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Review

Gas chromatography with spectroscopic detectors

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Abstract

In recent years, capillary gas chromatography (GC) with Fourier Transform Infrared (FT-IR) and/or mass spectral (MS) detection has become a primary analytical tool for qualitative and quantitative analysis of complex mixtures. Because of the wide range of applications, the analytical requirements have motivated a variety of chromatographic and detection developments. This review examines those, illustrating with applications that demonstrate the power of GC and multidimensional GC–MS, GC–FT-IR and GC–FT-IR–MS systems for solving a variety of analytcal problems. In addition, the article discusses the integrated performance of such analytical systems with the aid of recent sample introduction and computer data analysis advances.

Keywords: Detection, GC; Reviews; Mass spectrometry; Infrared detection; Instrumentation

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1. Background

At the outset, it should be acknowledged that the present review is a moderately updated version of an earlier review on the same topic, by the same authors published in the mid-1990s [1]. Recently, the senior author moved to the University of Arkansas, and all of the coauthors have gone their separate ways to professional appointments. Because the coauthors' prior contributions represent the substance of this review, it is certainly appropriate that they share in its authorship, even though they did not participate in its present incarnation. Thus, any problems with this Millenium volume review should be attributed to the senior author. However, most of the credit for it should accrue to the coauthors. The present version is a reasonable representation of the issues addressed in the field over the past decade and should provide a good basis for assessing the future prospects in this area of research as we enter the new century.

The fundamental idea of linking or 'hyphenating' analytical instrumentation is motivated by the intrinsic desire of chemists to identify and quantify chemical compounds in known as well as unknown mixtures. As a consequence of development of the type of linked analysis systems which are the topic of this review, powerful new mixture analysis strategies are now available. This review will consider the implications of some of the technological advances over the past 30 years, with emphasis on more recent developments. Specifically, it will address the combination of gas chromatography with structurally specific infrared (IR) and mass spectrometry (GC–Fourier Transform (FT) IR, GC–MS, and GC–FT-IR–MS) detectors. Because the accompanying other articles in this Millenium volume of the journal focus on related topics, it is expected that there will be some overlap. Here, we present our perspective on the issues involved in the still emerging and evolving field of multidimensional analytical techniques. At present, these types of instrumentation systems are more capable of compound *class* identification than of specific compound analysis, although there are numerous examples of success in the second category, as well. Many of the present approaches to analytical instrumentation focus on isolating either a class of compounds or a single mixture component using one or more separation methods followed by detection with non-specific detectors (e.g. flame ionization, electron capture, refractive index, etc.) sometimes in concert with more compound structure-specific detectors (e.g. IR, mass or nuclear magnetic resonance spectrometry). The latter trend has been made possible in large measure by the availability of inexpensive and fast computers.

Low and Freeman first suggested the potential usefulness of a linked gas chromatography–Fourier Transform infrared–mass spectrometry (GC–FT-IR–

MS) system in 1968 [2]. Following this suggestion, several separate innovations in the necessary elements of such an analytical system resulted in improvement of attainable practical detection limits from hundreds of micrograms to a few nanograms per component. Those advances, which have allowed development of routine GC–FT-IR–MS measurement systems have been summarized and discussed in several review articles appearing in the late 1980's [3–5]. In addition, commercialization has led to low-cost dedicated instruments capable of performing routine mixture analysis where components are present at the 10–100 ng level. Detection sensitivity improvements have focused more on the FT-IR link than the MS because of the well-known lower sensitivity of IR detectors. However, more recent improvements in FT-IR detection technology have reduced detection limits to 100 pg or less for strong IR absorbers. Further developments in IR detector technology and collection optics could ultimately result in detection limits comparable to those of full-scan MS or other non-structure-specific detection methods like flame ionization detection (FID). In addition to improved instrumentation, corresponding improvements in automated GC–FT-IR and GC–MS data analysis are also occurring. This aspect is becoming increasingly important because, in combined systems, a very large quantity of data generated is generated during complex mixture analysis. Thus, automated data analysis is essential. A section of this review will deal with the current status of such software. For convenience, each of the elements of linked gas chromatography–spectroscopy systems will be discussed separately.

2. Sample introduction techniques

Sample introduction methods play a crucial role in successful implementation of linked systems such as the ones discussed in this review. The details are among the key areas which are necessary to understand if one plans to use GC-based multispectral detection systems. Of course, the ultimate goal of any sample introduction protocol is injection of a volatile or semi-volatile mixture into the GC column directly or via a heated injection port. Steps prior to this may involve extraction of analytes from matrices that are unsuitable for injection onto the column. In cases where analyte focusing on the column is important, modified injection techniques are required and are particularly important for high-speed GC analysis. In this context, cryofocussing is an especially useful approach. Primary methods for extracting volatile and semi-volatile components from matrices which cannot be directly injected onto a GC column are head space sampling, purge and trap, liquid–liquid extraction, supercritical fluid extraction and solid-phase extraction. Secondary extraction or sample introduction techniques include use of coupled separations including GC, liquid chromatography, and supercritical fluid chromatography (SFC): GC–GC, LC–GC, SFC–GC. In a very broad sense, the difference between the two types of sample introduction systems is that in the primary techniques, the number of compounds injected onto the column may be very large depending upon the sample. However, with the secondary techniques, valving systems can provide the ability to select the number of compounds transferred to the second-stage column.

2.1. Primary techniques

2.1.1. Trapping methods

Koester and Clement [6] have critically reviewed trapping methods. Here the most recent applications and advances defined by those authors as emerging methods for volatile and semi-volatile organic compounds are covered. The most popular and well documented means of enriching volatile organics is the purge-and-trap method (PT) [7–9]. In addition to PT, the spray extraction technique has been developed to circumvent foaming and bubble formation in liquid matrices that contain 1–3 $g\,l^{-1}$ of surfactants [10–13]. This system works by spraying water-containing analyte into a closed extraction chamber that has a steady flow of carrier gas. The spray produces very small droplets described as having 37 μm [12] or 100 μm [13] diameters. Extraction efficiency is dependent upon the size of the spray droplet. The spray-and-trap (ST) efficiency initially reported by Baykut was in the range of 10–15% [10,11]. However, a modification of the spray nozzle increased the efficiency to more than 50% for the set of volatile organic compounds reported in these

experiments [12,13]. In addition, the reduced droplet size considerably reduced the amount of water necessary for analyte extraction. The proposed advantage of this system compared to that of PT is its portability, which would allow rapid onsite evaluations of environmental samples. Also, the proposed method reduces flooding of the adsorbant or trap by water, thereby circumventing a trap drying step which may result in analyte loss.

2.1.2. Miscellaneous methods

One alternative to PT and ST for volatile and semi-volatile organic compounds is introduction of large sample volumes using an on-column interface, to enhance trace analysis performance for GC–FT-IR analysis [14,15]. Other alternatives include vacuum distillation [16], hollow fiber membrane extraction [17–20], membrane permeation [21], and headspace solid-phase microextraction (SPME) [22,23]. The use of membrane technology is quite old, but novel applications to solventless direct extraction of volatile organic compounds (VOC's) for GC–IR–MS analysis may be the trend of the future. For example, in several papers Mitra et al. described a useful on-line membrane extraction technique using a microtrap to interface to gas chromatography [24–27]. Pawliszyn et al. developed hollow fiber membrane technology for analysis of VOC's and polar organics [17–20]. The systems include a multiplex GC system for VOC's [19], high-pressure extraction for semi-volatiles [18], and a non-multiplex cryotrapping sorbent interface for sample enrichment [20]. The unique advantage of the sorbent interface system is that at least six different configurations can be implemented for extraction of analytes. A comparison of the different configurations was carried out using trichloroethene as the analyte at concentrations ranging from 1 $\mu g\,l^{-1}$ to 1000 $\mu g\,l^{-1}$. Only one configuration, the flow-through module, showed the presence of analyte at the lowest tested concentration. Detection limits of other configurations ranged from 10 to 50 $\mu g\,l^{-1}$. Shoemaker et al. at the US Environmental Protection Agency (EPA) have followed up by developing a method that combines flat sheet membranes, instead of hollow fibers, for the extraction of polar compounds from water [21]. In this method, a flat sheet of membrane is interposed between two grooved mirror-image Plexiglas plates. In one of the grooves, the water sample containing the analytes is passed, and in the other, the flowing carrier gas transports the organics that diffuse through the membrane to a Tenax trap. In addition to the hollow fiber method, Pawliszyn and his students studied the SPME technique [22,23]. This method uses solid or liquid coating on a silica fiber to absorb organics from different matrices via direct contact or headspace. The absorption step is followed by placement of the coated fiber into a heated injection port, which subsequently deposits the analytes onto the column for further analysis. Optimization of direct extraction [23] and a comparison of direct and headspace extraction [22] have been reported. There are a great many possible applications of this technique. The advantages of these systems are their compactness, simplicity and high sensitivity. More recently, Pawliszyn has authored a very useful book that systematizes solid-phase microextraction, with attention to both theory and practical details [28].

In addition to the methods described above for analysis of effluents in water, there is progress in on-line and direct injection of analytes in water. The advantages are reductions in enrichment times, analyte loss, and contaminants. Mol et al. have determined the applicability of using an open tubular column with thick coating (5 μm) of dimethylsiloxane as a method of enrichment [29]. However, one problem with this technique is the swelling of the stationary phase and subsequent sample breakthrough. A different approach used by Muller et al. involved testing of suitable adsorbents in a programmed-temperature vaporizer (PTV) injector for direct injection of large volumes of water [30]. It was shown that Tenax TA was the best choice among the several adsorbents studied. Mol et al. continued their investigations in large volume sample injection in a series of papers [31–34]. One of their papers is devoted to environmental applications of the method [35]. An alternative approach, that does away with the PTV injector for large volume organic solvent injection has been demonstrated by Suzuki et al. [36]. This method requires some form of prior extraction of analytes from the water matrix, followed by direct splitless injection of 100 μl sample aliquots using an unmodified injector port. Solvent diversion is based upon the difference in trapping

efficiency of the two identical phase coated capillary columns after the mixture passes through a 3 m deactivated cold trap column at 40°C. For most of the tested compounds, the reproducibility in peak area was good and the relative standard deviations (RSD's) were within a factor of 2–4 in comparison to those obtained when 1 μl injections were employed.

Solid-phase extraction (SPE) and liquid–liquid extraction are not discussed here because these methods are well described in Koester and Clement's review [6] and in two contemporary books [37,38]. These same authors have also produced several excellent reviews on environmental analysis which include applications utilizing many of the sample acquisition and preparation methods discussed above [39–41]. One recent example of on-line GC coupled with SPE, is found in the paper by Louter et al. [42]. When analytes are present at levels below high ppb or low ppm, more specific detectors, such as GC–FT-IR generally require enrichment by a factor of 100 or more for qualitative analysis. This constraint may limit the utility of some of the extraction techniques for single capillary column systems. However, coupling these methods with a secondary technique, such as multidimensional gas chromatography, can provide greatly improved qualitative and quantitative information on low concentration analytes.

2.2. Secondary techniques

2.2.1. Multidimensional gas chromatography

Of the secondary methods mentioned earlier, only GC–GC and parallel dual column GC will be discussed here. The continued interest in multidimensional gas chromatography was noted in a recent review article, where the topic was discussed at some length [43]. Other articles in this journal will cover multidimensional chromatography other than GC–GC topics. The underlying theory is also covered in other articles in this journal. The proliferation of multidimensional separation techniques is in response to the lack of chromatographic resolving power when one attempts single column analyses of complex mixtures. In any complex mixture analysis 'complete' separation and detection of components is the fundamental goal. To date, the most extensive published attempt to exhaustively separate a complex mixture, containing several thousand compounds, was reported by Venkatramani and Phillips [44]. The question to be addressed is what level of separation and detection defines 'completeness'. As detection limits continue to decrease, the numbers of compounds detected can increase dramatically. For this reason, it is essential to continue to pursue improved separations strategies.

