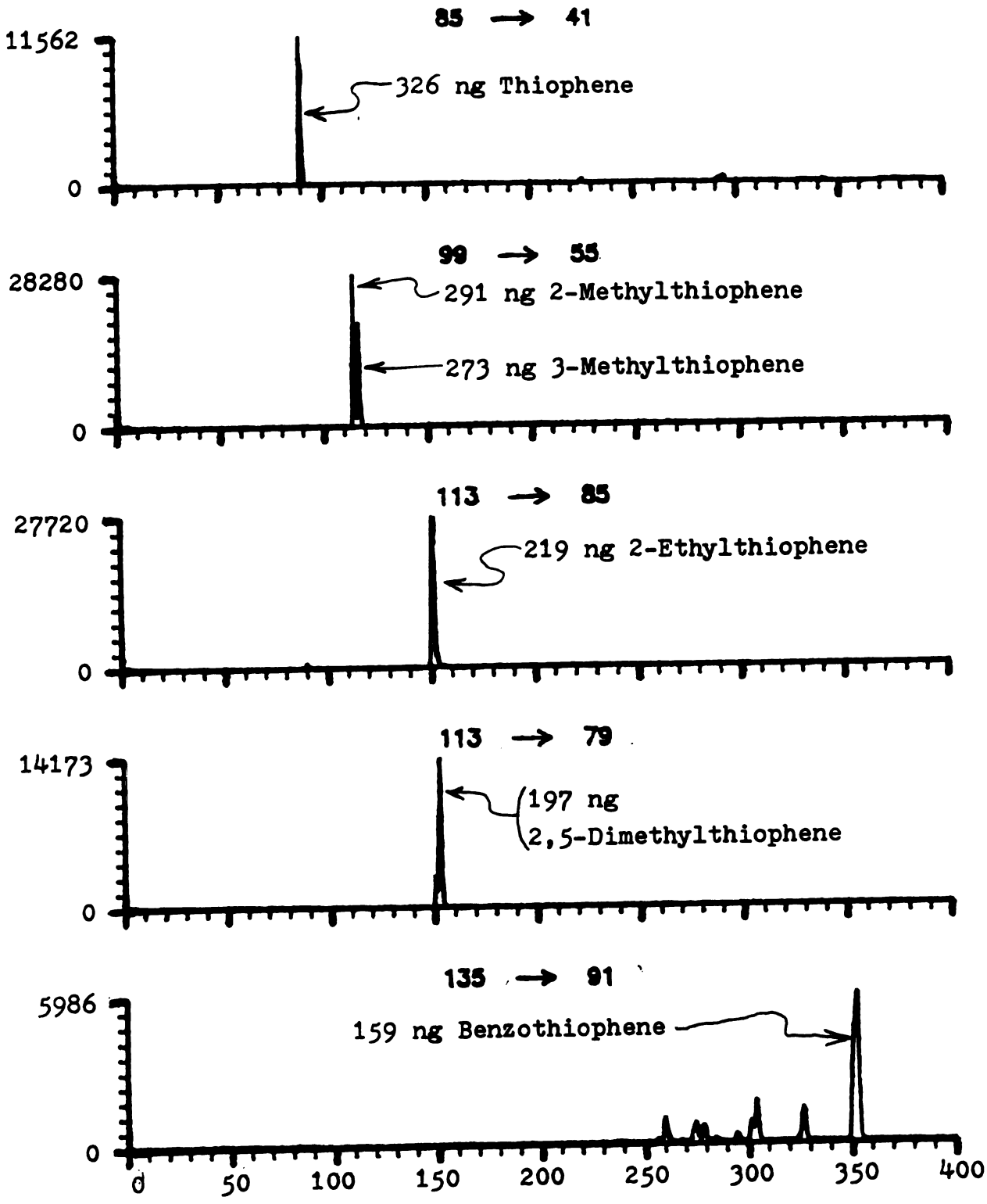


Figure 6.3a Detection of Thiophenes in Jet A

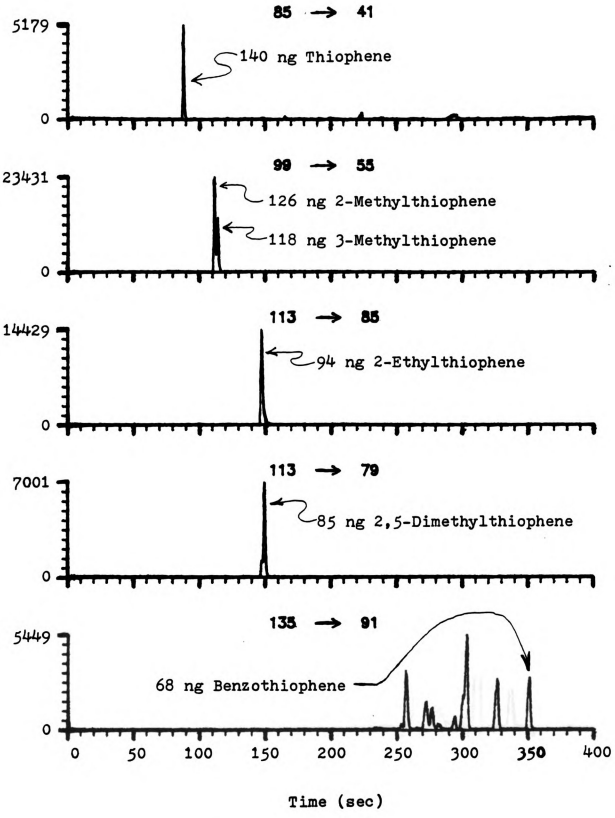


Figure 6.3b Detection of Thiophenes in Jet A

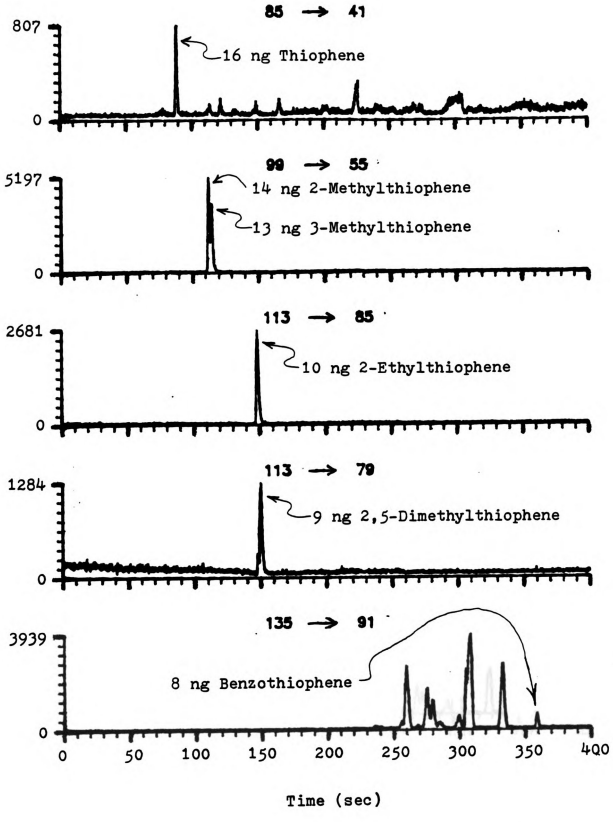


Figure 6.3c Detection of Thiophenes in Jet A

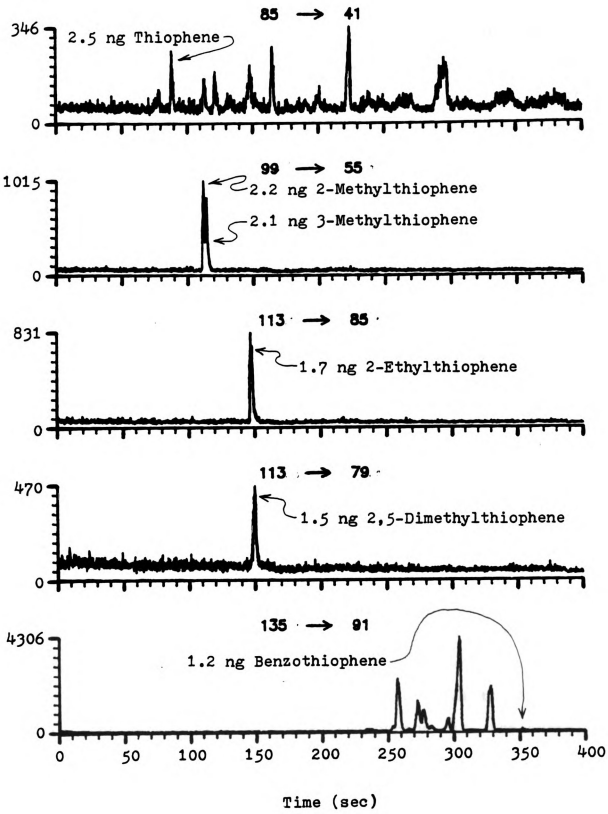


Figure 6.3d Detection of Thiophenes in Jet A

At the highest spike level (Figure 6.3a), the reactions for native thiophene and the C_1 and C_2 substituted thiophenes are observed to be virtually free of interferences from the Jet A hydrocarbon compounds. Several interfering peaks, however, are observed in the reconstructed single reaction chromatogram for benzothiophene. The use of capillary gas chromatography sufficiently separates these potential interferences, attributable to the isobaric C_4 substituted benzenes, so as not to be a problem. The chromatography also serves to easily resolve the 2-methyl- and 3-methylthiophene peaks, while the choice of reactions for the 2-ethyl- and 2,5-dimethylthiophene insures that they are readily distinguished.

As the thiophenes spike level is decreased (going from Figure 6.3a to Figure 6.3d), the relative abundance of species reactions interfering with thiophene and benzothiophene determinations are observed to increase. (The source of the interfering $85^+ \rightarrow 41^+$ reactions are the isobaric $C_6H_{13}^+$ ions formed from fragmentation of alkane molecules present in much higher relative abundances than thiophene.) From this work, it is observed that the selectivity of this analysis is sufficient that the limiting factor for detectivity of the thiophenes is the overall instrument sensitivity. Thus, detection limits for this technique should improve directly with improvements in instrument design.

Conclusions

The detection of trace level sulfur compounds in a fuel is an especially difficult analytical problem not only due to the complexity of the matrix, but also due to the fact that these compounds are isobaric with hydrocarbons, and their fragments, which are normally present in much higher abundances. Existing studies of the effect of thiophenes on jet fuel stability have found them to be deleterious at the parts per thousand level (54, 55). These levels correspond to detection limits for the thiophenes using batch MS with chromatographic pre-separations or capillary GC/MS (56).

Batch MS/MS at unit mass resolution has tremendous potential for dramatic improvements in both detectivity and analysis time, but will fail in cases where isobaric species also undergo isobaric neutral losses (e.g. benzothiophene and the C_4 substituted benzenes). BEQQ instruments would appear to have interesting potential in this area for target compounds which can be resolved at 10,000 to 20,000 resolution.

The use of capillary GC/MS/MS, demonstrated in this work, allows for detection limits on the order of 25ppm (for native thiophene) where previous techniques had been limited to parts per thousand. Application of this work will allow testing to determine whether or not thiophenes effect fuel stability at levels down to 25ppm. This ability to determine the existence of such threshold values would be of

tremendous economic impact in the establishment of workable specifications for future fuels refined from low-grade hydrocarbon sources.

Chapter 7.

Selective Ionization, Gas Chromatography, and Triple Quadrupole Mass Spectrometry as Tools for the Characterization of Fuels

The research presented in this dissertation has encompassed several aspects of bringing a powerful and elegant analytical instrument together and applying it to a specific problem. The GC/TQMS instrument together with its control and data systems and other accessories, however, is expensive and, to take full advantage of its capabilities, requires an analyst with more knowledge and skill than would be required for a single-stage GC/MS. The obvious and pragmatic question then becomes "How often is all of this power really necessary?"

Method development for complex mixture analysis can be viewed as a process of tying a series of "selectivity elements" together until a sufficient number of interferences have been removed that the signal of interest may be reliably measured. Depending on the particular problem, these "selectivity elements" may include such tools as chromatographs, mass analyzers, and monochromators, physical separations, such as sieveing, or chemical procedures, such as solvent extraction or derivatization reactions. An

understanding of the sample at hand and the goals of the analysis enables the analyst to choose the most effective set of tools to apply to the problem.

For the problem of determining part per million levels of thiophenes in jet fuels, the combined use of capillary gas chromatography, selective chemical ionization, and two stages of mass analysis separated by collisionally activated dissosciation (CAD) were deemed necessary. Each of these individual selectivity elements made a unique and necessary contribution to the analysis.

