JOURNAL OF MASS SPECTROMETRY, VOL. 33, 103–108 (1998)

A Historical Perspective and Commentary on Pioneering Developments in Gas Chromatography/Mass Spectrometry at MIT

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Klaus Biemann has helped shape many aspects of the field of modern mass spectrometry. This paper reviews and comments on some of his innovative contributions to gas chromatography/mass spectrometry (GC/MS) during the 1960s and 1970s with particular emphasis on the molecular separator, a comprehensive plan for the acquisition and processing of GC/MS data and the application of GC/MS to the analysis of drugs in emergency cases. © **1998 John Wiley & Sons, Ltd.**

J. Mass Spectrom. **33**, 103–108 (1998)

KEYWORDS: gas chromatography/mass spectrometry; Massachusetts Institute of Technology; Biemann

INTRODUCTION

In the early days of gas chromatography/mass spectrometry (GC/MS),[1] progress in the technology was greatly hampered by the dynamics of vacuum systems and data recording devices on most mass spectrometers. Mercury diffusion pumps were commonplace and frequently designed barely to keep up with modest leaks in various flanges so that proper operating pressure could be maintained in the mass analyzer. Chemical ionization had not yet been discovered, so the technique of differential pumping was not commonly available. The ion source, analyzer and detector regions were all evacuated by a single diffusion pump. The insult of even a modest gas flow (<1 ml min^{-1}) split from the effluent of a typical packed column (flow rate commonly in the region of 40–60 ml min^{-1}) was likely to shut down the mass spectrometer.

The light beam oscillograph[2] was in use at the time, but it had not been 'pushed' for rapid data acquisition as most samples were admitted through a batch inlet system to give a constant source pressure, and the mass spectrum was acquired leisurely over a period of 15–60 s. Whereas the light beam oscillograph did have adequate frequency response to acquire mass spectra much more rapidly, the magnetic mass spectrometers available at the time were not capable of scanning more quickly than 10–15 s per decade. (This Achilles' heel of the magnetic instrument was about to be the point of attack of the quadrupole mass spectrometer.[3]) And then there was the problem of a cardinal rule in mass spectrometry: 'Thou shalt not alter the partial pressure of the analyte in the ion source of the mass spectrometer whilst the mass analyzer is being scanned.' Because peak intensities in the mass spectrum are normalized to that of the base peak, any change in the partial pressure of the analyte during acquisition of the data would cause the corresponding portion of the mass spectrum to be altered relative to its 'normal' appearance, and thus the mass spectrum would be skewed to the extent that it might not be recognizable relative to the corresponding standard spectrum. Hence the concept of GC/MS is, in fact, a violation of this cardinal rule in mass spectrometry, as a gas chromatogram is by definition a recording of changes in the partial pressure of components of a sample with time. The only way to deal with this violation was to scan the mass spectrum as quickly as possible so as to minimize the relative change in partial pressure of the sample, and thus minimize the aberration in the 'normal' relative intensity pattern, or to record the mass spectrum photographically across the complete mass range simultaneously, thereby integrating changes in peak intensities.

Gohlke[4] first demonstrated GC/MS by connecting a gas chromatograph to a time-of-flight (TOF) mass spectrometer via a modified Nupro metering valve to obtain the mass spectra of methanol, acetone, benzene and toluene using a Polaroid camera to photograph the display on an oscilloscope. Lindeman and Annis[5] allowed 5% (2–3 ml min^{-1}) of the effluent from a packed GC column (10 ft column of 29% SF96 on crushed firebrick) to flow over a conventional gold leak to the ion source of a magnetic cycloidal mass spectrometer which they scanned from m/z 55 to 120 in 40 s while analyzing mixtures of isomers of hydrocarbons ranging from C_5 to C_8 and also toluene and naphthalene. Ebert[6] used a complicated array of toggle valves in combination with a metering valve as the interface to a

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CCC 1076–5174/98/020103–05 $17.50

Received 15 December 1997
Accepted 31 December 1997

TOF-MS system which was evacuated by a getter ion pump to detect traces of CS_2 in cyclohexane and also to obtain the mass spectra of di- and trichloroethane. Henneberg[7] used a capillary restrictor in his GC/MS interface during the analysis of mixtures of isomers of saturated and unsaturated hydrocarbons ranging from C_5 to C_7.