In gas chromatography, the methods utilized for multidimensional separation can be categorized as: (a) Deans-type switching; (b) valve based switching; and (c) on-column thermal desorption ([44], and references cited therein). In Deans-type switching [45] analytes do not come into contact with any chemically active surfaces between the injection port and the detector. This technique is very well established and commercial instruments employing this approach in various forms are available. For valve-based switching, components pass at least once across surfaces other than glass. In the third technique, components eluting from an initial separation stage are trapped on-column for a short period before being thermally desorbed onto the next column [44]. Advantages of this system are the comprehensive nature of the separation and its compactness. Unfortunately, detection is restricted to a non-structure-specific detection method such as FID. Coupling this particular comprehensive separation technique to structure-specific detectors like mass or IR spectrometers has yet to be demonstrated.

It is theoretically possible to construct a comprehensive GC–GC separation instrument using a valve-based approach. Fig. 1 shows the concept of a parallel cryogenic trapping multidimensional GC system. In this figure, several heartcuts from an initial separation can be taken and trapped, and subsequently, each of the trapped sections can be independently separated on a second column of differing stationary phase selectivity. This technique can be considered comprehensive if heartcuts are taken across the entire initial separation and each cut is separated a second time. However, in most potential GC–GC applications, analyses are carried out to determine the presence or absence of targeted compounds and to quantitatively analyze those present. A valve-based parallel cryogenic trapping system currently under development for both target and

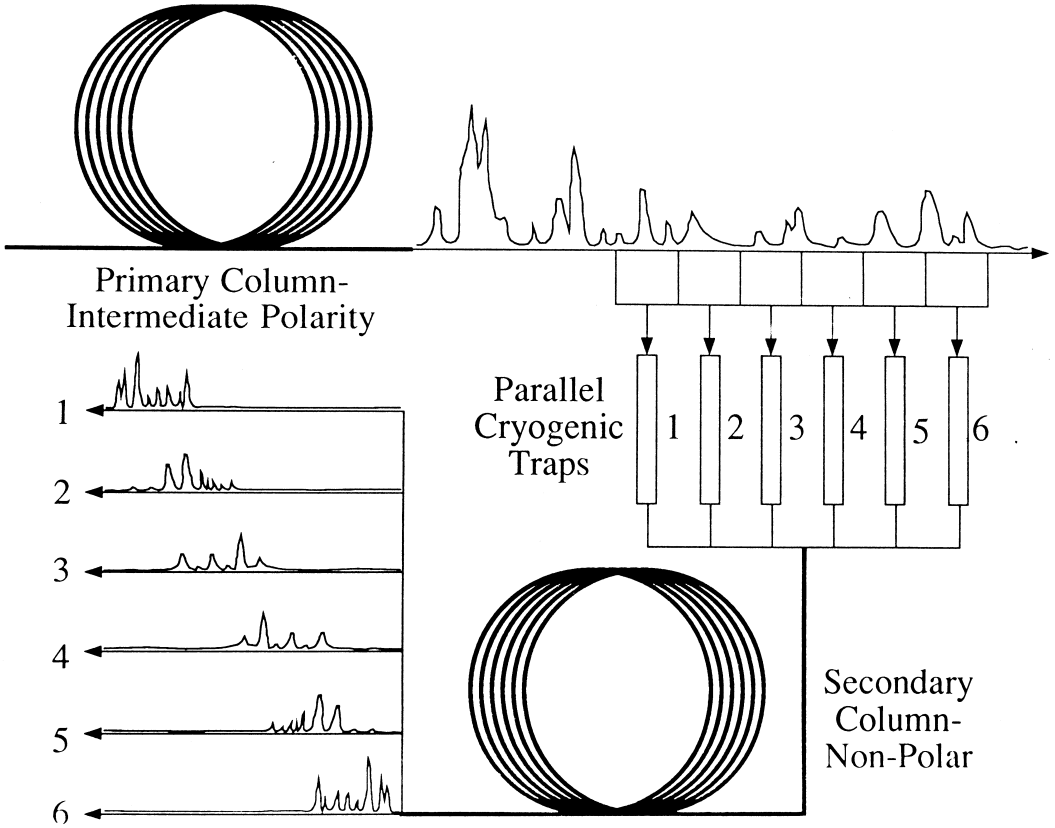

Fig. 1. Schematic diagram illustrating the concept of multidimensional GC utilizing parallel cryogenic traps. (Used with permission from Ref. [48].)

general analyses will be discussed later in this section [46–51] Other aspects of GC–GC techniques are detailed in a separate review by Phillips and Xu in this journal [52] and, therefore, will not be discussed here.

The motivation for development of the parallel cryogenic trapping technique can be understood by considering the results presented by Gordon et al. on the analysis of tobacco essential oil using a heartcutting GC–GC procedure [53]. That analysis required 23 individual heartcuts and 48 h of instrument time. Separations were carried out by injecting the sample, cold trapping the required section for a secondary-stage separation, back flushing the remaining eluent (or forward flushing), and finally performing a secondary-stage separation. The flushing and secondary-stage analysis were done simultaneously. If each primary separation sequence took 30 min, at least 6 h could have been saved by using six traps in parallel, rather than one. With a larger number of traps, the possibilities for time savings are even greater. This is not the only advantage with a parallel trapping system. Other advantages include the abilities to obtain precise adjacent cuts, simultaneous enrichment of six different sections, and the possibility of carrying out GC^n, $n=3–6$, for multiple chosen sections using a single sample injection. A possible disadvantage of such an approach is the use of mechanical valves to direct the effluent flows. This factor, in the case of evaluation of unknown mixture, could result in uncertainty about the composition because of the possibility that reactions could take place on the valve surfaces. However, several recent reports indicate that the valves usually

function as well as their valveless counterparts [46]. The qualitative aspects of such a system will be discussed later in the present review.

2.2.2. Parallel dual column gas chromatography

A recent interesting paper describes the advantageous use of the speed of time-of-flight mass spectrometry to allow parallel column gas chromatography with a single time-of-flight (TOF) detector [119]. This approach allows an increase in selectivity, without increasing analysis time, by doubling the number of peaks in the chromatographic dimension. By use of two separate columns with different stationary phases, separation information is maximized. When combined with chemometric interpretation of the resulting data, many of the same goals realized by multidimensional GC for analysis of complex mixtures may be achieved.

2.2.3. Fast gas chromatography

Another method which can be used to decrease the analysis time in complex mixture analysis is incorporation of fast primary-/secondary-stage separations. Although Desty suggested that high-speed GC separations were possible in the early 1960s [54], it was not until the advent of faster electronics and computers that this area was extensively explored. When interfacing high-speed separations to spectral detectors, many factors need to be considered. Most of these are related to instrumental dead volume and data acquisition rates. The many factors affecting fast GC performance, as well as examples of their application are discussed in recent review articles [43,55]. Speed of data acquisition is the limiting factor when interfacing fast separations to MS. Although fast GC has been interfaced with scanning mass spectrometers, such as quadrupole [56,57] and ion trap [58] instruments, their scan rates limit their ability to provide high-quality spectra for eluents from fast separations. For very high-speed separations, a high-speed detector, such as a TOF mass spectrometer, is necessary to successfully acquire mass spectral data [59]. Such fast GC–TOF-MS systems are now commercially available.

Other approaches to fast GC interface with various detectors are under development in many laboratories in different forms and applications [44,60–67]. Fast separations are typically performed with non-structure-specific detectors, such as flame ionization detectors. One area of current interest involves examining the feasibility of coupling fast separations to IR spectroscopy with an attempt to implement some of the features of parallel cryogenic trapping system. Preliminary results indicate that a matrix isolation IR spectrophotometer can be successfully utilized for detecting components separated under fast chromatographic conditions [65]. This method involves trapping effluents from the primary separation stage column by means of a single cryogenic trap maintained at 90–100 K. Subsequently, the trap is resistively heated by a current pulse to desorb the components for separation. The carrier gas utilized for these experiments was helium. Test mixtures were a Grob mix and early eluting sections of eucalyptus and cascarilla bark oils. The second-stage separation times for the Grob mix were less than 2.5 min and approximately 1 min for the hydrocarbons. In this study, the reconstructed IR chromatograms were similar to that observed for the FID chromatogram. Because both the primary and secondary columns were housed in the same oven, multidimensional analysis similar to that carried out by Venkatramani and Phillips was not possible [44]. The success of this technique primarily depends on the ability of compounds in complex mixtures, such as the essential oils, to survive the rapid thermal desorption process. In the research described here, it was demonstrated for the compounds investigated, decomposition is negligible. However, the behavior of the more polar components of these oils has not yet been investigated.

More recent reports by Borgerding and Wilkerson [68,69] describe approaches to cryofocussing for fast GC sample introduction, either trapping analytes onto bare metal tubes or using a microloop injector, similar to that used by Krock et al. for multidimensional GC applications [47–49].

Recently, Marriott and Kinghorn have reviewed the topic of cryogenic solute manipulation in gas chromatography with respect to a method they term longitudinal modulation [165]. In this method, which incorporates a cryogenic trap which can oscillate along the capillary GC column to alter the travel of a solute along the column, a liquid nitrogen trap is moved either by a pneumatic driver or a solenoid with a spring return. This can be done rapidly, with a

time of ca. 20 ms estimated to move from one position to another. These workers have published a number of recent papers describing investigations using this novel approach to cryogenic sample trapping [166–168]. Fig. 2 is a diagram of their system.

Another area of intense recent investigation is development of portable high-speed gas chromatography instruments. One way in which such devices have been used is for rapid on-site analysis [70,71]. An important component of this recent development is instrument miniaturization. Small gas chromatographs have been produced using micromachining and integrated circuit processing techniques with Si [72–74]. More recently, Sheya et al. reported the novel miniaturization of a tandem GC–GC instrument [75]. There is no doubt that these trends will continue as the need for field portable instruments expands, particularly in the environmental analysis area.

2.3. Summary

It is clear that in order to obtain the maximum amount of information from a separation coupled to multispectral detectors, the material provided to the detector needs to be both pure and present in sufficient quantity. The methods discussed here demonstrate the advantages of several types of injection, collection, preconcentration, and separation methods. As analyses require lower detection limits and higher qualitative accuracy, the future widespread application of these techniques to multispectral detection systems appears imminent.

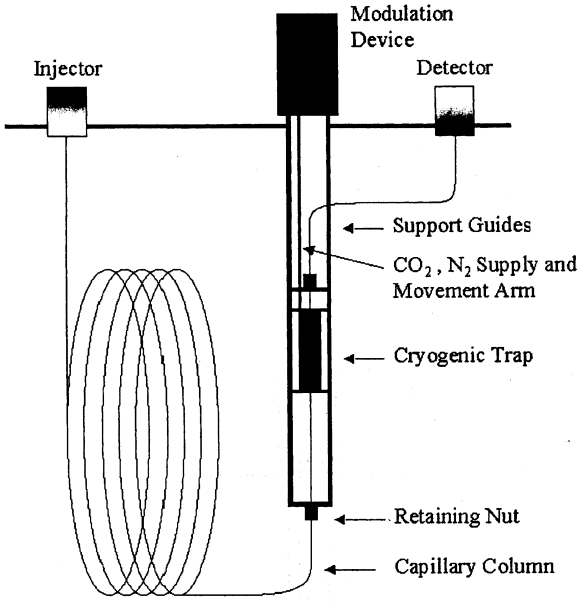

Fig. 2. Diagram of a longitudinally modulated cryogenic system located in a gas chromatographic oven. (Used with permission from Ref. [165].)

3. Gas chromatography–mass spectrometry

Gas chromatography coupled with mass spectrometric detection (GC–MS) is one of the most widely utilized analytical techniques. The explosion of applications stems from the excellent qualitative information obtained the high sensitivity inherent with mass spectrometric detection. So great has been this impact on the practice of GC that a recent comprehensive review of the field stated: ''Thus, for the first time mass spectrometers are reviewed in this section along with other standard, routine gas chromatographic detectors'' [43]. The great majority of GC–MS applications utilize capillary GC with quadrupole MS detection and electron ionization (EI). Nevertheless, there are substantial numbers of applications utilizing different types of mass spectrometers and ionization techniques coupled with multidimensional high-speed and pyrolysis–gas chromatographic methods. The following section of this review will consider the present power of this technique by discussing some selected examples of recent research that has utilized or aided in the development of the unique abilities of GC–MS.