Gas Chromatography as a Selectivity Element

The combination of gas chromatography with mass spectrometry has been immensely successful not only for the temporal dispersion of the sample, but also for the reproducibility of sample introduction. This comes at a price, however, of having to analyze a time-varying sample, limiting the available sample size, and increasing the analysis time. The use of MS/MS often reduces the demand on the chromatography to that of providing for reproducible sampling while using the primary mass filter to distinguish co-eluting species. This approach falls down, however, when it is necessary to distinguish co-eluting components which are isomeric or, in quadrupole MS/MS, isobaric.

An excellant example of this problem occurs in the work reported in Chapter 6. In that instance, the use of capillary gas chromatography was essential in preventing the butylbenzene isomers from interfering with the detection of benzothiophene. Not only do the butylbenzenes have the same molecular weight (134 amu) as benzothiophene, they also exhibit virtually identical MS/MS activity. In both cases the MH^+ ion (the only significant peak in the primary CI mass spectra of these compounds) readily undergoes a neutral loss of 44 amu to form identical daughter ions -- the highly stabilized 91^+ species. There are no other significant CAD products for either compound type.

Mass Analysis as a Selectivity Element

From a chromatographer's viewpoint, the addition of a mass spectrometric detector to a chromatograph adds a second data dimension which can enable positive identification of solutes as they elute from the column. In addition, the mass spectrometer can frequently aid in deconvoluting incompletely resolved chromatographic peaks through interpretation of appropriate single-ion chromatograms. This second point effectively relaxes the demand placed on the chromatographic resolution. Consequently, detection of lower concentrations of analytes are possible because lower demand on chromatographic resolution generally allows for the injection of larger samples. Additionally, decreased

demand for chromatographic resolution frequently allows for more rapid analyses.

As a practical matter, mass spectrometric deconvolution of partially resolved chromatographic peaks requires the existence of at least a distinctive peak in the mass spectrum of each of the incompletely resolved components and that signals for the components be reasonably close in magnitude. Thus, this approach becomes especially difficult when attempting to distinguish isomeric or, at unit mass resolution, isobaric compounds.

These limitations on mass spectral deconvolution of chromatographic peaks create an especially difficult situation for the determination of trace thiophenes in fuels. Not only is it exceedingly difficult to obtain complete chromatographic resolution for all the matrix components, the hydrocarbons tend to form ions at virtually every unit mass position in the spectrum. Many of these peaks may be quite small, nevertheless, they can be sufficiently large to interfere with the detection of isobaric trace components. (The alkylthiophenes are isobaric with the alkenes and the cycloalkanes; the benzothiophenes are isobaric with the alkylbenzenes.)

Where there are significant chemical differences between the analytes and the matrix, chemical ionization and MS/MS can help to alleviate these problems. By simplifying the mass spectra, chemical ionization can reduce the incidence of isobaric peaks in the mass spectra. Selective

CI reagents can be of further service by increasing the signal of the analyte relative to that for the matrix. Tandem mass spectrometry approaches the problem from the opposite end, taking advantage of the fact that even isobaric ions are likely to form unique fragment ions as a result of the CAD process. Chapter 6 demonstrated the power of the simultaneous application of selective chemical ionization and MS/MS by detecting part per million levels of thiophenes in spiked jet fuels.

Chemical Ionization as a Selectivity Element

Chemical ionization, with an appropriate choice of reagents, can be a powerful selectivity tool in cases where there is a difference in chemistry between the analyte and the matrix which can readily be exploited. The analytical goal of determining trace level polar compounds in a non-polar matrix is a perfect case for selective ionization. Polar compounds, with their relatively high proton affinities, can be ionized by a weak proton donor, such as protonated methanol, while minimizing ionization of non-polar compounds, which have relatively low proton affinities. An entirely different approach would have been required had the goal been to detect trace level non-polars in a polar matrix (e.g., hydrocarbons in a fermentation broth.) Further, selective ionization processes would not work at all when the analyte and the matrix are chemically

similar (e.g., the determination of a particular alkane isomer in a fuel sample.)