Although he did not make the initial report of GC/MS, Klaus Biemann at the Massachusetts Institute of Technology (MIT) proposed and developed aspects of interfacing a GC with a mass spectrometer and designed features for data acquisition and processing that greatly accelerated the development of GC/MS to the indispensable analytical tool that we know today. In particular, introduction of the Watson–Biemann separator[8] made a quantum leap in the applicability of GC/MS to the analysis of high-boiling, polar molecules such as steroids (C_{19}) and carboxylic esters (up to C_{18}).

THE WATSON–BIEMANN SEPARATOR

The earliest reports[4–7] of GC/MS showed the feasibility of introducing a minor fraction of the effluent from a GC column into an MS system. The power of the mass spectrometer in identifying individual components and even partially resolved components of a mixture was demonstrated with non-polar, thermally stable, small organic molecules. It was well known that metal surfaces in the chromatographic inlet system could cause thermal and/or catalytic degradation of many polar highly functionalized organic molecules.[9] Hence the earliest GC/MS systems that relied on standard metal micro-valves or metallic splitters were not suitable for the analysis of these highly functionalized compounds because of sample degradation and/or artifact formation at temperatures high enough to avoid condensation.

In late 1961, Klaus Biemann told me that it would be a good idea to develop an interface that could achieve molecular flow conditions that would preferentially remove the helium from the GC effluent so that a residual gas stream enriched in the organic components would enter the mass spectrometer. [Molecular flow through an orifice (effusion) is achieved when the mean free path is at least 10 times the diameter of the orifice.[10] Because some of the mathematical equations describing effusion are similar to those in Graham's law, effusion and diffusion are frequently confused.[11] Under molecular flow conditions (effusion), the probability that a molecular species will pass through an orifice (such as that of each of the pores in the wall of a fritted glass tube[8]) is inversely proportional to the square root of its mass and directly proportional to its mole fraction.] The interface should be all glass so as to provide an inert surface to thermally sensitive molecules. Further, there could be no valves involved in the interface as they would present a metallic surface somewhere along the path between the gas chromatograph and the mass spectrometer.

After a successful feasibility study with a crude design module incorporating the fritted glass disk of a Buchner funnel as the discriminating interface, a more suitable design (Fig. 1) having a lower dead volume was developed.[8] The real headache in the operational development of this design was the requirement that there be no valves. As the system operated on an effusion principle, it was necessary to establish conditions for molecular flow within the internal volume of the fritted glass tube; this pressure condition was controlled by the diameter of the capillary orifice between the gas chromatograph and central volume of the separator. Construction of the glass separator and 'manual adjustment' of the capillary orifices at the entrance and exit to the separator required establishing a friendly relationship with a cranky glass blower in the MIT Chemistry Department. The essential component of the separator, the fritted glass tube, had to be fused to the solid-walled capillaries. This was a nightmare for the glassblower because air trapped in some of the components of the sintered-glass wall would cause micro-explosions upon heating, thus greatly complicating the construction of the central portion to the molecular separator.