3.1. GC–quadrupole mass spectrometry with electron ionization

As previously mentioned, the most common configuration for a GC–MS experiment is a single capillary GC column directly coupled to an EI quadrupole mass spectrometer. The proliferation of this type of system results from its relatively low cost, low maintenance, high sensitivity, high information content, and the ready availability of reliable commercial instruments. A brief survey of

the literature over the last five years shows that papers describing GC–MS applications number on the order of several thousands. Ongoing trends apparent from reading the literature are a constant and impressive improvement in sensitivity and detection limits. Furthermore, the breadth of applications is quite diverse and includes medicine, environmental chemistry, flavor and fragrance chemistry, as well as many others. A recent review article [76] considers many of these applications and highlights a trend toward very fast separations, which is discussed below.

A large number of reports have resulted from research being done in environmental chemistry. There is no doubt that this entire article could be devoted to discussion of examples of such applications. However, only a selected representative few will be mentioned here. Not unexpectedly, a major part of these applications involve speciation of the materials analyzed. The reader is referred to the most recent comprehensive review of environmental analyis for an in depth coverage of the air, water, and soil applications [41]. Because of its impressive sensitivity, GC–MS allows chemists to detect extremely small quantities of environmental contaminants in water, soil and air. One of the main areas of interest to environmental chemists is the identification and quantitation of organic substances, such as chlorinated compounds, polycyclic aromatic hydrocarbons, and pesticides, in water and air [66,77–81] Volatile chlorinated organic compounds such as trihalomethanes (THM's) in chlorinated waters have been the focus of much of the research on chlorination products in water in recent years. However, there is also increased awareness that halogenated acetic acids (HAA's) in natural waters, especially chloroacetic acids (CAA's), make up a large fraction of the non-volatile chlorinated compounds in water (Tables 1–3).

Recently, Ozawa reported the analysis of directly derivatized HAA's by GC–MS with detection limits in the low $\mu g\,l^{-1}$ level [81]. In this report, several chloro- and bromoacetic acids were analyzed following a difluoroanilide derivatization to improve sensitivity, and the recoveries for 10 $\mu g\,l^{-1}$ of analyte in natural waters (lake and sea waters) were greater than 85% for all but one of the analytes. These investigators also used the well known method of single or selected ion monitoring (SIM) mode, to improve sensitivity. This mode improves sensitivity by limiting the mass of the ions detected to one or more specific fragment ions of known mass. As a consequence, it is highly selective and it eliminates a large portion of the noise inherent in full-scan

Table 1
Summary of GC–MS applications and detection limits

Mass spectrometer	Ionization technique	Sample(s)	Detection limit	Reference
Quadrupole	Electron ionization	Monochloro-acetic acid derivative	0.5 $mg\,l^{-1}$	[81]
		Polynuclear aromatic hydrocarbons	0.2–1 $ng\,l^{-1}$	[78]
		Organophosphorous and triazine pesticides	0.04–0.13 $mg\,l^{-1}$	[79]
		Atrizine	50 $ng\,l^{-1}$	[79]
		Dibromomethane	1.0 ppt	[77]
		Dimethyl sulfide	2.4 ppt	[77]
		11-Nor-9-carboxy-Δ^9-tetrahydro cannabinol	0.5 $mmg\,l^{-1}$	[85]
Quadrupole ion trap	Chemical ionization	Toluenediamine	0.05 $mg\,l^{-1}$	[93]
	Electron ionization	11-Nor-9-carboxy-Δ^9-tetrahydro cannabinol	0.3 $mg\,l^{-1}$	[85]
		cocaine	50 $ng\,g^{-1}$	[116]
Tandem ion trap	Electron ionization and collision induced dissociation	Morphine	1–20 ppb	[124]
		Cocaine	5–30 ppb	[124]
Double focusing	Electron ionization	Atrizine	0.2 $ng\,l^{-1}$	[80]

Table 2
List of compounds identified in Fig. 12 by IR and MS; the IR and MS libraries contained approximately 3000 and 42 000 compounds, respectively (adapted with permission from Ref. [51])

Peak no.	Peak compound identification
1	*cis*-1,3-Dimethylcyclopentane
2	2, 2-Dimethylhexane
3	*trans*-1,3-Dimethylcyclopentane
4	1,2-Dimethylcyclopentane
5	1,2-Dimethylcyclopentane
6	Cyclohexene
7	Straight chain hydrocarbon, alkane
8	Cyclic hydrocarbon
9	Alkene
10	Heptane
11	Straight chain hydrocarbon, alkane
12	Alkene
13	Benzene
14	Straight chain hydrocarbon, alkane
15	Heptane isomer
16	Hexene, heptene isomers

Table 3
Component identifications for Fig. 13 supported by both IR and mass spectra (adapted with permission from Ref. [47])

Peak no.	Peak identification
1	α-Pinene
2	1,8-Cineole
3	Terpinolene
4	*p*-Cymenene
5	Linalool
6	Fenchyl alcohol
7	*trans*-Pinocarveol
8	4-Terpineol
9	α-Terpineol
10	Geraniol
11	α-Terpinenyl acetate
12	Aromadendrene
13	Camphor
14	α-Thujene
15	Camphene
16	Sabinene
17	β-Pinene
18	β-Myrcene
19	Decane
20	α-Phellandrene
21	3-Carene
22	α-Terpinene
23	Limonene
24	γ-Terpinene
25	Carvomenthene
26	β-Phellandrene
27	*p*-Cymene

detection mode. For example, by monitoring the molecular ion peak with m/z 205 for the monochloroacetic acid (MCAA) derivative, a detection limit of 0.5 $\mu g\,l^{-1}$ could be achieved.

As previously mentioned, environmental chemistry is not the exclusive application of GC–MS. Another of the areas in which GC–MS applications have dramatically increased is clinical or medicinal chemistry. In the past few years, several drug analysis methods by quadrupole GC–MS have been developed [82–85]. One recent example is an intermethod comparison between quadrupole and ion trap GC–MS systems for detection of the marijuana metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (TA) in urine [85]. In this comparison, much like the environmental applications, both detectors were run in SIM mode, detecting ions with mass-to-charge ratios between m/z 305 and 385 in order to improve the sensitivity of the method. Detection limits and reproducibility data for the detection of TA were very similar for the two detectors. For the quadrupole detection limits were 0.5 $\mu g\,l^{-1}$ (ppm), while they were slightly lower, 0.3 $\mu g\,l^{-1}$, for the ion trap. Although the main source of the sensitivity of these methods is the SIM mode, analyzing samples in the SIM mode obviously precludes the collection of full mass spectra. Thus, by opting for maximum sensitivity, the analyst must forego one of the main advantages of having a spectrometric

detector—its ability to perform qualitative as well as quantitative analyses.

Gleispach et al. reported a study which is a good example of GC–MS usage for qualitative analyses. They utilized a full-scanning quadrupole GC–MS system to qualitatively identify steroids and organic acids in urine, and following the identification process, operated the system in SIM mode for quantitative analyses [86]. The impressive qualitative power of GC–MS over non-spectrometric detection is clearly demonstrated in one of the examples in the study. In one of the urinary steroid chromatograms from a patient, a very large peak was identified by its mass spectrum to be dehydroepiandrosterone (DHEA), a metabolite indicative of an adrenal carcinoma. Surgical intervention showed the presence of the tumor which was be removed. Had it not been for the critical identification of the metabolite by GC–MS, the surgery might not have been recommended. Other quantitative analyses utilizing the SIM mode were performed to monitor the urinary level of *N*-acetyl-L-aspartic acid, which is used for the diagnosis of Canavan's disease. *N*-Acetylaspartic acid was found to be present in both healthy and diseased human urine, but the diagnosis of Canavan's disease was confirmed by the fifteen-fold elevated excretion of the acid in the diseased patient.

In some qualitative identification cases with extremely complex mixtures, the resolution limits of a standard single column GC–MS analysis are exceeded, and in these cases, the most common option is to utilize multidimensional GC (MDGC) or GC–GC coupled to the MS detector. The reason for utilizing a more powerful separation technique is the need to acquire mass spectra of pure components rather than spectra of mixtures of two or more components. Highly accurate qualitative identification assignments can only be made from spectra of pure components, and spectral subtraction or chemometric methods do not always provide the correct spectrum for the unknown component of interest. Therefore, the easiest method to improve qualitative accuracy is to improve the separation and acquire pure spectra in the first place. Representative of the types of samples that require MDGC are essential oil or environmental samples.

One of the most thoroughly investigated applications of MDGC–MS or GC–GC–MS is analysis of essential oils. Identification and quantitation of enantiomers in these types of oils can provide information as to the chemotaxonomy of the oil [87], as well as the effects the enantiomeric ratios have on smell and taste properties [88]. Hennig et al. described an investigation of the composition of *Pinus peuce* needle oil by GC– and MDGC–MS [87]. Because of the biological activity of monoterpenes in the interrelation between forestry insects and the host trees, a method was required to identify the reasons behind the diminishing populations of the *Pinus peuce* in Germany. Direct enantiomeric separation of the monoterpenes was achieved by using modified cyclodextrins as the GC stationary phase. Utilizing the retention times and mass spectra, chromatographic peaks could be identified, and the quantitative abilities of the MS were used to develop the enantiomeric ratios from the β-cyclodextrin analytical column. The ratios of α-pinene, camphene, β-pinene, limonene and β-phellandrene were determined for the two oil samples that were studied. Unfortunately, a larger population of samples must be analyzed before conclusions about the ratios can be drawn, but the method does demonstrate the ability and ease of adapting MDGC techniques to mass spectrometric detection.

As for the environmental applications of MDGC–MS, typical recent applications are the identification of fatty acid methyl ester as minor components in fish oil [89] and the separation and determination of coplanar polychlorinated biphenyl (PCB) congeners [90]. The latter is an area of intense research because of the toxicity of certain PCB congeners. The difficulty in this type of analysis is the complete chromatographic separation and identification of the highly toxic PCB congeners from the less toxic congeners [90]. MDGC–MS provides the separation power and the sensitivity to detect and quantify the small amounts of the congeners of interest. However, unlike the previous examples, ionization by EI in the SIM mode did not provide the sensitivity required for the analysis [91]. As a consequence, the authors utilized negative chemical ionization (NCI) in conjunction with SIM mode detection to achieve detection limits of the order of 10 fg to 1 pg of material, or at the low ppt level, depending on the degree of chlorination. It was also determined that the sensitivity of the system with NCI increases with

increasing number of chlorine substituents, and the range of linearity is 3–4 orders of magnitude [92]. As this example points out, in some cases simple EI does not provide adequate sensitivity, and this conveniently introduces another method of ionization which can improve not only sensitivity but selectivity: chemical ionization.

3.2. GC–quadrupole mass spectrometry with chemical ionization

For some types of analyses, it is desirable to use a 'softer' form of ionization than EI, which typically causes extensive fragmentation. In these cases, chemical ionization (CI) may provide an adequately soft ionization technique. CI usually produces molecular ions (M^+ or M^-), adduct ions (M+CI reagent)$^{+/-}$, and fragment ions, but the degree of fragmentation can be controlled somewhat by the nature of the reagent gas. CI reagents vary from application to application, but the most popular are methane and ammonia. In most cases, CI is chosen to provide better selectivity and sensitivity than EI experiments. However, due to the somewhat specialized requirements of CI-capable instruments, the instrumentation required to perform GC–CI/EI-MS experiments is more expensive than an exclusively EI-based instrument, and, subsequently, the number of instruments and applications are relatively low compared to EI.