The research presented in Chapter 5 quantitates an instance of the sensitivity and selectivity implications inherent in the selection of ionization conditions for a mass spectrometric analysis. The work also illustrates the trade-off between sensitivity and selectivity when using methanol chemical ionization. An understanding of this relationship, taken together with knowledge of the selectivity of the rest of the analytical system, allows the analyst to essentially "tune" the methanol pressure to a value which supplies a sufficient contribution to the selectivity of the overall method while retaining the maximum possible sensitivity. Extending this approach to an entire multi-dimensional analysis method allows the development of highly selective techniques which do not require a severe sensitivity trade-off at any one element of the system.

The methanol work developed trends in the ionization process which can be developed by varying the extent of cluster ion formation. Future work covering additional analyte functionality and the effects of varying source temperature may offer extended insights into the process. Study of cluster ions formed by different polar molecules may reveal different selectivity patterns to be exploited for different analytical problems.

Tandem Mass Spectrometry as a Selectivity Element

As a tool for mixture analysis, tandem mass spectrometry with CAD can frequently facilitate the distinction of isomeric and isobaric ions by differences in their fragmentation patterns. By programming a triple quadrupole mass spectrometer to monitor specific CAD reactions, the analyst can obtain a highly selective analysis of chromatographic effluents with signal-to-noise advantages analogous to that for selected ion monitoring in single stage GC/MS. Additional modes of selectivity are available through choice of collision energy, collision pressure, and the use of reactive collision gases. In the analysis of thiophenes in jet fuel, the chromatographic resolution and ionization conditions provided sufficient selectivity that the collision conditions could be tuned for maximum sensitivity.

Conclusion

This dissertation has demonstrated a specific problem which has become more tractable for routine analysis because of the availability of GC/TQMS. The use of this instrument allowed part per million level determination of thiophenes in jet fuels without requiring any lengthy preparative scale liquid chromatographic separations as would traditionally be employed. One can readily imagine equally complex problems

in the analysis of such complex matrices as biological systems and hazardous wastes, as well as other petroleum-based products and intermediate streams, where such an approach would be equally valuable.

The thiophenes work presented an example of the analysis of polar components in a non-polar matrix. This suggests testing the applicability of GC/TQMS to the analysis of non-polar analytes in a polar matrix, or the even more difficult problem of non-polar targets in a non-polar sample.

The latter challenge would be of particular value in the petroleum industry, where the traditional mass spectrometric type analyses fail to provide sufficient detail to accurately model petroleum refining processes. Specific problems would be distinction between isomeric types such as the alkenes and cycloalkanes and 5-member and 6-member cyclic systems.

This work demonstrates GC/TQMS to be a powerful tool that is available to solve some very difficult analytical problems. Truly, one crucial mark of a skilled analyst is an awareness of all the tools at his or her disposal and the ability to choose the simplest one which meets the sensitivity, selectivity, precision, accuracy, speed, and cost constraints placed on obtaining the desired information.

APPENDICES

Appendix A

Inter-Processor Synchronization for Multi-Processor Data Acquisition

The power of a multiple microprocessor control system comes from the ability to divide the control tasks among the different processors. This division of tasks allows for higher speed operation by performing different time-critical activities in parallel instead of intermingling them on a single processor. Applying this approach to simultaneous instrument control and data acquisition, however, requires that these independent processors be closely coordinated. This appendix serves as a follow up to chapters three and four, providing a detailed discussion of how the multi-processor data acquisition software achieves the needed synchronization.

Sweeps and Scans

The most basic form of data acquisition on the multiple microprocessor control system is the sweep. A sweep records ion current as function of the voltage applied to any single element of the ion path while everything else is held

constant (quadrupole masses are a special case in that mass is being swept rather than voltage). The control system stores sweep data as ordered pairs of raw voltage (or mass) and intensity; that is, no peak-finding or any other data treatment is performed. The operator is responsible for commanding the quadrupoles for the desired RF-only or DC/RF mode. Additionally, the current software does not allow for any sweeps with linked device. Since raw data is being retained, sweep width is limited to 1024 steps.

Scans refer to the traditional mass spectrometric data acquisition mode, where ion intensity is monitored as a function of mass and mass-intensity pairs are saved only for peaks. This allows for a virtually unlimited scan width, the sole limitation being 1024 peaks per scan (this number should be quite adequate even for a 2000 amu quadrupole, assuming unit mass resolution). Five scan types are defined --

1SCAN - the first quadrupole is scanned and the other two are placed in RF-only mode,

3SCAN - the third quadrupole is scanned while the first two quadrupoles are kept in RF-only mode,

PSCAN - parent scan, the third quadrupole is set to transmit a fixed mass, the first quadrupole is scanned, and the center quadrupole is kept in RF-only mode,

DSCAN - or daughter scan, the first quadrupole is set to transmit a single mass, the third quadrupole is scanned, and the center quadrupole is placed in RF-only mode, and

NSCAN - neutral loss scan, the first and third quadrupole are scanned simultaneously with a fixed mass offset and the second quadrupole is retained in RF-only mode.

In each of these modes any RF-only quadrupoles are linked to the scanning quadrupole, scanning at one-half the RF power level of the scanning quadrupole.

The settings and scan windows for each of the ion path elements are maintained in the Variable Device Parameter Table (VDPT). The VDPT allows entries for a default value, start and end values, and step size for up to thirty-two ion path elements, with a complete set of these values being memory resident for each of the four defined ionization modes. Entries in this table are normally modified by means of the Parameter Editor, **PED**, and sixteen of these tables, with titles, may be maintained on the disk. It is through the VDPT that the user specifies the scan limits and step size to be used for any device to be swept or scanned, and the values at which all of the other ion path elements are maintained.

Four additional parameters necessary for data acquisition are set through the ASET (acquisition set) command. These are the threshold, minimum peak width, maximum peak width, and scan rate. The scan rate may be set to one of thirteen different nominal values ranging from 1 to 10,000 points per second (corresponding to 0.1 to 1000 amu/sec for a mass sweep or scan at 10 steps per amu). The other three parameters are used by the "on-the-fly" peak finding algorithm. All four of these parameters are downloaded to the appropriate slave processors whenever they are changed.

All of the actual data acquisition code resides on the three slave processors -- ion path, reduction, and detection. At the start of a sweep or scan, the master processor downloads the necessary parameters onto the parameter stacks of each slave immediately prior to giving them the commands needed to perform the selected type of scan or sweep.

Ion Path Processor

The ion path processor is responsible for setting the appropriate RF/DC status of each of the quadrupoles for the selected scan type (or do nothing for a sweep) and scanning the appropriate mass or voltage. During a scan, the ion path is also responsible for scanning the RF power on the RF-only quadrupoles. At the start of a sweep, the master has placed the number of the device to be swept, the scan limits and the step size on the ion path slave's parameter stack. For a scan, the master places the scan limits and

step size on the stack but the device number is not needed. In either case, the ion path clears its software status byte, stops the fast scan task, initializes the AMU Timer (57), and converts the scan limits and step size into a starting DAC value, a step size in DAC units, and a number of steps, and sets any scanned devices to their initial values. The AMU Timer is a dedicated piece of hardware which provides a timed synchronization signal to approximate the nominal scan rate. It is initialized using the scan rate parameter which was defined by ASET.

After a settling time of roughly 10 milliseconds, the ion path slave can enter the scanning loop. At this point, the current DAC value of the scanned device is stored in a processor register. During a sweep, a second register contains the address of the device to be scanned. Each of the five scan routines has encoded in it the addresses of the appropriate quadrupole mass DAC's.

The first step in each loop iteration is to check that the detection slave has finished sampling the previous data point. This is done by testing a register on the Link and Sync Output Module (58) going to the detection slave. After this has been verified, the ion path can write the new values out to the DACs (for the first step this will just be a repeat of the initial value). For scans, the values to send to RF-only quadrupoles are calculated by dividing the mass of the scanned quadrupole by two (a single shift right). After writing out the new data values, the ion path

then waits for the signal from the AMU timer and then uses the Link and Sync Output module to inform the detection slave that it can now proceed with acquisition of the next data point. The current value of the scanned device can now be sent to the reduction slave, again using the Link and Sync Output Module. The last step in the loop is to increment the register containing the current data point by the step size to calculate the value of the next data point. This loop is then repeated for each step.