The all-glass effusion molecular separator, especially when silanized (a chemical treatment to reduce the polarity of active sites on glass[12]), presented a remarkably inert surface for the interface between the GC and MS, components, and allowed the analysis of highly polar, thermally labile molecules such as alkaloids and steroids.[13] Use of a photographic plate permitted the acquisition of high-resolution mass spectra in a double-focusing Mattauch–Herzog instrument as illustrated in Fig. 2, which shows the superposition of the mass spectrum of PFK calibration compound together with that of individual alkaloids [represented by peaks in the

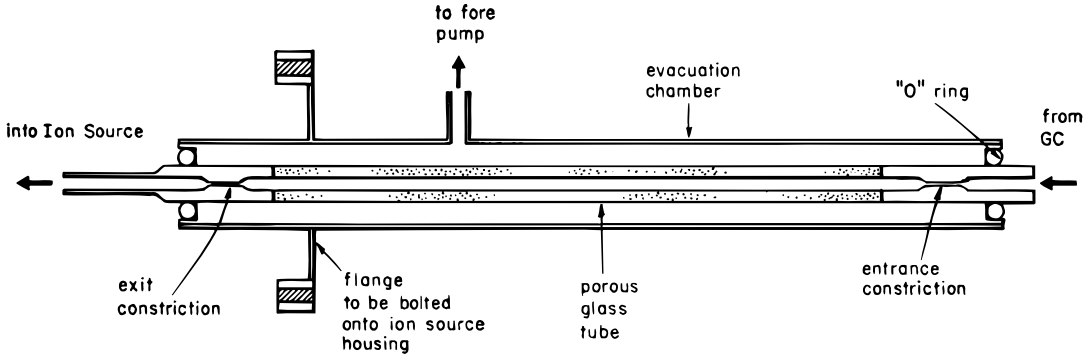

Figure 1. Schematic diagram of the Watson–Biemann separator, an effusion-based interface between GC and MS. Reprinted from Ref. 13 with permission from the American Chemical Society.