There have not been as many publications describing use of CI, compared with the thousands for EI reports, however, there is still extensive use of the chemical ionization method. Many papers focus on the use of CI for the differentiation of isomers by GC–CI-MS in environmental, biological and petroleum analyses. Typically, isomer differentiation by EI is difficult because often the mass spectra of isomers are quite similar (or identical) and differ only in subtle ways. However, with CI, the reactions of analyte with reagent gas may produce dramatically different spectra for the isomers due to differing reactivities of the isomers towards the reactant gas. A good example of this was demonstrated by Slater and Manville who differentiated thiocyanates and isothiocyanates by GC–CI-MS using ammonia as the reagent gas [92]. They found that EI mass spectra of allyl and alkyl thiocyanates and isothyocyanates do not permit unambiguous identification, and CI mass spectra with methane and butane also precluded identification because both isomers yielded the same spectrum. However, the use of ammonia as the reagent gas produced two very different spectra for the allyl thiocyanate and isothiocyanate, and the differences were enough to allow accurate differentiation of the isomers.

Another recent application of CI-MS utilizing the SIM mode is determination of toluenediamine (TDA) isomers in human urine and blood [93]. This method also used ammonia as the chemical ionization reagent gas, but in contrast to the previous example, ions were analyzed in the negative ion mode. Differentiation of the isomers was achieved by varying the ammonia pressure, temperature and electron energy and monitoring ions with m/z 394 and 374, corresponding to the $(M\text{-}20)^-$ and $(M\text{-}40)^-$ ions of the toluenediamine pentafluoropropionic anhydride derivatives. This method was reported to have a detection limit of 1–5 fg of injected material for each of the five TDA isomers, corresponding to less than 0.05 $\mu g\,l^{-1}$ of TDA in human urine or plasma. It was also reported that these limits could be improved by a factor of 40 if an enrichment step was included. However, even before an enrichment step, the detection limit of the method is better than most of the previous EI examples, clearly showing the possible improvements that CI can provide over electron ionization techniques.

In terms of isomer complexity and content, petroleum products are probably the most complex mixtures. Total qualitative analyses of petroleum products is virtually precluded, even with standard GC–EI-MS techniques. However, a recent report using nitric oxide chemical ionization to analyze reformulated gasolines and their blending components has demonstrated a new approach to improve the qualitative understanding of these mixtures [94]. Utilization of nitric oxide CI provides simplified and more selective mass spectra compared to EI, and the simplified spectra can offer more information about the identity, hydrocarbon type by boiling region or carbon number, and normal/iso paraffin splits in petroleum products. This report is a good example of the use of a specific reagent gas to provide a specific type of ionization which can then be used for more general qualitative information.

3.3. GC–ion trap mass spectrometry

In analyses requiring high sensitivity full-scan mode operation, quadrupole GC–MS in full-scan mode is insufficiently sensitive to perform at levels lower than tens of nanograms. Further improvements in the sensitivity must be done by improving the mass spectrometric hardware rather than by adjusting the quadrupole parameters. One type of mass spectrometry which is well suited for high-sensitivity chromatographic applications is quadrupole ion trap detection (ITD). Recent advances in ITD technology for GC–MS, such as improved sensitivity, trap designs, and understanding of the trapping parameters [95], have opened up several applications in many areas of analytical chemistry. Applications utilizing these enhancements range from laser pyrolysis–GC–MS [96,97] to environmental analyses applications. Examples are analysis of hexenes emitted to air from gasoline [98], determination of herbicides and their metabolites [99], and fragrance analyses, such as the identification and quantification of the chemicals responsible for the malodor of beet sugar [9].

The medical and biochemical fields provide ample opportunities for utilizing GC–ITD-MS. In many potential applications, high sensitivity is a main requirement. Recently, the need for additional qualitative information has necessitated the move to a highly sensitive full-scan detector like the ITD. One good example of this was recently demonstrated by Hernandez et al. when they analyzed autopsied human brain tissue for cocaine and its metabolites by GC with full-scan ITD [100]. Traditional drug analysis is performed on blood or urine. However, in cases where a possible drug-related death has occurred, it has been reported that the concentration of cocaine in blood changes significantly between death and autopsy [101]. Confirmation of a drug-related death can only be done with qualitative data such as mass spectra, so the ability to obtain full spectra of possibly small quantities of cocaine and its metabolites is imperative. With the method developed for the analysis of brain tissue, detection limits on the order of 50 ng g^{-1} or 50 ppt in the full-scan mode were determined. This detection limit is similar to the limit obtained for toxaphene utilizing electron-capture negative ion (ECNI) mode quadrupole detection [102]. However, ITD allows the collection of the entire mass spectrum at this level, and ECNI-MS is a SIM mode detection technique which limits its qualitative use. Another use of the highly sensitive qualitative power of ITD was reported by Ghoos et al., who analyzed volatile organic compounds produced by fermentation products released by bacterial degradation in the human colon [102]. Utilizing an off-line, closed-loop trapping system as a means of preconcentration, the reported method was used to identify many of the volatile organic components in human urine and feces with the hope that a greater understanding of the metabolite formation due to anaerobic metabolism could be gained.

3.4. GC–ion trap tandem MS

One additional ITD method, not previously mentioned, but which has very powerful qualitative potential, is the sequential mass measurement technique (MS–MS). This technique conveniently introduces the topic of tandem mass spectrometry. In this ITD technique, an ion of interest (parent ion) can be selected by ejecting all unwanted ions, and the parent ion is then typically fragmented by means of collision with a neutral gas. However, there has been a recent report of fragmentation induced by application of random noise on the end-cap electrode to effect collisional fragmentation [103]. Other types of secondary fragmentation can be induced by laser photodissociation [104] or charge exchange and CI [105]. The resulting mass spectrum is called a product ion spectrum, and it is characteristic of the secondary fragmentation process. MS–MS techniques provide an additional dimension of spectroscopic information, and this subsequently provides a higher degree of qualitative accuracy. Recent examples of the use of tandem ion trap applications include a paper reporting analysis of polychlorobiphenyls [106,107] and several analyzing dibenzodioxins and dibenzofurans [106–109]. Computer simulations showed that non-resonant excitation can provide an extremely rapid way to increase ion kinetic energy for MS–MS purposes [110].

A report by Traldi et al. demonstrated the ability of GC–ITD to perform MS–MS for the investigation of morphine and cocaine in the hair of drug addicts

[111]. In this report, pure morphine and cocaine were subjected to collisionally activated or induced dissociation (CAD or CID, respectively) with the carrier gas, typically helium, in the ion trap, and, as expected, the resulting product ion mass spectra were found to be characteristically matched to the differing CID fragmentation pathways of the compounds. Detection limits for the method were determined by spiking blank hair extracts with morphine and cocaine. In this way, it was determined that detection limits were 1–20 ppb for morphine and 5–30 ppb for cocaine. Subsequently, hair sample extracts from addicts and non-addicts were analyzed, and it was determined that the ITD-MS–MS technique could be applied to drug abuse investigations. However, it was also found that although morphine was readily detectable in heroin abuse cases, cocaine metabolites were not as easily detected, and the best method for cocaine abuse confirmation was the identification of cocaine itself in the hair samples.

3.5. GC–tandem mass spectrometry

The ability to perform tandem mass spectrometry is not restricted to ion trap mass spectrometers. Probably the majority of tandem mass spectrometry is carried out with triple quadrupole, Fourier Transform, or sector mass spectrometers. Triple quadrupole or sector instruments can be operated in several modes to obtain information on the product ions, parent ions, neutral losses and reaction ions. The most widely used mode is the product ion scan mode, and, for example, in a triple quadrupole instrument used for product ion MS–MS, the first quadrupole acts as a mass selective filter for one or more ions of interest (the parent ions). The second quadrupole is used as the collision cell where the ions selected from the first quadrupole are fragmented, using a collision gas such as helium or argon. Finally, the last quadrupole is operated in the full-scan mode to obtain the complete mass spectrum of the product ions. Applications of tandem mass spectrometers are typically those which require isomer analysis or high accuracy qualitative confirmation. The use of tandem mass spectrometric techniques for the clinical and forensic diagnosis of illicit drug use by ITD has already been mentioned [111], but there are recent reports utilizing triple quadrupole mass spectrometers to accomplish the same task [112] as well as other biologically related analytical challenges [113–115]. Other recent applications of tandem mass spectrometry have appeared in the field of environmental analyses ranging from the trace analysis of toxic organophosphorous compounds in the presence of interfering hydrocarbons [116] to the identification and verification of chemical warfare agents [117,118].

The recent report by Black et al. examines the applicability of GC–MS in the SIM mode and GC–tandem MS to the analysis and identification of Iran–Iraq chemical warfare (CW) samples which were expected to contained sarin, sulfur mustard and their degradation products [118]. Samples ranged from bomb crater soil to burial clothing from two deceased Kurdish villagers. GC–MS in SIM mode was used for screening purposes, and GC–tandem MS was used for the confirmation of the nerve agent sarin. While the grave samples were determined to be free from nerve agents, sulfur mustard or their hydrolysis products, several of the soil samples contained sulfur mustard and/or its hydrolysis products. The extract from one of the metal shrapnel fragments taken from a bomb crater was suspected of containing sarin due to a relatively weak response at the retention time of sarin, and re-analysis by GC–tandem MS matched the responses corresponding to the standard. The total amount of sarin extracted from the fragment was estimated to be 170 ng which corresponds to low ppt level detection and identification. The presence of sarin on the metal fragment was reported to be somewhat of a surprise due to the sample's exposure to the environment for four years, but the reason for the presence of sarin could only be traced to the green paint that was unique to the metal sample. This suggests that paint analysis may be a useful tool in future analyses of suspected or alleged CW use. Ultimately, the use of GC–MS and tandem MS provided a confirmation of the CW use in Iraq, and it provided some insight which may be useful for future analyses.

3.6. Fast GC–time-of-flight mass spectrometry

Typical analysis times for a standard GC–MS experiment can easily range from 0.5 h to well over 1 h. In an attempt to reduce the analysis time while

maintaining the superior qualitative information of a mass spectrometric measurement, chemists have examined the applicability of faster GC separations in combination with fast MS detection. A relatively recent review of mass spectrometry observed that, ''A major trend in GC/MS development has been toward very fast separations, . . . '' [76]. For example, recent experimental developments in high-speed gas chromatography with FID have led to observations of peak widths (FWHH's) ranging from 20 to 200 ms [44,60,61,67,120,121]. In all these cases, the GC analysis involved either isothermal or temperature programmed GC. A novel method proposed for rapid separation that is different from the above is thermal gradient programmed GC [122]. In this technique, elution is facilitated in three dimensions, (i.e. a function of time, distance and temperature) rather than in one (isothermal) or two (programmed temperature) dimension. In any event, reconstructing peaks with widths in this range with spectrometers requires a fast detection system capable of producing at least 10 measurement points per peak. Mass spectrometry has the potential to accurately measure peak widths in this time range. However, this technique requires matching several technologies together. The first consideration is choice of a sample introduction method capable of producing a narrow injection bandwidth. Second is the choice of an appropriate column size to generate the necessary theoretical plates, and finally, a fast detector is required to match decreased peak widths. The first aspect has been partially addressed in the previous section. A review by Peters et. al. has dealt in depth with the requirements for sample introduction for high-speed separations [120]. Column choice is beyond the scope of this review. This section will deal with the final aspect of instrumental advances in mass spectrometric detection for fast gas chromatography.