The ion path slave now informs all the other processors of the completion of the scan by setting its software status byte to a 1. It then waits for the detection slave to indicate that it is done with this last data point and then restores all of the ion path devices to their initial values. When the reinitialization is complete, the ion path slave can return to its idle state of monitoring the command FIFO from the master processor.

Detection Processor

The detection slave functions identically for both scans and sweeps. This processor is responsible for controlling the data acquisition board (DAq) (59), transmitting intensity values on to the reduction slave, and maintaining synchronization with the ion path processor. At the start of a scan or sweep, the detection slave executes a hardware reset of the DAq and sets the number of conversions that it is to average for each data point. This number of

ADC conversions is determined by the rate parameter requested by the user through ASET. Scan initialization is completed by indicating to the ion path that it is ready for the next data point; this is done through the use of a Link and Sync Output Module.

The scan loop begins with the acquisition processor then monitoring the Link and Sync Output module, waiting for a signal from the ion path slave indicating that the ion path has been properly configured for the next data point. The acquisition processor then gives the start command to the DAq and waits for the it to indicate completion. This slave then informs the ion path processor that it is through with the current data point (using the Link and Sync Output module), reads the value of this data point from the DAq, clears it, and transfers the value on to the display/reduction processor by means of a Link and Sync Output module. The last step in the data acquistion loop has the acquisition slave check the software status byte from the ion path slave to see if it has been set to the value 1, which would indicate the end of the sweep or scan. If the end of scan is indicated, the DAq is reset and the acquisition processor returns to monitoring the command FIFO from the master processor. If there is no end of scan indication, the detection slave loops back to the point where it was waiting for the ion path ready signal on the Link and Sync Input module.

Reduction Processor

The processor most affected by whether this is a scan or a sweep is the reduction slave. For a sweep, it only needs to read the x-axis value from the ion path slave and the y-axis value from the detection slave and place them into the data buffer. For a scan, rather than putting all of the raw data into the data buffer, it must perform "on-the-fly" peak-finding and store only the actual peaks. Scan and sweep initialization are identical. The master places the device number of the scanned device on the parameter stack of the reduction slave. During scan initialization, this number is stored for later use, the data buffer and peak-finding parameters are initialized, and the processor performs a read from the Link and Sync Input Module (60) from the detection slave to inform that slave that it is ready to receive the first data point.

For a sweep, the run-time loop begins by reading the x-axis value sent from the ion path slave via a Link and Sync Input Module, and the corresponding intensity value is read from the Link and Sync Input Module of the detection slave. These values are then stored in the next available location in the data buffer. The last step in the loop is to check the software status byte from the ion path slave. If this byte has been set to 1, the scan is complete. If not, the processor returns to the start of the loop.

At the end of the scan, the reduction slave must convert each of the x-axis values which were sent from the

ion path slave in DAC units to physical units. During the process of performing these conversions, the reduction slave also determines the maximum intensity value and the total ion current for the sweep. When this is done, the slave sets its software status byte to a 1, informing the master that data is now available for uploading. Following this, the ion path's software status byte is reset to zero, the sweep data are transferred to the graphics buffer, and the peak-finding variables are re-initialized. When all of this is complete, the reduction slave can return to monitoring the FIFO with commands queued from the master.

Selected Ion Monitoring/Multiple Reaction Monitoring

Because of the hierarchical design of the TQMS computer systems (61), the tasks comprising the MRM package were divided between the multi-microprocessor "control system" and the PDP-11/23 "data system". In keeping with this overall system design, the control system was given responsibility for instrument set-up, data acquisition, storage, real-time graphics, and generation of simple data listings and display. The data system was given responsibility for generation of experimental summaries, advanced graphics output, and archival storage. On both systems, the MRM tasks were, insofar as possible, designed to be compatible and logical extensions of programs developed for the handling of full scan data.

Because the control system code was written in FORTH (62, 63, 64) it was a straight-forward matter to create a family of related routines to perform selected ion monitoring and multiple reaction monitoring. This family includes commands to perform SIM with just one of the quadrupoles, leaving the other two in RF-only mode, SIM with one of the quadrupoles fixed at selecting a single mass while jumping the other mass-resolving quadrupole, and MRM. The two single-quadrupole scan modes are invoked by the commands 1SIM and 3SIM. These command names were chosen to stress their analogy to the single quadrupole full scan commands 1SCAN and 3SCAN, which are used to scan the first quadrupole and the third quadrupole, respectively, while leaving the two non-scanned quadrupoles in RF-only mode. Similarly, the two cases of leaving one quadrupole fixed and jumping the other while performing CAD in the center quadrupole, are given the commands PSIM and DSIM as analogies to the parent ion scan, PSCAN, and the daughter ion scan, DSCAN, commands. There is no NSIM command provided as an analog to the NSCAN mode because, as will be explained later there was no experimental advantage over selecting the same reactions in the MRM mode.

The control system portion of the SIM/MRM software package can be divided into three parts -- experiment definition, run time, and post-experiment. The experiment set-up phase consists of defining the ions/reactions to be cycled (which will be referred to as elements), the cycle

frequency, a scale-factor to be used for the real-time display, and the length of time to collect data. The run time phase consists of a command for each of the five data modes (1SIM, 3SIM, PSIM, DSIM, and MRM). Each of these commands performs data acquisition, storage, and real-time graphics. The third phase, post-experiment, consists of subroutines to the general-purpose system commands for generation of Data LISTings (DLIST) and simple graphics DISPlays (DISP).

Definition of the elements to monitor is done with a pair of screen editors, SIMED and RED, the Selected Ion Monitoring Editor and the Reaction Editors, respectively. These names were chosen to go with the Parameter Editor, PED, the Method Editor, MED, and the Sequence Editor, SED. In addition to selecting a list of up to eight elements to monitor, the editors allow for specification of a parameter set to be used with each element. Further, up to thirty-two sets of ions and sixteen sets of reactions may be stored on the disk for future use (using the SIMSAVE, SIMGET, RSAVE, and RGET commands). The current version of the software, however, does not provide for different sets of elements to be chained to cover different portions of a GC run.

Additional experiment definition takes the form of prompted input in the SSET (for SIM set-up) command. Within this command, the user defines the cycle period to be employed (six values, ranging from 0.1 sec/cycle to 4

sec/cycle are allowed), the length of time to acquire data, and a scale factor for the real-time display.

Based on the information entered in the experiment set-up phase, all five of the run commands (1SIM, 3SIM, PSIM, DSIM, and MRM) check that there is sufficient disk space available to handle the cycle period and experiment duration specified. Additionally, the number of ADC samples to average for each data point is determined by the cycle frequency, number of elements to monitor, and whether this is a SIM or MRM mode. For a given sampling rate and number of ions to monitor, the SIM modes can generally perform more averaging than would be possible using the corresponding MRM conditions. This is because the SIM modes are only concerned with the jumping of one quadrupole (remembering that one quadrupole is static in the PSIM and DSIM modes). This explains why an NSIM mode is not implemented; it would require that two quadrupoles be jumped, making it little more than a special case of MRM where all of the neutral losses happen to be the same.

Each of the five run commands performs data acquisition, storage, and simple real-time graphics. The interprocessor synchronization required to perform these functions is illustrated schematically in Figure A.1. Again, the inherent modularity of the FORTH language allows each of the five commands to be constructed from common subroutines, some of which are the same as those used for scan and sweep data acquisition. Because of FORTH's