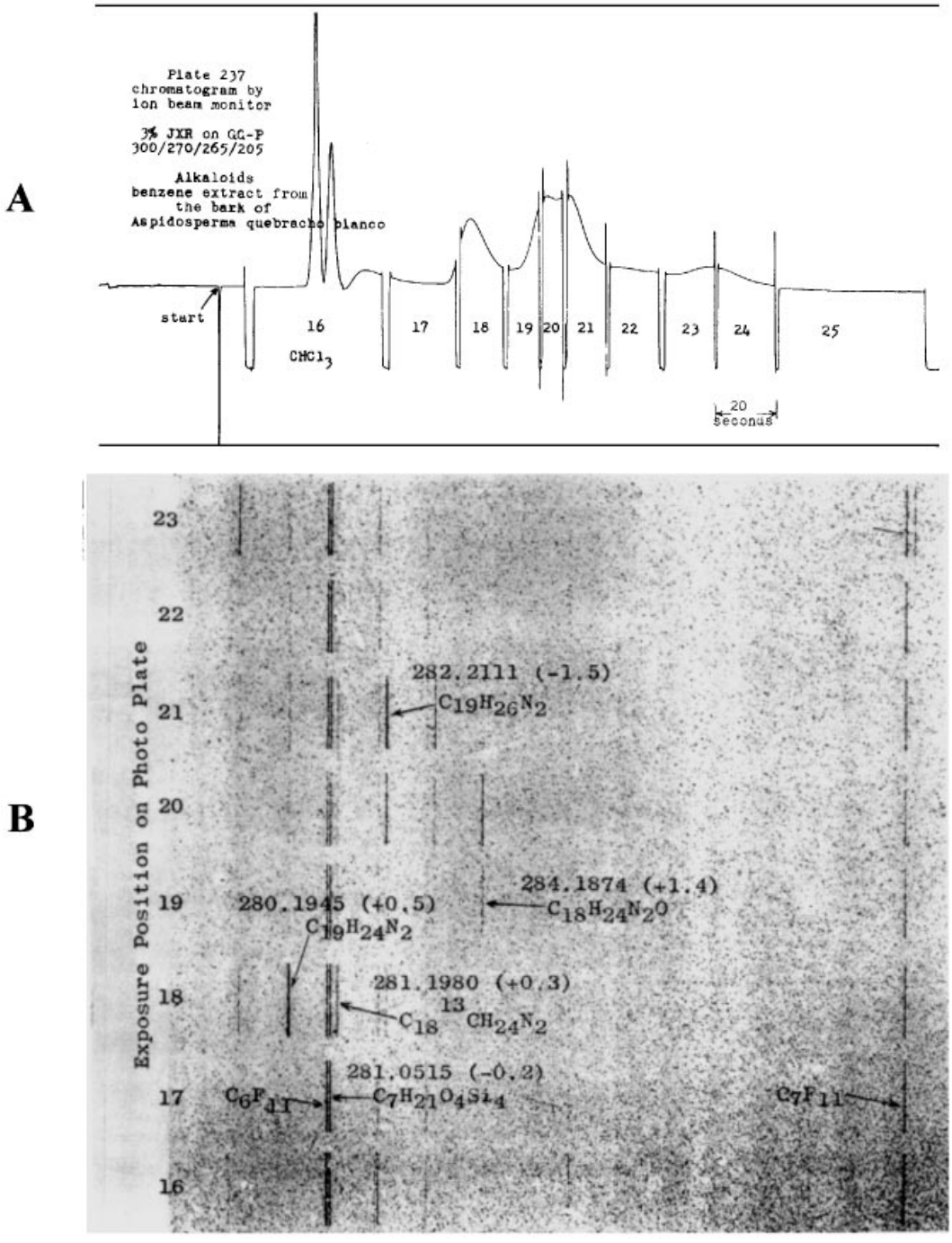

Figure 2. GC/high-resolution MS of an alkaloid extract. (A) Tracing of total ion current showing the chromatogram of a portion of the extract of *Aspidosperma quebracho blanco* bark; the numbers along the abscissa of the chromatogram correspond to the exposure numbers on the photographic plate in (B), negative excursions in the TIC indicate times at which the ion beam was deflected while the photoplate was moved to a new exposure position. (B) Magnification of a portion of a photoplate containing the high-resolution mass spectra of the alkaloids separated in the gas chromatogram in (A); the exposure position along the ordinate corresponds to mass spectra obtained at times corresponding to sections of the chromatogram in (A). Reprinted from Ref. 13 with permission from the American Chemical Society.

total ion current (TIC) trace in Fig. 2(A)] as indicated by the vertical lines in discrete horizontal rows on the photographic plate in Fig. 2(B). The all-glass separator enjoyed considerable acceptance in the field during the late 1960s. To some extent this was because the details of the jet orifice separator developed simultaneously by Ryhage in Sweden,[14] and also used successfully in the analysis of thermally labile compounds, were shrouded in secrecy for patent reasons. [The jet orifice separator operates on the principle of diffusion in the viscous flow regime (mean free path comparable to the diameter of the orifice) and is based on earlier work by Becker[15] for the separation of isotopicaly labeled gases.]

The fragility of the all-glass separator was always a concern. The flexibility and remarkable selectivity of the membrane separator[16] (the precursor of modern applications of MIMS[17]) soon became much more popular in the early 1970s. Extinction of all separators came in

the early 1980s with the advent of fused-silica capillary columns and bigger and more versatile vacuum systems, especially those adapted for differential pumping.

A DATA SYSTEM FOR GC/MS

Although many other workers[18–20] made valuable contributions to the development of data systems, Hites and Biemann[21] in 1967 laid out a comprehensive plan for the acquisition, storage, processing and display of a complete data field for GC/MS that remains a template for today's modern data systems. Their pioneering work was done with an IBM 1800 mini-computer, and it is interesting to note that the individual mass spectra were recorded on microfilm using a movie camera mounted in front of the oscilloscopic display (monitor) on the computer system. A valuable and useful feature of the data system was the reconstruction of the total ion current (Fig. 3) from consecutively recorded mass spectra, which provides a 'user-friendly' means of interacting with a huge array of mass spectra in a way that allows the most relevant data to be readily selected for study.