Mass spectrometry in the TOF configuration with a high-speed recorder and an optimized ion extraction technique provides the fastest way to acquire a mass spectrum [123,124]. The time to acquire a mass spectrum is limited by the flight time for the highest mass under analysis. For GC–TOF-MS purposes, a full spectrum can be collected in under 100 μs. However, a single spectrum will not the possess the required signal-to-noise ratio (S/N). In order to improve the S/N, several spectra must be averaged. A research group at Michigan State University has developed a time array detection scheme with an integrating transient recorder for averaging spectral acquisition on a rapid time scale [125–127]. The maximum number of transients that can be collected in one second with this system is reported to be 5000 using a 200 MHz, 8-bit A/D converter. For an accurate and clean spectrum, at least 100 transients have to be averaged. This summing results in 50 data points/s. Thus, GC peaks having a 200 ms width at the base can be represented by 10 full mass spectra. In two preliminary analyses of this system, the maximum rate demonstrated was 20 scans/s and the test mixtures were a Grob mix [126] and charcoal lighter fluid [125]. However, it was noted that 50 scans/s is possible by using a single component as an example. The demonstration of the effect of various scan rates on chromatographic resolution was carried out using a nine component test mixture [66]. In this investigation, the effect of rates from 1 to 50 scans/s are shown. As expected, increases in scan rate resulted in better resolution. It was reported at the 1994 American Society for Mass Spectrometry (ASMS) conference in Chicago, that data could be collected using a 14-bit A/D with a maximum scan rate of 1000 spectra/s [128]. With this instrument in conjunction with an on-column cryofocussing device, a GC–MS peak width of ~120 ms was observed for the earlier eluting components of the test mixture employed. A scan rate of 100 scans/s, with each scan an average of 50 transients, was utilized for chromatographic reconstruction. In addition to this latest report, Wollnik et al. demonstrated the feasibility of a high-speed chromatographic system with TOF as the detector [59]. The data recording system was based on a commercially available transient recorder connected to a personal computer. The recorder can add the 8-bit data collected every 5 ns to a summation memory. The slowest step, according to the authors, is the time taken to transfer averaged spectra from this recorder to the host computer. The current dead time for this process is ~10 ms; during this time no data can be collected. This dead time results in a maximum scan rate of 100 scans/s. They have presented data up to a scan rate of 65 scans/s with a peak width capability of 100 ms.

3.7. Fast GC–miscellaneous fast mass spectrometries

A time-of-flight mass spectrometer is not the only instrument capable of high-speed mass detection. Two high-speed GC–MS systems, one with multichannel array detection and another with ITD, were reported [58,129,130]. In one of the reports, the fastest integration used for a spectral point was 100 ms with a range of 50–500 u [129]. The novelty of this instrument is in its portability and miniaturization for on-site analysis. The other report concentrated on the spectrometer sensitivity with single ion monitoring [130]. The reported peak widths under these conditions were between 50 and 100 ms. Finally, a commercial ion trap system was investigated to evaluate its performance for high-speed GC–MS analyses [58]. From existing literature, it seems that the method of choice for high-speed GC–MS analyses would be restricted to the development of TOF-MS instrumentation because of its inherent high spectral generation speed. However, there does appear to be a possibility for other types of mass spectrometers to become fast GC detectors. If these developments in mass spectrometry are complemented by developments in either off-line or high-speed on-line IR measurements, a very fast and useful linked system could be implemented.

3.8. Summary

Recent developments and applications of coupled GC–MS have demonstrated the trend towards lower detection limits and increasingly accurate identification abilities. Hardware developments have provided increased simplicity and decreased costs, and because of these improvements, there have been a large number of reports covering a very broad range of applications. Additionally, applications of fast separation techniques with recent advances in mass spectrometry have provided the ability to perform GC–MS experiments in a fraction of the time it has traditionally taken. However, in some cases, the qualitative abilities of GC–MS, regardless of the experimental equipment, may be compromised. When this type of application is encountered, it may be useful to examine the complementary information obtained from IR spectroscopy.

4. Gas chromatography–Fourier transform IR spectroscopy

In the early years of GC–FT-IR instrumentation, the analytical community was reluctant to accept this technique as a common method for routine and/or target analysis of mixtures. The main reasons were that GC–MS was a well established technique with substantially better detection limits and the availability of large databases of mass spectra for identification of components via spectral searching. It was not until the demand for identifying increasingly smaller amounts of material in more and more complex matrices did the analytical community look to other methods to overcome some of the limitations of mass spectral detection. Fig. 3 demonstrates one of the main limitations of mass spectrometry: the inability to distinguish closely related isomers because they have very similar mass spectra. For example, many drugs have very similar or identical EI mass spectra, making identification difficult at best. One alternative is to use CI mass spectrometry, as previously described. Because CI is a softer ionization method, higher mass ions are produced, providing more information about the intact molecule. However, as Fig. 4 shows, FT-IR spectroscopy provides information on the intact molecular structure rather than the fragments, resulting in a unique spectrum for each molecule. Because IR spectroscopy gives information on the intact molecule, similar structures such as isomers can be distinguished. The fingerprint region, 1600–900 cm^{-1}, is where most organic molecules exhibit the majority of their individual characteristics. These properties make FT-IR an alternative and complementary method to mass spectrometry for GC detection.

Two major developments in FT-IR spectroscopy in the 1970s led to the current popularity of GC–FT-IR instrumentation [131]. The first of these was the introduction of the narrow-range mercury cadmium telluride (MCT) photodetector. The specific detectivity (D^*) of this detector exceeded that of the triglycine sulfate (TGS) detector, which was the standard detector supplied with most FT-IR spectrometers, by at least an order of magnitude, thus making the detection of the small sample quantities eluting from a GC column more feasible. The second breakthrough was the construction of gold-coated

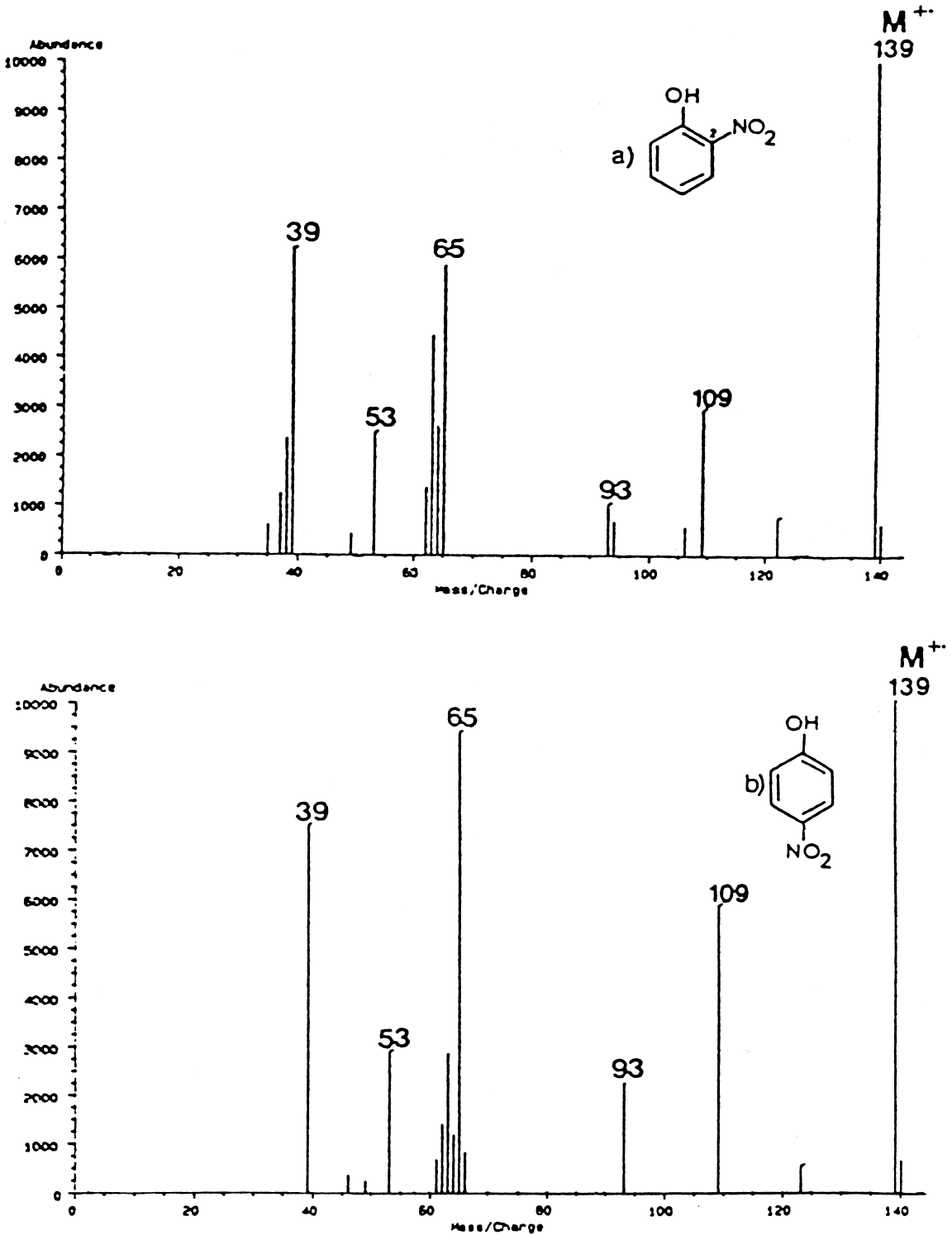

Fig. 3. Mass spectra of: (top) 2-nitrophenol; (bottom) 4-nitrophenol. (Used with permission from Ref. [141].)

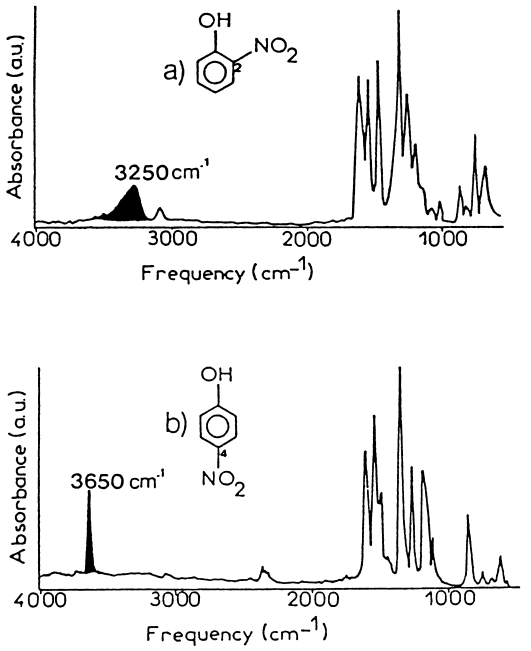

Fig. 4. IR spectra of: (a) 2-nitrophenol; (b) 4-nitrophenol. In the former, note the broad and not very intense ν(OH) band at 3250 cm^{-1} compared to the sharp and intense ν(OH) band at 3650 cm^{-1} of the 4-nitrophenol. (Used with permission from Ref. [141].)

borosilicate glass, or 'lightpipes' by Azarraga [132]. Since the introduction of the lightpipe instrument, two other methods of IR detection of GC effluents have been developed, leading to three basic types of GC–FT-IR instruments: (a) lightpipe, (b) matrix isolation, and (c) subambient trapping. Only brief descriptions of each type will be given here. For further details, the reader should refer to the appropriate sources [133].

4.1. GC–FT-IR instrumentation

The most common and simple GC–FT-IR instrument is the flow through cell or lightpipe instrument [132,133]. As mentioned above, a lightpipe is narrow bore (about 100–200 μm I.D.) borosilicate capillary with a smooth, thin layer of gold coated on the inside surface. By reflecting the IR beam through the lightpipe via the gold coating, the path length of the cell is increased by a factor of ten or more with respect to the actual length of the glass capillary. As Beer's law indicates, absorbance is a function of path length, so the detection limits of the instrument are also increased accordingly. The simplicity of the instrumental design requires little operator interaction, allowing the system to be automated for continual analyses with minimal supervision. The main limitations of the lightpipe interface are the degradation of the chromatography due to the lightpipe cell volume, the throughput degradation at higher temperatures, and the relatively high detection limits, generally in the low nanogram range. Cryogenic trapping methods were developed to help overcome these problems.

Matrix isolation (MI) FT-IR was first introduced in the mid-1980s [134]. This technique involves mixing a gaseous sample with an inert gas, usually argon, and cryogenically freezing it onto a rotating gold disk maintained at liquid He temperatures to form a solid matrix trace approximately 300 μm wide. Reflection–absorption spectra are obtained from the deposited effluent after the GC separation is completed. Matrix isolation and cryogenic trapping of the molecules reduce the rotational broadening as well broadening due to intermolecular interactions. The resulting spectra therefore exhibit very sharp and narrow lines (usually <0.5 cm^{-1} FWHH for small molecules). These narrow spectral features are responsible for MI–IR spectroscopy's most significant feature: distinguishing positional isomers. Concentration of the sample to a small spot size increases the sensitivity of the instrument. Also, since the GC effluent is cryogenically frozen onto a cryodisk, postrun signal averaging may be utilized to further increase the quality of the spectra and detection limits. The detection limits of a matrix isolation instrument are in the tens of picogram range, which exceeds the limits of a lightpipe instrument by at least two orders of magnitude.