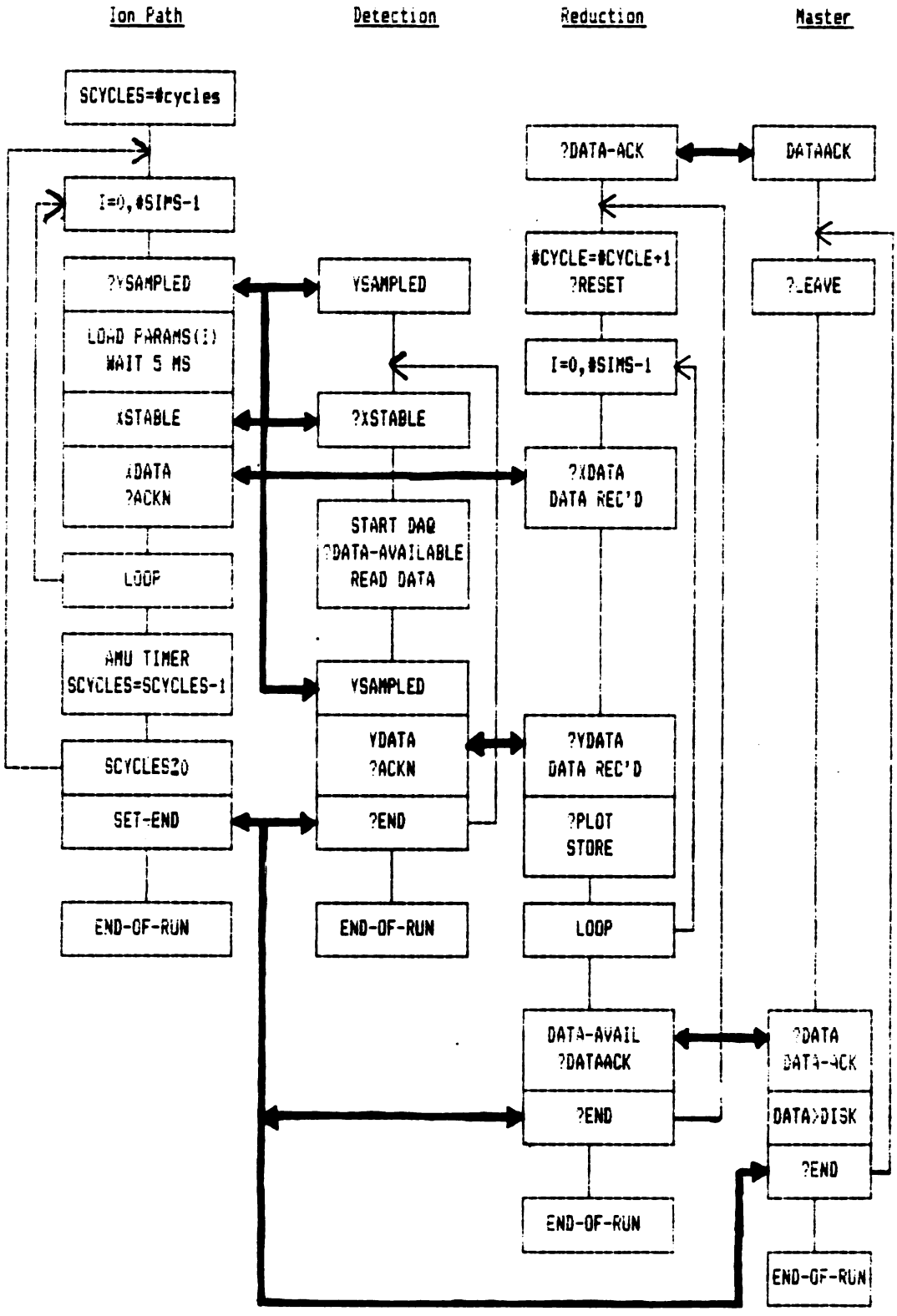


Figure A.1 Logic Flow for Multiprocessor SIM/MRM

threaded dictionary structure and linking mechanisms, a single copy of each of these shared subroutines is available for use by any higher level command. This allows applications as complex as the entire TQMS control system to be entirely memory resident, a major factor contributing to the overall speed of the system.

Detection Processor

The run time code for the data acquisition processor, slave 3, is the simplest of the four processors involved in the multi-micro system. Actually, it can use the exact same code as is used for data acquisition in sweep and scan modes. Acquisition for SIM and MRM begins with commanding a hardware reset of the Data Acquisition Board (DAq) and setting the number of ADC conversions that the DAq hardware is to average for each data point. The acquisition processor then monitors the Link and Sync Output module waiting for a signal from the ion path slave indicating that the ion path has been properly configured for the next data point. The acquisition processor then gives the start command to the DAq and waits for the DAq to indicate completion. Slave 3 then informs the ion path slave that it is through with the current data point (using the Link and Sync Output module), reads the value of this data point from the DAq, clears it, and transfers the value on to the display/reduction processor by means of a Link and Sync Output module. The last step in the data acquistion loop has the

acquisition slave check the software status byte from the ion path slave to see if it is indicating the end of the experiment. If the end of experiment is indicated, the DAq is reset and the acquisition processor returns to monitoring the command FIFO from the master processor. If there is no end of scan indication, slave 3 then loops back to the point where it was waiting for the ion path ready signal on the Link and Sync Input module.

Ion Path Processor

During run time, slave 1, the ion path processor, is responsible for cycling the quadrupoles through the elements being monitored, activating the appropriate ion path parameter set for each element, counting the number of cycles which have been executed so as to determine when the end of the experiment has been reached, and control of the time period for each cycle by synchronization with the AMU Timer module.

At the beginning of the experiment, the ion path slave is initialized by having the master processor download the list of elements for monitoring, the parameter sets to be switched in, the number of elements to be monitored, the number of cycles to execute, and a code for the cycle period. All of the parameter values as well as the masses to use are converted into DAC units prior to the start of the analysis. Slave 1 stores the number of cycles to execute in a memory location known to the master and does a

decrement and test for zero at the end of each cycle. This arrangement allows the master to overwrite the current value if the operator requests an early termination of the experiment.

The code for the cycle period is used to set up the AMU Timer. Unlike scans and sweeps, where the AMU Timer is used to effect a step-by-step time synchronization, SIM and MRM use the AMU Timer to synchronize cycles. Since the AMU Timer can only be set up for a small set of specific and discrete time intervals, this approach makes it possible to optimize the amount of averaging performed by the DAq not only for the selected cycle period, but also on the basis of the number of elements being monitored, and the type of monitoring being performed. For a series of experiments, this feature allows the operator to add or subtract elements from the list while maintaining a constant cycle period. This becomes especially significant at short cycle times, where only a limited amount of averaging can be performed; the operator has the freedom to trade off the amount of data to be obtained (i.e. the number of elements to monitor) in a run against the degree of averaging to be performed.

The actual data acquisition code consists of a singly-nested loop structure. The outer loop is executed for each cycle and the inner loop is executed for each element within a cycle. Each pass through the outer loop initializes and executes the inner loop, waits for the synchronization signal from the AMU Timer, decrements the cycle counter, and

exits when the cycle counter comes down to zero. The inner loop begins by monitoring the Link and Sync Output module from the detection slave, waiting for the signal indicating that it is done with the previous data point. Upon receipt of this signal, the ion path slave sets the quadrupoles as needed for the next ion or reaction, switches in the appropriate set of parameters, and allows a five millisecond settling time. At the end of the settling period, the detection slave is informed that the ion path is now stable by means of the Link and Sync Output module. At this point, the ion path processor also uses the Link and Sync Output module to pass the index number of the current element to the display/reduction processor. The inner loop is then repeated for each element which is being monitored. After all of the cycles have been executed, the ion path slave sets a bit in its software status byte to inform the other slaves of completion and restores the ion path to its state prior to the experiment. When these tasks are complete, the slave returns to monitoring the command FIFO from the master processor.

Display/Reduction Processor

The third slave, the display/reduction processor combines the x-axis information being sent it by the ion path slave and the y-axis information being sent it by the detection slave. This combined information is then used to provide real-time graphics and is relayed back to the master

processor at the end of each cycle. This slave is initialized by reading the number of elements being monitored, clearing its software status byte, and setting up the graphics screen. During run time, the display/reduction processor also uses a singly nested loop structure, the outer loop being executed once for each cycle, and the inner loop being executed for each element within a cycle.

The outer loop begins by checking whether the master processor has finished reading the data from the previous cycle. After this has occurred, as indicated by the master clearing slave 2's software status byte, the inner loop is executed. On completion of the inner loop, the software status byte is set, informing the master that data is now available, and the display/reduction processor checks the software status byte of the ion path slave to see whether there are additional cycles to be executed. If there are additional cycles to execute, the cycle number is checked to see if it is an integral multiple of 1000. If it is, the graphics screen is re-initialized. When the end of the experiment is indicated, the display/reduction processor returns to monitoring the command FIFO from the master.

On each cycle, the inner loop is executed once for each element being monitored. This loop begins by reading the index of the current element, which is passed to it by means of the Link and Sync Input Module coming from the ion path slave. Next, the corresponding intensity value is read from the Link and Sync Input module coming from the detection

processor. This intensity value is stored in an array indexed by the value which had been provided by the ion path. If this index is less than or equal to five, the intensity value is scaled and plotted for the appropriate element in the real-time display, otherwise the slave loops back for the next element.