Another valuable feature, invoking an inherent selective attribute of the mass spectral data, was the introduction of the mass chromatogram,[22] which is a presentation of the relative importance of a particular peak in an array of mass spectra as a function of scan number as illustrated in Fig. 4. The mass chromatogram concept led to an even more sophisticated approach to data processing, called 'reconstructed mass spectra.'[23] This allowed the deconvolution of overlapping mass spectra of chromatographically unresolved components. The deconvolution is achieved by performing a peak profile analysis of mass chromatograms across the spectrum. Only the mass spectral peaks that maximize in intensity at a particular scan number are selected for the reconstructed mass spectrum at that point in time as illustrated in Fig. 5. For example, consider the intensities of the peaks at *m/z* 179, 256 and 310 at the stage of scan No. 117 in Fig. 5; at this point, the intensity of

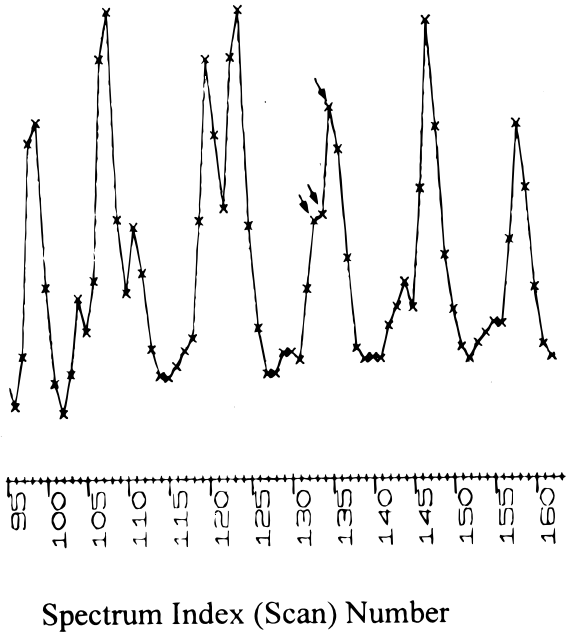

Figure 3. Reconstructed total ion current chromatogram. Reprinted from Ref. 21 with permission from the American Chemical Society.

m/z 310 is at a maximum, and so the reconstructed mass spectrum prepared from scan 117 will contain a peak at *m/z* 310, whereas it will not contain a peak at *m/z* 179 or at *m/z* 256 as that ion current did not reach a maximum in the corresponding mass chromatograms.

Once it had become possible to record the hundreds of mass spectra produced during a single GC experiment, one had to deal with the vast amount of data produced. Although the aforementioned methods produced the number of mass spectra that most correctly represented each component of the mixture, manual interpretation of each was time consuming and tedious. At that time a sizable collection of mass spectra had been converted to digital form thrugh a cooperative effort by JPL (Pasadena, CA, USA), the mass Spectrometry Centre in Aldermaston (UK) and MIT. The next logical step was to devise an algorithm that was capable