Subambient trapping, sometimes referred to as direct deposition (DD), is similar to the matrix isolation method in that the GC effluent is cryogenically frozen onto a surface [135–138]. The differences are that in the subambient trapping method, the effluent is frozen onto a moving IR transparent window, usually constructed out of zinc selenide (ZnSe). Because there is no matrix gas present, the molecules are not matrix isolated. IR spectra are collected shortly after the effluent is

deposited so that the spectra are collected in real-time. As with MI–FT-IR, because the effluent is cryogenically frozen to a surface, post-run signal averaging can be utilized. Detection limits of the subambient trapping method are comparable to those obtained with the matrix isolation method. As Fig. 5 demonstrates, one advantage of subambient trapping is that the IR spectra obtained can be searched against standard KBr spectra. Because KBr reference spectra libraries are larger than either the matrix isolation or vapor-phase libraries, the probability of finding a match for a spectrum of an unknown compound is increased.

4.2. Applications of GC–FT-IR spectroscopy

Many different types of analyses utilizing GC–FT-IR can be performed, ranging from biological to environmental to forensic applications. Although the most common use of GC–FT-IR is for the qualitative separation and identification of components in a mixture, quantitative results can be obtained. It should be noted, however, that quantitation by GC–FT-IR is complicated by many uncertainties associated with both the chromatography and spectroscopy [139]. The main problem with spectroscopy is the inability to accurately determine how much material is in the lightpipe during the measurement. A percentage can be estimated by using the lightpipe volume, carrier gas flow-rate, GC peak elution time, and spectrometer scan rate. The estimate of the percent of material in the lightpipe is not required to be too precise to be within the total error. Errors due to sample preparation and GC errors are both larger than those associated with the spectrometer. The main errors associated with the chromatography include injector discrimination, irreversible column adsorption, and incomplete solute focusing on the head of the column. The FT-IR method utilized for an analysis depends on the information desired.

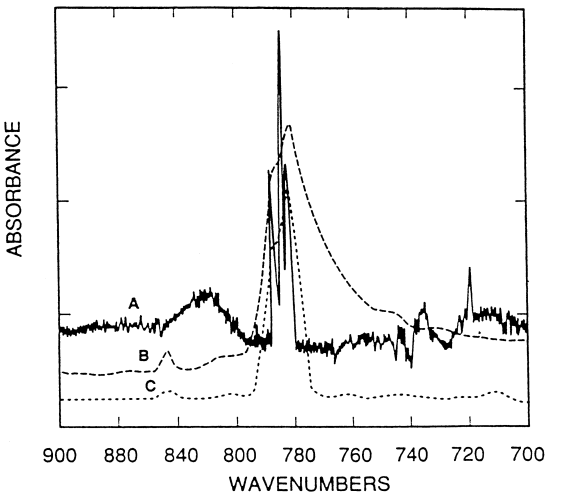

Fig. 5. Comparison of IR spectra of naphthalene obtained (A) under matrix isolation conditions in nitrogen at 12 K, (B) in KBr at room temperature, and (C) at ≈77 K using low-temperature trapping. (Used with permission from Ref. [157].)

Jackson et al. recently performed an intercomparison of the three types of GC–FT-IR systems [140]. They examined the sensitivity of each system by collecting data on differing concentrations of a caffeine standard. They reported that the detection limits of the systems were approximately 10 ng, 40 pg and 35 pg for the lightpipe, matrix isolation and subambient trapping methods, respectively. Because they were studying the sensitivity of high-resolution GC–FT-IR systems and the lightpipe detection limits were so much higher than the limits of either of the two cryogenic trapping methods, they continued their study utilizing only the MI and DD methods. In the remainder of their study, Jackson et al. analyzed a Grob test mixture, a commercial sample of isoheptanol isomers, and a standard pesticides mixture. They reported that the two cryogenic methods were successful at separating and identifying the components in the Grob mixture. The alcohol and pesticide mixtures presented some problems, both with the chromatography and the IR detection. Through manipulation and optimization of the GC parameters and the speeds at which the deposition surface travelled, separation and spectra of the alcohol isomers were obtained. The resolution of the IR reconstructed chromatogram was equivalent to that obtained with a separate, optimized GC–FID instrument. The pesticide sample presented a few more problems than the other two samples. The FT-IR chromatograms were not sufficient to locate all components present, as determined by other detection methods (FID and MS). Because sensitive signal-averaged spectra can be obtained, the ability to obtain IR spectra of each component is limited not as much by the detection limits of the instrument, but

rather by the analyst's ability to locate the peak or presence of the compound from the IR chromatogram. This problem can be circumvented by the use of more sensitive parallel detectors to locate the retention times at which a component is present so that IR spectra can be obtained.

Budzinski et al. investigated the use of a lightpipe instrument for analysis of many environmentally important compounds and also studied the limits of detection (LOD's) of the GC–FT-IR system [141]. By analyzing compounds which had ambiguous mass spectra, the data presented demonstrated the complementary nature of IR spectroscopy with respect to mass spectrometry. The standard compounds they used to characterize the instrument were organochlorinated compounds *o,p′*- and *p,p′*-DDD and DDT, two nitrophenol isomers, and five aromatic methylphenanthrenine isomers. After running standards to verify that the GC–FT-IR system is capable of distinguishing each compound from the other closely related ones, they used the system to analyze 'real world' samples. A PCB industrial sample was analyzed for target compounds PCB 118 (2,3′,4,4′,5-pentachlorobiphenyl) and PCB 119 (2,2′,3,4′,5′,6-hexachlorobiphenyl), which often coelute on apolar columns. Each of the IR spectra of the compounds are unique and can lead to unambiguous identification of the compounds. Although the two compounds coeluted in the chromatography, the IR spectrum of the GC peak showed characteristics of both of the compounds, leading to the conclusion that both PCB 118 and PCB 119 were present in the sample.

In their study of detection limits, Budzinski et al. concluded that the LOD for the lightpipe system is in the mid-nanogram range, depending on the molar absorptivity of the analyte. For strong IR absorbers such as phenols, the LOD is about 25 ng. This limit increases to about 50–80 ng for medium absorbers such as pesticides, and to about 100 ng for weak absorbers like methylphenanthrenes. If the analytes are strong IR absorbers in a region with a specific and intense band of vibration, then the LOD can drop as low as 10–15 ng.

The results of a study by Krock and Wilkins [142] on the minimum identifiable quantities (MIQ's) of a number of EPA-regulated compounds agreed with Budzinski's findings. Krock and Wilkins studied three classes of compounds: a nine-component phenol mixture, a ten-component chlorinated hydrocarbon mixture, and a sixteen-component polynuclear aromatic hydrocarbon (PAH) mixture, for a total of thirty-five compounds analyzed. The results showed that the MIQ's of the three classes were in the range 1–10 ng. PAH's had the highest detection limits, 5–20 ng, with an average of about 5 ng. The relatively high MIQ could possibly be due to the necessity of maintaining the lightpipe at higher temperatures relative to those required for the other two classes to prevent the higher boiling point compounds from condensing in the lightpipe. The higher temperature increases the background noise, thus increasing the detection limits and the MIQ's. The phenol mixture had MIQ values ranging from 1 to 10 ng, with only one of the nine having a value greater than 5 ng. The high volatility of the chlorinated hydrocarbons, which allowed the lightpipe to be operated at lower temperatures, contributed to those compounds having the lowest MIQ values. The values ranged from 1 to 7 ng, with the majority being below 2.5 ng. The relatively low detection limits for these classes of compounds demonstrate the feasibility and utility of using GC–FT-IR lightpipe systems for the target analysis of trace (low ppb) semivolatile organic samples.

Because of recent environmental legislation, measurement of benzene and total aromatic hydrocarbons in reformulated gasoline has become important. Diehl and others used lightpipe GC–FT-IR for the qualitative and quantitative analysis of benzene, toluene, ethylbenzene and xylenes (BTEX) in gasoline [143]. The authors used selective wavelength absorbance reconstructions to collect the IR chromatogram. The 600–798 cm^{-1} range is selective for BTEX. Using deuterated standards to create a calibration curve of the compounds, the authors were able to quantify the amount of BTEX present in the gasoline. This calibration curve was linear over a wide range, about 0.5% (w/w) for benzene to 25% (w/w) for toluene. The detection limits ranged from about 50 ng for benzene to 200 ng for *m*-xylene. With a standard deviation and percent accuracy of 0.8% and 0.5%, respectively, the authors concluded that GC–FT-IR was a good method for determining, both quantitatively and qualitatively, the amount of BTEX in gasolines.

Although the introduction of the lightpipe system

demonstrated the feasibility and utility of GC–FT-IR as an analytical tool, its high detection limits relative to GC–MS continued to be a limiting factor. To overcome this problem and increase the applicability of GC–FT-IR, cryogenic trapping methods for IR detection were developed. As mentioned earlier, GC–MI-FT-IR has the advantage of producing spectra with narrow features, yielding more detailed information about the molecule. The limiting factors in the analysis are the detection limits of the IR detector and how well it can maintain the chromatographic resolution.

A study characterizing the environmentally significant brominated and bromo/chloro-*p*-dioxins and dibenzofurans by GC–MI-FT-IR was conducted by Childers et al. [144]. They claim that high-resolution GC–MS is the most sensitive analytical technique for detecting these halogenated compounds, which can be identified by their characteristic bromine or bromine–chlorine isotope ratios of molecular ions, as well as GC retention times. However, accurate determination of the large number of different isomers can be difficult utilizing GC–MS data only. Therefore, the complementary method of GC–MI-FT-IR was used to yield additional information. The substitution of a bromine for a chlorine in tetrahalogenated dibenzodioxins and dibenzofurans changes the relative intensities of some bands and causes others to shift to lower frequencies. By using the fifteen most intense IR absorption bands for the compounds, the various isomers were identified.

Klawun et al. have developed a method to improve the chromatographic resolution of the MI-FT-IR detector [145]. The cryodisk on the commercial MI-FT-IR instrument rotates at a fixed rate. By designing a method to vary the speed at which the disk rotates depending on how closely together the peaks elute, the ability of the instrument to resolve closely eluting peaks is improved. At its original speed, peaks with retention times that differ by less than about 5 s cannot be resolved in the IR chromatogram. However, increasing the rotation speed by a factor of two or more resulted in resolution of such peaks, allowing the analyst to obtain a spectrum of a pure compound instead of that of a mixture. This speed manipulation program also allows IR detection of fast GC separations, which will be discussed later in further detail.

Another limitation of the commercial GC–MI-FT-IR instrument addressed by Rodriguez et al. is that the sample introduction system allowed only typical liquid sample introduction [146]. Analysis of gases, solids and some liquids require a different or modified injection port. To overcome the limitations of the commercial sample inlet, the authors developed an injector/trap (I/T) sample inlet which permits introduction of gaseous, liquid or solid samples onto the GC column for separation and IR detection. This inlet also allows preconcentration of samples, as well as analysis of large liquid sample volumes (100 and 1000 μl). The authors were able to characterize, both qualitatively and quantitatively, a hydrocarbon standard consisting of C_9–C_{16} hydrocarbons. They also used the I/T to analyze a perfume sample, both by headspace analysis and liquid injection. Because of the complexity of the perfume sample, two-dimensional GC was used to perform the separation. By using GC–MI-FT-IR with the I/T introduction system, the authors were able to identify the main components responsible for the desired scent of the perfume sample.

As mentioned earlier, the direct deposition cryogenic trapping technique has an advantage over the matrix isolation technique in that the IR chromatogram and spectra are collected in real time and the resulting spectra may be searched against a standard KBr reference library. Many researchers have used this technique to detect the presence of drugs or other toxins, both in humans and animals. Visser et al. used GC–DD-FT-IR for analysis of β-agonists in cattle [147]. These compounds are a group of *N*-phenylethanolamines illegally used as repartitioning agents in veal and cattle. In this study, tissue samples were analyzed for the presence of clenbuterol, mabuterol and salbutamol, all of which were trimethylsilyl-derivatized before the analysis was performed. Because the structures of the three compounds are similar, EI mass spectra were insufficient for identification, although CI spectra could be used. After running reference spectra of the derivatized substances, the authors were able to detect and identify the trimethylsilyl-derivatized clenbuterol, mabuterol and salbutamol in extracts of biological samples. The limit of detection was about 1–2.5 ng μl^{-1}.