The run time tasks for the master processor include reading data values from the display/reduction slave as each cycle is executed, formatting these data into a proper record for the data file, writing this data out to disk, and monitoring the keyboard for requests for early termination of the run. This is a unique aspect of MRM and SIM data acquisition; the master has no run-time tasks during sweeps or scans.

All five of the SIM/MRM run commands behave in essentially the same manner. The first step is to download required information to each of the slave processors. In the case of the parameter tables and the lists of elements to monitor, this is done by direct memory transfers. The remaining information is downloaded through the command FIFOs, pushing the information onto the stack of the appropriate slave. The downloading process is followed by commands to start each of the slaves. Next, the master clears the display/reduction slave software status byte, updates the current experiment header, creates a new scan header for the active data file, and enters its data acquisition loop. This loop is executed once for each cycle

and begins with the master monitoring the keyboard to see if the "Q/q" key has been struck. If that occurs, the cycle counter on the ion path slave is set to 1, so that execution will terminate at the end of the current cycle. In any case, the master breaks out of the keyboard monitor loop when slave 2, display/reduction, sets its software status byte to indicate that a full cycle of data has been acquired. The master then reads the intensity values from the slave by means of interprocessor reads and clears the slave 2 software status byte when it's done. These values are then formatted into a data record which includes the mass settings of the first and third quadrupoles for each element (storing a -1 for an RF-only quadrupole). The data record is written out to disk at the end of the active data file and the master increments its cycle counter. The last step in the loop is to test the software status byte from the ion path slave to see if it's indicating the end of the experiment. When the end of the experiment is indicated, the master updates the experiment and scan headers in the data file to reflect the number of data records which were actually obtained. Once this is completed, the master is done executing the MRM/SIM and can return to accepting new commands from the user.

At the end of a SIM/MRM experiment, the data are stored as a scan within the most recently defined experiment in a file maintained by the FORTH Database Management System (65). This scan is stored in a manner which is compatible

with the data structures used by sweep and scan data. This allows all of the data file utilities, such as experiment directories, scan directories, data listings and display commands, to be used in essentially the same manner as they are for scans and sweeps. Directories, experiment deletion, and scan deletion are entirely identical. Data listings are generated by a special subroutine to the normal command, **DLIST**, and no additional commands are required. Displays are also generated by a special subroutine, this time to the normal display command, **DISP**. The greater flexibility required by the display subroutine, however, requires a special display set-up command **CSET** (chromatogram set-up) to be used instead of the **DSET** command which is used to format sweep and scan displays. The CSET command allows the user to specify which elements to display (to a maximum of five at one time) and over which time interval. Each chromatogram is separately normalized over the time interval used for the current display. Additionally, there is a command **NEXT** which allows the user to display a set of elements as a series of displays containing successive time windows of constant width, without having to return to the CSET command.

As is the case with sweeps and scans, archiving of data and generation of high quality plots is performed on the PDP-11/23 data system. Data may be uploaded to the data system by means of a 16-bit parallel link. Unlike scans and sweeps, however, MRM/SIM data cannot be conveniently

incorporated into the MSU-LLNL Multi-Dimensional Database structure (66). This is because the data points cannot be represented as an ordered, x-y pair, as required by the multi-dimensional database. Instead, they must be represented as ordered triples, composed of quadrupole 1 mass, quadrupole 3 mass, and intensity. Thus, a new set of utilities had to be written to process these data on the PDP-11/23 data system.

Data from the control system are uploaded to the data system as "experiments". Each experiment, a series of 64-byte records, consists of some header information, user-supplied comments, and headers and data for one or more "scans", which are presumably related. Each scan corresponds to an MRM/SIM run and consists of a header record, the full set of parameter values for each element monitored, and one data record for each cycle (regardless of how many elements were actually monitored). The control system uploads one experiment at a time up the 16-bit parallel link to the data system with transmission of a checksum for each record. The receiving task on the data system, UPLOAD (67), creates a specially formatted file consisting of each 64-byte record as it is received. A pair of tasks, GCTRAN and GCXFER, were written to take the file created by UPLOAD, and separate it into one direct-access file for each of the scans in the experiment. Each one of the scan files contains the scan header, all of the comments for the experiment, the scan parameter sets, and all of the scan

data. In addition, the user is prompted for any additional comments to go into each individual scan file as it is being generated.

Appendix B

Program Listings for Single and Multiple Processor Scans and Sweeps

This appendix is included to provide partial listings of the programs discussed in Chapter 3.

Sweeps

Single Processor

Register Usage -

```
0     - scratch
1     - counts steps
2     - current x value
I     - address of swept device
W     - pointer to next slot in data buffer
```

Labels -

```
AMUF/F     - address to reset amu timer
STEP       - step size, in DAC units, for swept device
TESTMUX    - connects CPU test input to amu timer
daqbase2   - address of lowest data byte from data
                acquisition board
daqstat    - address of data acquisition board status
                byte
startdaq   - address to start data acquisition
```

Code for SWEEP loop -

```
BEGIN
     2 I) MOV                    Write out the new x value
     1 # TESTMUX MOV B           Select the amu timer
     WAIT                        Wait for the amu timer
     AMUF/F STA B                Reset the amu timer
     0 0 SUB
     0 DEC
     startdaq STA B              Start the data
                                      acquisition board
     2 0 MOV                     Get the current x value
     STOS                        And place in data buffer
     0 0 SUB
     STOS                        Flag word of data buffer
     BEGIN
          daqstat LDA B          Get data acquisition status
          04 #B 0 TEST           And test for completion
          0= NOT                 Exit loop when bit is set
     END
     0 0 SUB                     Clear highest byte of
intensity double word
     daqbase2 4 + LDA B          Get second highest byte
     STOS                        Store high word of intensity
     daqbase2 LDA B              Lowest byte of intensity
     daqbase2 2+ 0 HI MOV B      And next lowest byte
     STOS                        Store low word of intensity
     daqf/fclr LDA B             Reset data acquisition board
     STEP 2 ADD                  Increment x value
LOOP                             Repeat until end of sweep
```

Multiple Processors

Ion Path Processor

Register Usage -

```
     0     - scratch
     1     - count number of steps
     2     - store current x value
     W     - address of device being swept
```

```
Labels -

      AMUF/F     - address to reset amu timer
      STEP       - the step size, in DAC units, for swept
                   device
      TESTMUX    - multiplexes CPU test input to amu timer or
                   Link and Sync module
      XDATA      - address to write x value to reduction micro
      XSTB       - strobe to transmit x value to reduction
                   micro
      YF/F       - address to reset ok from detection micro
      xstable    - address to send ok to sample to detection
                   slave


Code for SWEEP loop -

CREATE ?YSAMPLED          Subroutine checks for detection
                               micro done sampling previous
                               point
   0 0 SUB                Clear accumulator and select
   TESTMUX STA B          The Link and Sync module
   WAIT                   Wait for an ok from the detection
                               processor
   YF/F STA B             Clear the y status flip-flop
RET                       And return to calling program


CREATE XSTABLE            Subroutine syncs with amu timer and
                               gives an ok to sample to
                               detection micro
   1 # 0 MOV
   TESTMUX STA B          Select the amu timer
   WAIT                   Wait for the amu timer
   AMUF/F STA B           And reset it
   xstable STA B          Send ok to detection slave
RET                       And return to calling program


CREATE >PEAK              Subroutine to send current x value
                               to reduction micro
   2 XDATA MOV            Write the current x value
   XSTB STA B             Strobe the value over to the
reduction                      micro
RET                       And return to calling program
```

```
BEGIN
   ?YSAMPLED CALL        Check that detection slave is done
   2 W) MOV              Write the new x value out to the
                              swept device
   XSTABLE CALL          Sync with the amu timer and send ok
                              to sample to the detection
                              micro
   >PEAK CALL            Send current x value to the
                              reduction micro
   STEP 2 ADD            Calculate the next step value
LOOP                     Loop through the desired number of
                              steps
```

Detection Processor

Register Usage -

0 - scratch, high word of intensity value
2 - low word of intensity value

Labels -

PF/F	- address to clear the reduction sync. flip-flop
SSTAT	- base address of processor software status bytes
TESTMUX	- multiplexes CPU test input to the synchronization flip flops of the ion path and reduction micros
XF/F	- address to clear ion path sync. flip-flop
YDATA	- address for sending a data word to the reduction micro
YSTROBE	- address to transmit data to the reduction micro
daqbase2	- address of low data byte from the data acquisition board
daqf/fclr	- address to clear the completion flip-flop from the data acquisition board
daqstat	- status byte of the data acquisition board
startdaq	- address to start the data acquisiton board