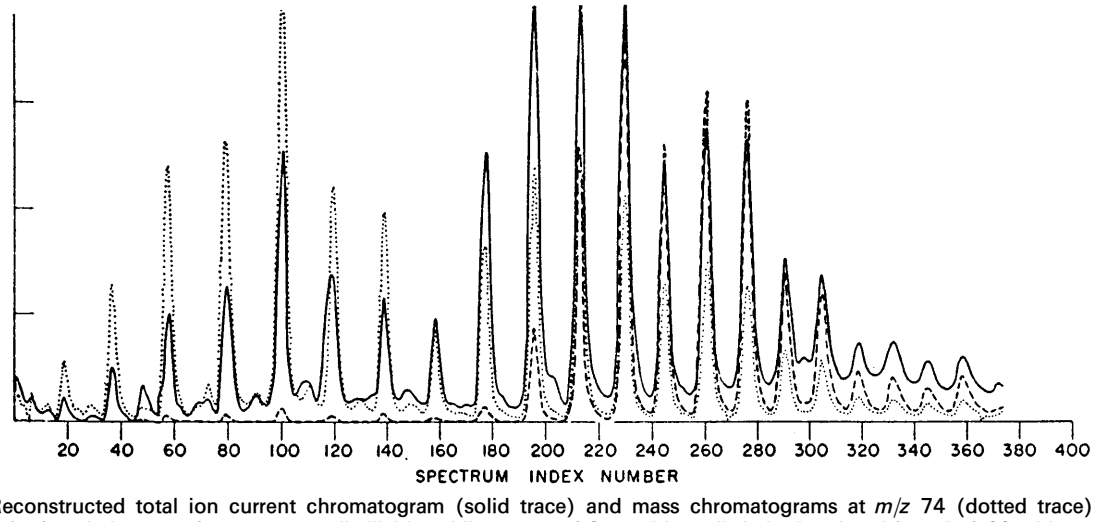

Figure 4. Reconstructed total ion current chromatogram (solid trace) and mass chromatograms at *m/z* 74 (dotted trace) and *m/z* 98 (dashed trace) of methyl esters of a non-steam distillable acidic extract of Green River oil shale. Reprinted from Ref. 22 with permission from the American Chemical Society.

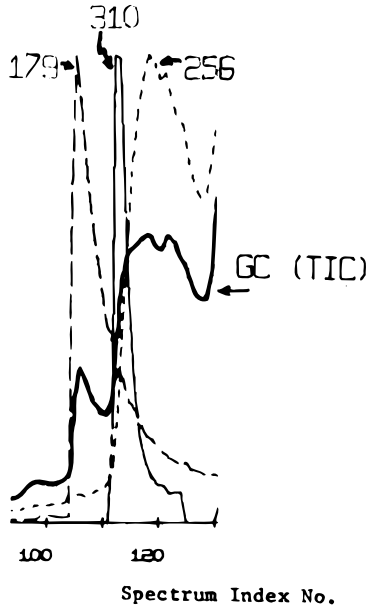

Figure 5. Mass chromatograms at *m/z* 179, 310 and 256 together with reconstructed total ion current chromatogram (solid trace) showing mass chromatograms maximizing at different scan numbers. Reprinted from Ref. 23 with permission from Marcel Dekker.

of comparing the mass spectrum of an unknown with all those in the collection ('library') and identify the one that fitted the data best.

The limited speed, memory and storage capacity of computers of the 1960s required compression of the data. The few previously proposed algorithms (alluded to in Ref. 24) used the 5–10 highest peaks in the spectra. This approach often failed, particularly with aliphatic compounds which all exhibit the same strong signals at low *m/z* values, but can be differentiated by distinct, although weaker, signals at higher *m/z* values. Selecting the most intense signals in consecutive 14 u windows remedied this problem and led to a much more reliable matching process. The principle of this concept was presented by Ron Hites (then a graduate student at MIT) in his first oral presentation at the International Mass Spectrometry Conference held in Berlin (September 1967); a more refined version was finally published in 1971.[24] Most commercial data systems then incorporated versions of this approach ('library search'), which became increasingly more sophisticated and powerful with the rapidly increasing power of smaller and less expensive computers.

ANALYSES OF BODY FLUIDS FROM OVERDOSE VICTIMS BY GC/MS

The serendipity by which the Biemann group contacted the medical community, and then pioneered the use of GC/MS for assisting in the timely biomedical activity of drug analysis, is revealed by the following short story from Professor Biemann himself:

> "Sometime in 1970, Jim Althaus, one of my graduate students, met at a cocktail party Dr Hedley-White, an anesthesiologist at one of the Harvard Medical School Hospitals. His expertise was in maintaining patients who were in a coma and promoting their recovery. He chatted with Jim and asked him about his research project. Jim replied that he was determining the structure of alkaloids by GC/MS. Dr Hedley-White used GC to follow the decrease of the coma-causing agent in the blood of his patients, but had not heard of mass spectrometry. A few days later, Dr Hedley-White called me to report that he had observed a new peak in the GC of one of his patient's blood, which probably represented a metabolite of the drug (the patient had overdosed on a sleeping pill which was a chlorinated hexane derivative). So he collected the fraction from the gas chromatograph and sent it over to MIT. We obtained its mass spectrum, which looked like that of benzyl alcohol. The library search confirmed it, but how could a seven-carbon compound be a metabolite of a hexane derivative? Dr Hedley-White said he would run some experiments to investigate the problem. The next Sunday he called to tell me 'the rest of the story.' He had switched the blood-sample stabilizing agent from coumarin to heparin, and when he finally read the fine print on the label of the heparin bottle, it said 'stabilized with 1% benzyl alcohol.' Our results convinced him that we had a machine that could identify a compound that was completely unexpected. The patient recovered from the coma after 23 days (not because of our information, but because of Dr Hedley-White's medical care and expertise). Thereafter, he sent us a blood sample from each of his overdosed patients, to identify the causative agent.
>
> "Word soon got around, particularly to the staff at Children's Hospital where they often had to deal with small children who had gotten into their parent's medicine cabinet. If it happened at night, the emergency room had a list of home telephone numbers of all the people in my laboratory who could be called. That person then drove to the laboratory while the hospital sent a blood or urine sample over by taxi. Samples were extracted with dichloromethane, the extract concentrated and injected into the GC/MS system. The causative agent was identified and reported back by telephone to the attending physicians. Dr Cathy Costello, then an NIH postdoctoral trainee in my laboratory, soon took over this project, which lasted for about 3 years. By then my students and postdocs who had always been enthusiastic about the project because of its potentially lifesaving aspects got tired of getting telephone calls in the middle of the night. At that point, the methodology was sufficiently established that commercial MS laboratories were glad to take it over, and their clients (mainly hospitals in the Greater Boston area) were already 'hooked.' For NIH, it was a good example of how their MS facilities contribute not only to biomedical research, but also to sometimes lifesaving patient care."

Some of the early applications of GC/MS to the problem of separating and identifying toxic agents and metabolites in physiological fluids were published by the Biemann group.[25,26] In one early example,[25] a 24-year-old woman had been admitted to Beth Israel Hospital, Boston, in critical condition, suspected of ingesting an overdose of the drug Librium. A urine sample (200 ml) was extracted with dichloromethane to yield separately the acidic, neutral and basic fractions; another portion (100 ml) was evaporated to dryness. High- and low-resolution mass spectra were obtained on each of the four fractions ('neutrals,' 'acids,' 'bases,' 'evaporated') by introduction of a sample directly into the ion source of the mass spectrometer. Because none of the mass spectra indicated the presence of chlorine,

Librium could be ruled out because its metabolic products were known to retain the chlorine attached to the phenyl group. Alternative analysis of the fractions by GC/MS showed several unusual components for normal physiological fluids.[25] Processing of the copious GC/MS data to generate reconstructed total ion current chromatograms and mass chromatograms with their newly designed data system allowed the MIT group eventually to deduce that Darvon was the causative agent, based on detection of diphenylbutene derivatives in the sample and on reference data from metabolic studies of Darvon.

This and other emergency applications of GC/MS made the Biemann search algorithm a necessary invention for the efficient (and thus sometimes lifesaving) purpose of identifying an unknown toxic agent by comparison of its spectrum with a library of mass spectra of drugs and drug metabolites.

CONCLUSION

The Biemann group at MIT played a major role in shaping the field of GC/MS. Their innovative developments made a significant impact in large measure because they were conducted in the context of solving realistic and important problems. The Biemann approach to the acquisition and processing of mass spectra during GC/MS is a particularly valuable contribution as it optimizes the analytical utilization of a potentially overwhelming quantity of data.

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