In forensic toxicology, the identity of a compound

of interest must be confirmed by at least two independent methods (i.e. two techniques that are based on differing chemical or physical properties) with the confirmation methods being specific to the analyte of interest. The most common choice for positive identification of drugs is GC–MS, due largely to its sensitivity. However, a 1989 survey of 150–200 forensic laboratories, showed that 94 false-positive and 304 false-negative results had been reported for a set of standard samples. Kalasinsky et al. have conducted studies on the feasibility of GC–DD-FT-IR for the analysis of amphetamines in the forensic laboratory [148,149]. One area where GC–MS analysis is challenged is in the detection and identification of amphetamines. Amphetamines are difficult to distinguish from methamphetamines and in post-mortem analyses, the inability to distinguish amphetamines from the amines produced from the degradation of the sample can result in false-positive results. The Kalasinsky study included a comparison of the ability of three techniques —GC–MS in SIM mode, lightpipe GC–FT-IR, and GC–DD-FT-IR— to detect, identify and quantify derivatized amphetamines. Not surprisingly, they found that the GC–MS had the lowest detection limits and the lightpipe instrument the highest, at 5 and 25 ng ml^{-1}, respectively; the direct deposition IR instrument had a respectable limit of <10 ng ml^{-1}. Although the LOD of DD-FT-IR is greater than GC–MS (SIM), the IR method has a similar LOD and an identification certainty which is comparable to that of full-scan MS. The limits of linearity (LOL) were 10–6000, 100–25 000 and 10–2500 for GC–MS (SIM), vapor-phase IR, and direct deposition, respectively. The LOL for the lightpipe instrument is the largest due to the adherence to Beer's law until the flow cell or GC column becomes overloaded. The LOL for the DD instrument is relatively small due to the sample spot size (ideally about 100 μm) becoming larger than the IR beam, which is optimized for a 100 μm spot. Because the DD instrument was optimized for low level determinations, it overloads at concentrations greater than a few nanograms per sample deposit, resulting in the small range of linearity. The limits of quantitation for the three techniques are 10, 100 and 20 ng ml^{-1} for GC–MS (SIM), lightpipe IR, and DD-IR, respectively. It was found that, run for run, GC–FT-IR accurately identified more materials than GC–MS. If the analyte concentrations were at least 20 ng ml^{-1}, they could be detected 100% of the time. This percentage dropped to 90% and 50% for quantities of 10 and 5 ng ml^{-1}, respectively.

4.3. Summary

It has been well demonstrated that developments in the past few years have provided unprecedented abilities for GC–FT-IR. Between improvements in sensitivity and chromatographic resolution, GC–FT-IR has clearly shown that it can provide information about complex mixtures that was previously unobtainable with GC–MS. Additionally, as improvements in IR detection continue, lower detection limits and higher sensitivity will continue to help GC–FT-IR emerge as a powerful analytical technique. Although it is a useful technique by itself, linking FT-IR and MS detection together following a separation can further improve the information obtained from an analysis.

5. Linked GC–FT-IR–MS

It has been demonstrated that GC–FT-IR is a powerful technique for analysis of complex mixtures. Because IR and MS yield complementary information, a combined GC–FT-IR–MS instrument is an extremely versatile tool for many types of analyses. Other non-specific, yet sensitive detection methods, such as an FID may also be utilized. An initial problem with interfacing GC to IR and MS was the significant difference in sensitivity between the two spectral detectors (i.e. the IR spectrometer becomes the limiting detector) [150]. However, with the development of cryogenic IR methods, this mismatch in sensitivity is minimized, if not eliminated. As a consequence, commercial GC–FT-IR–MS instruments are available with both the lightpipe and matrix isolation IR detectors. By using parallel MS and IR detectors, it is possible to obtain much more information from a single experimental run. Although the MS spectral libraries are significantly larger than those available for IR spectra, the IR can confirm the identification of a component by providing functional group and structural backbone

information. Also, homologs not easily identified by IR spectral analysis can be differentiated by MS while the reverse situation holds true for structural isomers. Many researchers have used such instruments for analysis of complex mixtures.

5.1. Typical GC–FT-IR–MS analyses

De Jong et al. performed a comparative study on the identification of stimulants in drug testing utilizing both ion trap GC–MS and lightpipe GC–FT-IR [151]. The routine analysis of blood or urine for drugs such as stimulants is most often performed by GC–MS due to its excellent sensitivity and detection limits. However, many drugs have very similar EI mass spectra, making the task of identification reliant upon the chromatographic retention time, which is no better than using only GC for the analysis. However, by utilizing on-line FT-IR, i.e. a lightpipe system, information on the intact molecule can be obtained. To test the abilities of GC with EI-MS, CI-MS and FT-IR detection, the authors analyzed three different mixtures of stimulants often used by athletes: (1) compounds of different structures but similar EI spectra, including compounds with the same molecular mass but different substitution patterns; (2) amphetamines which produced the same base peaks, making those with the same molecular mass difficult, at best, to distinguish (some compounds had identical EI spectra); (3) ephedrines, which differ by one methyl group, yielding spectra that differ by a mass of 14 u in the base peak. The first challenge in the analysis of the mixtures was in the chromatography. This was relatively easy to overcome by derivatizing the compounds with tetramethylsilane and trifluoroacetic acid, which greatly improves the chromatographic properties of all the mixtures. Derivatization also affects the fragmentation, yielding more high mass ions. Because of the higher masses present, greater differences in the EI mass spectra are present, making identification using this data more accurate.

As stated earlier, the EI spectra of drugs, even with derivatization, can be similar. With CI-MS, the molecular ions of each compound in all three mixtures were observed in the spectra. The CI spectra of mixture one compounds were clearly different, even without derivatization, so that easy identification of the compounds could be obtained. In the second mixture, each amphetamine exhibited a unique spectrum, but ethylamphetamine and dimethylamphetamine could only be differentiated after derivatization. CI–MS proved to be a quick and easy confirmation method for mixture three, because each component differed by a mass of 14, which clearly was shown in the CI spectra. Even though IR spectra of amphetamines are very similar in the fingerprint region, the authors successfully identified the different stimulants in all three mixtures using the vapor-phase spectra. Derivatization of the compounds increased the sensitivity, but this increase was mainly due to the improved chromatographic properties of the mixture after derivatization.

The feasibility of identifying the isomers from mono- and dinitration of phenyl- and diphenylacetic acids utilizing GC–FT-IR and GC–MS was the focus of the research by Soják et al. [152]. Identifying the position and attachment of the nitro groups to one or two benzene rings was the problem. Mass spectrometry is usually unable to distinguish between ring-substituted isomers, especially between *meta*- and *para*-isomers. There are some differences, however, between mass spectra of the mono- and dinitro derivatives: the molecular ion is always absent in mononitro derivatives and all dinitro derivatives with one nitro group in the *ortho* position. By studying the IR data, mainly the stretching vibrations of the NO_2 group and the benzene ring, the position, *ortho*-, *meta*- or *para*-, could be determined. The asymmetric stretching frequency shifted to higher frequencies as the position of the NO_2 group changed from *ortho* to *meta* to *para*. Various other shifts and/or splitting patterns observed in the spectra contributed more information to aid in the identification process. Using the information obtained from the mass and IR spectra, the authors were able to successfully identify all isomers from the nitration and dinitration of mono- and diphenylacetic acid, demonstrating the complementary information that can be gained from by use of both IR and MS.

Coleman and Gordon [153] have demonstrated the utility of GC–MI-FT-IR–MS in the analysis of natural products such as flavors and fragrances and essential oils. Because stereochemical conformations, as well as the amount, of organic substances contribute to the flavor and smell of products such as

essential oils, the ability to characterize the complex samples, both qualitatively and quantitatively, is important. Often the chemicals responsible for the desired characteristics in a sample are not major components. Therefore, the analytical method must be able to detect and identify trace amounts of the relevant compounds. Coleman and Gordon demonstrated the ability of the MI-IR detector to distinguish individual conformational isomers at the nanogram level. One pair of compounds of interest were geraniol and nerol. These two compounds are isomers differing only in a *trans* vs. *cis* configuration of a double bond. Because they are isomers, they have very similar physical properties, yet are very different in terms of their flavor/fragrance properties and applications, making accurate assessment of their presence essential. The lightpipe instrument did not possess sufficient resolution to distinguish these two isomers. Likewise, GC–MS is not capable of providing information regarding the geometry of the molecule. However, because both geraniol and nerol do have differences in their matrix isolation IR spectra, the authors were able to identify and quantify the amounts of each in the essential oil samples studied.

Hedges and Wilkins identified many of the components in a sample of eucalyptus oil by utilizing lightpipe GC–FT-IR–MS [154]. The separation of the sample was a difficult task because essential oils are complex with hundreds of components. Therefore, as Fig. 6 demonstrates, overlap of peaks is impossible to avoid. Furthermore, 40–80% of the oil was comprised of 1,8-cineole, creating a dynamic range problem. GC–FT-IR–MS is suited to study such a sample because the separating power of capillary GC is the best relative to analysis time. Even though though some components remain unresolved, this problem can be solved by MDGC or utilizing spectral subtraction techniques when acquiring and analyzing the spectral data. Identification of the components was made by library search matches of both the IR and MS spectra and verified against literature sources when possible. Because the MS libraries are much larger than the IR libraries, the probability of a match between the compound of interest and a compound in the library was greater for the mass spectrum. The cases where searches on both the IR and MS searches agreed upon the identity of a compound were assumed to be the most accurate. In cases where the MS identified a compound that was not in the IR library, the IR spectrum was often used to verify functional groups and other structural information to help support the MS result. It was found that the IR search often returned closely related isomers, even if the actual compound was not present in the library. One problem encountered when using hit lists from two different libraries was that the libraries may list a different name for the same compound. To overcome this problem, Chemical Abstract Service (CAS) numbers were used for comparisons of hit lists. Other problems, which prevented the identification of some compounds in

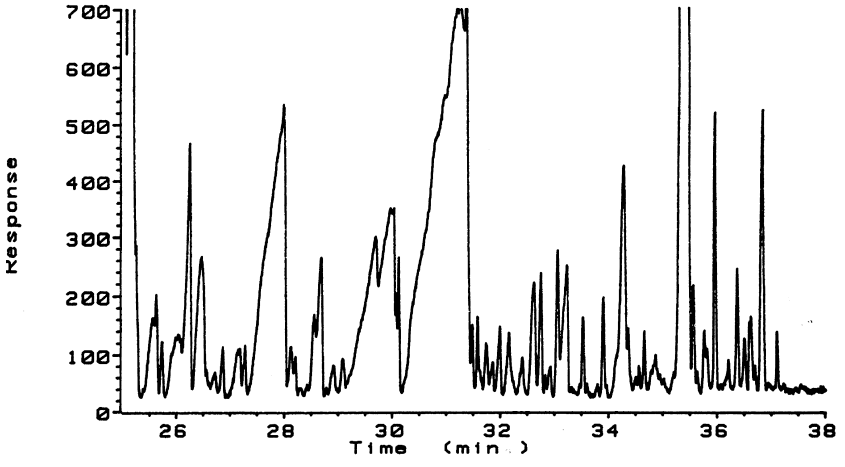

Fig. 6. Latter portion of an IR reconstructed total response chromatogram of *E. Australiana*. (Used with permission from Ref. [154].)

the sample included insufficient IR sensitivity for some components and loss of chromatographic resolution, especially in the MS due to the significant dead volume of the IR, which was positioned before the MS in the serially linked system. Irrespective of these problems, however, the authors found GC–FT-IR–MS to be a valuable method in the analysis of complex mixtures. In their analysis of eucalyptus oil, the authors observed 58 peaks in the chromatogram and were able to successfully identify 33 of the components.