Code for SWEEP loop -

```
CREATE ACQUIRE                  Subroutine to control the data
                                     acquisition board
   0 0 SUB
   0 DEC
   startdaq STA                 Send start command to the data
                                     acquistion board
   BEGIN
      daqstat LDA B             Get the status byte
      04 # 0 TEST B             Test the completion bit
      0= NOT                    Exit when bit is set
   END
   daqbase2 2 MOV B             Get the lowest byte
   daqbase2 2+ 2 HI MOV B       And the next lowest byte
   0 0 SUB                      Clear the top byte of the
                                     intensity
   daqbase2 4 + LDA B           Get the third intensity byte
   daqf/fclr LDA B              Clear the data acquisition
                                     board
RET                             And return to calling program


BEGIN                           Start of acquisition loop
   0 #B TESTMUX MOV             Select the ion path monitor
   WAIT                         Wait for ok from the ion
                                     path micro
   XF/F STA B                   And reset
   CALL ACQUIRE                 Obtain an intensity value
   YSAMPLED STA B               Give the ion path micro an ok
                                     to step the x value
   YDATA STA                    Send intensity high word to
                                     the reduction micro
   2 YDATA MOV                  Send intensity low word to the
                                     reduction micro
   YSTROBE STA B                Strobe the values across
   1 #B TESTMUX MOV             Select reduction micro monitor
   WAIT                         Wait for acknowledge from the
                                     reduction micro
   PF/F STA B                   And reset the the flip-flop
   SSTAT 1+ LDA B               Get the ion path micro's
                                     software status byte
   0 SHR                        Look at its low bit
   CS                           Carry set implies the SWEEP
                                     has been completed
END
```

Reduction Processor

Register Usage -

```
0     - scratch, x axis value, low intensity word
2     - high word of intensity
W     - pointer to next location in the data buffer
```

Labels -

```
RSTROBE   - address to strobe over intensity data from
              the detection slave
SSTAT     - base address of processor software status
              bytes
XDATA     - address of data sent from ion path slave
XF/F      - address to reset the ion path flip-flop
XSTROBE   - contains status of flip-flop indicating x
              axis data available
YDATA     - address of intensity data sent from the
              detection micro
YF/F      - address to reset the detection flip-flop
YSTROBE   - contains status of flip-flop indicating
              intensity data available
```

Code for SWEEP loop -

```
CREATE ?XDATA                  Subroutine to read the x axis
                                    value sent from the ion
                                    path slave
   BEGIN
      XSTROBE LDA B            Monitor the strobe flip-flop
      0 SHR B                  from the ion path micro
      CS                       Carry bit sent indicates data
                                    available
   END
   XF/F LDA B                  Clear the flip-flop
   XDATA LDA                   And read the x value
RET                            Return to the calling program
```

```
CREATE ?YDATA                    Subroutine to read the 32-bit
                                      intensity value from the
                                      detection slave
   BEGIN
      YSTROBE LDA B              Monitor the strobe flip-flop
      0 SHR B                    from the detection micro
      CS                         Carry bit set indicates data
                                      available
   END
   RSTROBE LDA B                 Strobe the data in
   YF/F LDA B                    Clear the flip-flop
   YDATA 2 MOV                   Read in the high word
   YDATA LDA                     Read in the low word
RET                              Return to the calling program


BEGIN
   ?XDATA CALL                   Get the x axis value
   STOS                          And store it in the data
                                      buffer
   0 0 SUB                       Clear the accumalator
   STOS                          And store in the flags word
   ?YDATA CALL                   Get the intensity value
   STOS                          Store the low word
   2 0 MOV                       Move in the high word
   STOS                          And store it
   SSTAT 1+ LDA                  Get the software status byte
                                      for the ion path slave
   0 SHR                         Check its low bit
   CS                            Carry bit set implies that the
                                      SWEEP is complete
END
```

Neutral Loss Scans

Single Processor

Register Usage -

- 0 - scratch
- 1 - loop counter, intensity low word
- 2 - scratch, intensity high word
- U - intensity high word
- S - stack pointer
- I - intensity low word, pointer
- W - pointer into data buffer

Labels -

(thld)	- the working intensity threshold
AMUF/F	- address to reset the amu timer
FRAC	- integer portion of the quad 3 mass step size
FRAC 2+	- fractional portion of the quad 3 mass step size
M1DAC	- address of the quad 1 mass dac
M2DAC	- address of the quad 2 rf-level dac
M3DAC	- address of the quad 3 mass dac
MASS1	- the quad 1 mass for the next step
MASS3	- the quad 3 mass for the next step
MASS3FRAC	- the fractional part of the quad 3 mass for the next step
STEP	- the step size for quadrupole 1 mass
TESTMUX	- multiplexes the amu timer to the CPU test input
daqstat	- the data acquisition board status byte
kflag	- flag cleared when on a peak tail
max-peak	- running intensity maximum for current peak
mwidth	- the maximum allowed peak width
pwidth	- the minimum allowed peak width
startdaq	- address to start the data acquisition board
xlast	- counts the peak width
xmax	- quad 1 mass value at the current peak maximum
xvalue	- current quad 1 mass value
ylast	- counts consecutive points above threshold
yprev	- intensity for the previous step

Code for NSCAN Loop -

```
CREATE NEWTHRESH                Subroutine to check for a new
                                     peak maximum
   max-peak 2+ 1 SUB            Is the current intensity value
   max-peak 2 SBB               greater than the current peak
                                     maximum
   0<                           -ve implies a new maximum
   IF
      xvalue LDA                Get the current mass value
      xmax STA                  And save it
      I max-peak 2+ MOV         Save the intensity low word
      U max-peak MOV            And the intensity high word
      U U OR                    Look if the high word is zero
      0=                        True if high word is zero
      IF
         I SHR                  Take half of the current
                                     intensity
         I (thld) MOV           And use it as the new
threshold
      ELSE                      High word is non-zero
         8000 # (thld) MOV      Use 32k as new threshold
      THEN
   THEN
RET                             Return to the calling program


CREATE ?RISING                  Subroutine to check for a
                                     rising peak
   yprev 2+ 1 SUB
   yprev 2 SBB                  Compare the current intensity
                                     to the previous point
   0< NOT                       ≥ 0 implies a falling peak
   IF
      I yprev 2+ MOV            Save the intensity low word
      U yprev MOV               And the intensity high word
   ELSE                         The peak is rising
      0 # kflag MOV             Clear the tailing peak flag
   THEN
RET                             Return to the calling program
```

```
CREATE ?PEAK                    Subroutine to perform peak-
                                     finding
   daqbase2 1 MOV B             Get lowest intensity byte
   daqbase2 2+ 1 HI MOV B       And the next lowest byte
   2 2 SUB                      Clear intensity high word
   daqbase2 4 + 2 MOV B         And get top intensity byte
   2 U MOV                      Make a copy of the high word
   1 I MOV                      And the low word
   (thld) 1 SUB
   0 # 2 SBB                    Test if current point is above
                                     the threshold
   0< NOT                       ≥ implies above threshold
   IF
      ylast INC                 Count number of points above
                                     threshold
      kflag LDA                 Check the tailing peak flag
      0 0 OR                    Zero implies peak was falling
      0=
      IF
         xlast INC              Counts points since start of
                                     peak
         xlast LDA              Get the peak width
         0 mwidth CMP           And compare it with the
                                     maximum peak width
         CS                     Carry set implies peak exceeds
                                     maximum width
         IF
            xmax # I MOV        Address of beginning of data
            4 # 1 MOV           Move 4 words into data buffer
            MOVS REP            Do it
            0 0 SUB             Clear the accumulator
            xmax STA            And re-initialize
            xlast STA                "
            max-peak STA             "
            max-peak 2+ STA          "
            1 # kflag MOV       Set the tailing peak flag
         ELSE
            NEWTHRESH CALL      Check if this is a new maximum
         THEN
      ELSE
         ?RISING CALL           Possible tailing peak
      THEN
```

```
   ELSE
      ylast 1 MOV              Get the current value
      0 # ylast MOV            And clear it out
      1 1 OR                   Check it for zero
      0= NOT                   ≠ implies previous point was
                                    above threshold
      IF
         xlast LDA             Get number of points over
                                    threshold
         0 pwidth CMP B        And compare to minimum peak
                                    width
         CS                    Carry set implies minimum
                                    width satisfied - save
                                    the peak
         IF
            xmax # I MOV       Start of data area
            4 # 1 MOV          Number of word to move
            MOVS REP           Move them into data buffer
            0 0 SUB            Put a zero in accumalator
            xmax STA           And re-initialize
            xlast STA                "
            max-peak STA             "
            max-peak 2+ STA          "
            1 # kflag MOV      Set the tailing peak flag
         THEN
      THEN
      0 # kflag MOV            Clear the tailing peak flag
   THEN
RET                            And return to the calling
                                    program
```

```
BEGIN
   MASS1 2 MOV                Get the quad 1 mass value
   2 M1DAC MOV                And write it to the dac
   MASS3 LDA                  Get the quad 3 mass value
   M3DAC STA                  And write it to the dac
   0 SHR                      Use half the mass 3 value for
                                   the quad 2 level
   M2DAC STA                  And write it to the dac
   1 #B TESTMUX MOV           Select the amu timer
   WAIT .                     And wait for it
   AMUF/F STA B               Reset the amu timer
   0 0 SUB                    Clear the accumulator
   0 DEC                      And decrement to -1
   startdaq STA B             Start the data acquisition
                                   board
   2 xvalue MOV               Save the quad 1 mass value
   STEP 2 ADD                 Add in the next step
   2 MASS1 MOV                And save the new value
   MASS3 LDA                  Get the current quad 3 mass
                                   value
   MASS3FRAC 2 MOV            Get the current quad 3
                                   fractional mass
   FRAC 2+ 2 ADD              Add in the fractional step
                                   size
   FRAC 0 ADC                 And the integer part, with
                                   carry out of the fraction
   MASS3 STA                  Save the integer part
   2 MASS3FRAC MOV            And the fractional part
   BEGIN
      daqstat LDA B           Get status of the data
                                   acquisition board
      04 #B 0 TEST            Test the completion bit
      0= NOT                  Bit set implies data is
                                   available
   END                        Loop until data available
   1 PUSH                     Save the loop counter
   ?PEAK CALL                 Do the peak finding
   1 POP                      Restore the loop counter
LOOP                          Execute the next step
```

Multiple Processors

Ion Path

Register Usage -

- 0 - scratch
- 1 - loop counter
- 2 - quad 1 mass
- I - fractional part of quad 3 mass
- W - integer part of quad 3 mass

Labels -

FRAC	- integer part of quad 3 mass step size
FRAC 2+	- fractional part of quad 3 mass step size
M1DAC	- address of quad 1 mass dac
M2DAC	- address of quad 2 rf level dac
M3DAC	- address of quad 3 mass dac
STEP	- step size for quad 1 mass

Code for NSCAN Loop -

Subroutines ?YSAMPLED, XSTABLE, and >PEAK are the same as used in the SWEEP loop.

```
BEGIN
   ?YSAMPLED CALL          Wait until the detection micro
                                is done with the previous
                                point
   2 M1DAC MOV             Set the new quad 1 mass
   W 0 MOV                 Get the new quad 3 mass
   M3DAC STA               And set quad 3 to this value
   0 SHR                   Divide it by two
   M2DAC STA               And set the quad 2 rf level
   XSTABLE CALL            Inform the detection micro
                                that the next step is
                                ready
   >PEAK CALL              Send the current quad 1 mass
                                to the reduction micro
   STEP 2 ADD              Calculate the next quad 1 mass
                                value
   FRAC 2+ I ADD           Calculate the fractional part
                                of the next quad 3 mass
                                value
   FRAC W ADC              Calculate the integer part of
                                the next quad 3 mass
                                value
LOOP                       Loop through the desired
                                number of steps
```

Detection

Same code as used for SWEEP

Reduction

Register Usage -

```
0     - scratch
1     - intensity low word
2     - intensity high word
U     - intensity high word
S     - stack pointer
I     - intensity high word, pointer
W     - pointer to data buffer
```

Labels -

```
(thld)     - the current intensity threshold
kflag      - flag set to indicate a tailing peak
max-peak   - intensity of current peak maximum
mwidth     - the maximum allowed peak width
pwidth     - the minimum allowed peak width
xlast      - counts peak width
xmax       - quad 1 mass at maximum intensity of current
             peak
xvalue     - current quad 1 mass
ylast      - counts points above threshold
yprev      - intensity for the previous step
```

Code for NSCAN Loop -

```
CREATE NEWTHRESH                    Subroutine to check for a new
                                         peak maximum
   max-peak 2+ 1 SUB                Is the current intensity value
   max-peak 2 SBB                   greater than the current peak
                                         maximum
   0<                               -ve implies a new maximum
   IF
      xvalue LDA                    Get the current mass value
      xmax STA                      And save it
      I max-peak 2+ MOV             Save the intensity low word
      U max-peak MOV                And the intensity high word
      U U OR                        Look if the high word is zero
      0=                            True if high word is zero
      IF
         I SHR                      Take half of the current
                                         intensity
         I (thld) MOV               And use it as the new
threshold
      ELSE                          High word is non-zero
         8000 # (thld) MOV          Use 32k as new threshold
      THEN
   THEN
RET                                 Return to the calling program


CREATE ?RISING                      Subroutine to check for a
                                         rising peak
   yprev 2+ 1 SUB
   yprev 2 SBB                      Compare the current intensity
                                         to the previous point
   0< NOT                           ≥ 0 implies a falling peak
   IF
      I yprev 2+ MOV                Save the intensity low word
      U yprev MOV                   And the intensity high word
   ELSE                             The peak is rising
      0 # kflag MOV                 Clear the tailing peak flag
   THEN
RET                                 Return to the calling program
```

```
BEGIN
   ?XDATA CALL                Get the quad 1 mass value
   xvalue POP                 And save it
   ?YDATA CALL                Get the intensity value
   2 POP                      The high word
   1 POP                      And the low word
   2 U MOV                    And make copies
   1 I MOV
   (thld) 1 SUB               Check if the intensity is
   0 # 2 SBB                  Above threshold
   0< NOT                     ≤ implies above threshold
   IF
      ylast INC               Count points above threshold
      flag LDA                Get the tailing peak flag
      0 0 OR                  Check it for zero
      0=                      0 implies not a tail
      IF
         xlast INC            Count the peak width
         xlast LDA            And get the value
         0 mwidth CMP         Test if we've exceeded the
                                   maximum width
         CS                   Carry set if wide peak
         IF
            xmax # I MOV      Start of data area
            4 # 1 MOV         Number of words to move
            MOVS REP          Move peak data into data
                                   buffer
            0 0 SUB           Get a zero
            xmax STA          And re-initialize
            xlast STA                "
            max-peak STA             "
            max-peak 2+ STA          "
            1 # kflag MOV     Set the tailing peak flag
         ELSE                 Peak is not too wide
            NEWTHRESH CALL    Check for a new maximum and
                                   adjust (thld)
         THEN
      ELSE                    Tail of a previous peak
      ?RISING CALL            See if data is rising again.
                                   If so, clear kflag
      THEN
```

```
   ELSE                          Peak is below threshold
      ylast 1 MOV                Get the count above threshold
      0 # ylast MOV              And zero the counter
      1 1 OR                     Check if the previous point
                                      was above threshold
      0= NOT                     ≠ 0 implies it was
      IF
         xlast LDA               Get the peak width
         0 pwidth CMP B          And see if it meets the
                                      minimum width
         CS                      Carry set implies peak is wide
                                       enough
         IF
            xmax # I MOV         Start of data area
            4 # 1 MOV            4 words to save
            MOVS REP             Transfer to data buffer
            0 0 SUB              Get a zero
            xmax STA             And re-initialize
            xlast STA                  "
            max-peak STA               "
            max-peak 2+ STA            "
            1 # kflag MOV        Set the peak tail flag
         THEN
      THEN
           0 # kflag MOV         Peak ended below threshold.
                                      Cancel the flag
   THEN
   SSTAT 1+ LDA B                Get the ion path micro
                                      software status byte
   0 SHR                         Get its low bit
   CS                            Carry set implies end of scan
END
```

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