Bicchi et al. identified the sulfurated compounds in *Tagetes patula* cv. *nana* essential oil utilizing GC with FT-IR, MS, and atomic emission spectroscopy (AES) detection [155]. The authors were trying to detect and identify the acetylenic thiophenes, which are quantitatively minor but of high biological interest. The classical methods of detection and identification, FID and EI-MS, respectively, are often insufficient for many reasons, including the complexity of essential oils, the small amounts of the compound of interest in the sample, chromatographic problems ranging from complexity of the chromatogram to coelution problems. GC with microwave-induced plasma (MIP) atomic emission spectrometric (AES) detection is multi-element sensitive, selective, qualitative, linearly quantitative, and provides information about the elemental formulae of compounds separated by gas chromatography. The information obtained from the AES results, combined with the GC retention times can help to identify sulfur-containing compounds. Although this method can provide information about elemental composition, structural information cannot be obtained, which is why FT-IR and MS detection were also used. FT-IR unambiguously identified the isomers in the sample which were indistinguishable by MS. However, only three of the seven acetylenic thiophenes could be detected by FT-IR due to the small amounts present. Thus, it was necessary to use MS for detection and identification of these compounds. By using GC–MIP-AES, GC–FT-IR and GC–MS to identify the acetylenic thiophenes present in *Tagetes patula* cv. *nana* essential oil, the authors demonstrated the utility of using a variety of chromatographic detectors to obtain complementary information in the analysis of a complex mixture.

Williams et al. identified and quantified 50 targeted phenolic compounds utilizing lightpipe GC–FT-IR–MS [156]. Because positional isomers exhibit very similar mass spectra, these compounds are difficult to unambiguously identify by GC–MS, especially without good chromatographic separation. Single ion monitoring can be used to some extent to 'resolve' overlapping peaks and obtain pure mass spectra. However, because the IR spectra of these isomers can be very different, 'pure' spectra can be obtained from overlapping chromatographic peaks via spectral subtraction techniques. After optimizing the chromatographic conditions, the authors used standards to create MS and IR libraries of the 50 target compounds for more rapid library searching. The GC–FT-IR–MS system was calibrated by preparing standard solutions and using the total ion chromatogram (MS) and total response chromatogram (FT-IR) to set up calibration curves. The detection limits were in the tens of ng μl^{-1} range and the linear dynamic range was between tens of ng μl^{-1} and a few hundred ng μl^{-1} for most compounds. The phenolic compounds were identified by retention time and the use of spectral library searches. Quantification of most compounds was determined by the total response of the IR and MS spectrometer. Special cases, however, required quantification by MS only, using single ion monitoring, or by IR only, using the absorption at a single wavelength. The precision of the analyses was better than 10%. These data indicate that GC–FT-IR–MS is a very powerful method for both qualitative and quantitative analysis of phenolic compounds, despite detection limits which are higher than those of GC–FID or GC–MS. Analysis was faster using MS data only. However, IR data were superior for identification of unknowns, especially isomeric compounds.

Gurka et al. used a lightpipe GC–FT-IR–MS instrument for quantitation of mixtures [139]. Mass spectral data were acquired in total ion chromatogram (TIC) and single ion chromatogram (SIC) modes while the lightpipe data were acquired in the Gram–Schmidt (G–S), maximum absorbance (MA) and integrated absorbance (IA) modes. In this study, the authors addressed the issue that, in most MIQ studies, pure analytes in pure solvents are used, representing only the most ideal case. If the ideal case holds true, then the lightpipe GC–FT-IR instrument should have the ability to identify and quantify

compounds at the low nanogram level in real time. However, the MIQ's for real samples are higher due to background materials, mainly hydrocarbons. Because hydrocarbons are weak IR absorbers, the MIQ for GC–FT-IR should be less affected by the hydrocarbon background species than GC–MS. To compare quantitative results of MS and FT-IR in the different modes, the mean regression correlation coefficients (R^2) were used as a measure of data scatter. It was found that scatter increases in the order TIC comparable to SIC<MA<IA<G–S. However, the G–S and IA results can be improved by omitting hexachloroethane and dibenzofuran because of scattered plots and coelution, respectively, so that the difference between the best and worst regression coefficients is only 5 ppt, indicating that the regression scatter is comparable for all five modes. In this context, it should be noted that small, highly halogenated compounds are typical environmental compounds of interest and that coelution is a common problem in chromatography. The authors also did a comparison on the relative molecular sensitivities of the IR and MS detectors. The TIC mode is the least sensitive to molecular structure with the G–S mode being the most sensitive and SIC, IA and MA all being about equal.

Baumeister et al. utilized a GC with parallel MI–FT-IR, MS and FID to study the isomer distribution of two mixtures of C-7 alcohols [150]. Each of the three detection methods gives different types of information: FID provides retention time and quantitative information, FT-IR spectra provide functional group and isomer-specific information, and fragmentation patterns and molecular masses can be obtained from the mass spectra. The alcohol samples investigated contained so many closely related isomers that GC–MS alone was not sufficient for identifying the components with a high degree of certainty and lightpipe FT-IR was not sensitive enough to detect the minor components. The authors determined that there were eight components present, six common to both mixtures and one unique to each of the mixtures. As expected, the mass spectra of all eight components were very similar, although the existence and relative intensities of many major peaks were helpful during the interpretation of the spectra. Although the IR spectra all contained the expected spectral features for a saturated $C_7H_{16}O$ alcohol, i.e. the O–H and C–O stretches at ca. 3660 cm^{-1} and ca. 1062 cm^{-1}, respectively, structural information was obtained. The degree of branching could be determined by studying the ratio of the CH_3 to CH_2 symmetric and antisymmetric modes, and the presence of an isopropyl or *tert*-butyl group could be determined by the appearance of characteristic multiple absorptions in the CH_3 bending region. By using the structural information obtained from the IR spectra along with the results from the library search of each spectrum, the eight different C-7 alcohol isomers were identified.

Smyrl et al. used simultaneous GC–DD-FT-IR–GC–MS for identification and quantification of isomers in a complex mixture [157]. Their system differed from most GC–FT-IR–MS systems in that the effluent from a single column was not split to the two detectors. Instead, after injection, the sample was split to two identical columns, one of which was connected to the MS detector and the other to the IR. By using this configuration, larger samples can be injected without degrading the chromatographic separation by overloading the column and greater flexibility in column selection for a particular application can be obtained. To demonstrate the power and utility of such a system, the authors analyzed a sample of structural isomers which was prepared by steam distillation/extraction on coal. Napthalene was used as a test compound to study the detection, identification and quantitation abilities of the instrument. The data presented showed the ability to identify a DD-FT-IR spectrum when searched against a standard KBr spectral library. To study the quantification capabilities of the system, two different methods were used: area or height of the chromatographic peak generated by measuring the absorbance in the predefined windows in the overall FT-IR spectrum [or by using the total absorption measured from the interferogram (Gram–Schmidt)], and by the absorbance of a particular band in the actual IR spectrum. Using the actual IR absorption band resulted in an increase in sensitivity by approximately two orders of magnitude when compared with using the peak area of the selected wavelength (functional group) chromatogram. However, using the area of the peaks in the MS total ion chromatogram provided an even more superior method of quantitation, especially in the case of some trace

components which the IR detector was unable to even detect. Mass spectral detection, due to the larger library, was better in many cases for identifying individual components. But, as previously mentioned, in the case of isomers, IR spectra were required to identify which isomer of the compound was present. By separating and identifying the different components in a coal extract, the authors demonstrated that the detection limits and abilities of IR and MS to distinguish isomers are complementary.

More recently, Amenta et al. recognized the value of hyphenated techniques in the chemistry community and developed ways to incorporate GC–MS and GC–FT-IR into freshman and sophomore chemistry labs instead of waiting until an instrumental analysis course to introduce students to these techniques [158]. However, because they did not have access to a combined GC–IR–MS system, they used separate GC–MS and GC–IR instruments for experiments in which students both monitored reaction progress and characterized the products of a ferrocene synthesis. Lightpipe-based FT-IR was used for the IR measurements employed by the students. One of the pedagogical advantages of this approach was that it allowed the students to see the relative simplicity of gas-phase IR spectra, compared with those of the solid products.

Basiuk and Navarro-González identified unusual products of silica-catalyzed amino acid condensation using GC–FT-IR–MS, as well as HPLC–particle beam MS [159]. For their GC–IR–MS analysis, they used the commercial lightpipe-based Hewlett-Packard GC–IR–MS system. Many of the products were first identified by interpreting their mass spectral fragmentation patterns with IR spectra being used to confirm the identifications. Additionally, computer-generated simulated IR spectra were employed for additional confirmation of assigned identifications. In another paper, Basiuk et al. also studied amino acids and their pyrolysis products using GC–FT-IR–MS, as well as NMR [160]. Again, initial identifications were based upon the mass spectral data with IR spectra, both acquired and simulated, used for confirmation. Both this and the previously cited paper are good examples of applications where the analyst is not sample limited, and can therefore tolerate the sensitivity limitations of on-the-fly GC–IR. These spectral data, combined with GC retention times, provide a good deal of information about the identities of mixture components. As expected, more information leads to more confidence in the ultimate identifications.

Guitton et al. used GC–MS and GC–FT-IR to identify fentanyl metabolites [161]. Fentanyl is a synthetic opioid used for surgical analgesia and sedation. Their goal was to develop an analytical method to simultaneously detect and identify its metabolites. The subjects of their study were seven patients from an intensive care unit who had been receiving fentanyl infusion for less than 3 days. Twenty milliliter aliquots of 24-h urine samples were analyzed. In the present study, two both stand-alone GC–MS and GC–IR instruments were used, in addition to a direct-linked GC–IR–MS system. Regardless of which system was used, the GC–IR spectra were obtained using a lightpipe interface. Interestingly, the primary use of the GC–IR–MS instrument, as reported here, was to establish the necessary correspondence between the mass and IR spectra for the same chromatographic peak. This observation highlights one of the primary advantages of an integrated system, which is the avoidance of any ambiguity regarding which mass and IR spectra represent those of the same material. Obviously, even though one attempts to make chromatography identical, with separate stand-alone GC–MS and GC–IR systems it can be difficult to be absolutely certain that the correct pairing have been made. The authors also noted that GC–MS had been previously used for this analytical problem, but that this was the first time GC–FT-IR had been used to successfully confirm identification of nine of the ten previously identified metabolites. Because of the lack of metabolite standards against which the previous GC–MS data could be compared, the confirmatory evidence provided by GC–IR–MS was exceptionally useful.

As mentioned earlier, FT-IR is very useful for isomer analysis. Sommer et al. used this advantage to study polychlorinated dibenzo-*p*-dioxins (PCDD's) and polychlorinated dibenzofurans (PCDF's) extracted from municipal fly-ash using GC–FT-IR–MS analysis [162]. For this investigation, a linked GC–IR–MS system was used. The authors noted some difficulties resulting from the much different flow-rate requirements of the IR vs. the MS detector. This

could be compensated for experimentally. FT-IR spectra are critical in this study because certain isomers are of toxicological importance. Furthermore, because many assignments for dioxin vibrations are uncertain in the literature, ab initio calculations were used on some dioxins and furans to obtain simulated IR spectra. These spectra coordinated well enough with measured to allow definite assignment of the most important vibrations. Because the IR detector employed a lightpipe instrument, sample preconcentration was necessary for adequate detection and detection limits of 10–20 ng were obtained, depending upon the analyte. One of the primary conclusions of this paper was that FT-IR spectroscopy was a useful aid in dioxin and furan analysis, but that it could not replace GC–MS due to its lower sensitivity. It was also noted that the gas-phase IR spectra were more susceptible to interferences from the fly-ash matrix than were literature MI-FT-IR spectra used for comparisons.

5.2. Multidimensional GC–FT-IR–MS

As has been previously stated, the purpose of linking both IR and mass spectral detectors together is to provide more accurate qualitative information about a complex mixture. Unfortunately, in systems with a single chromatographic column and a complex mixture, the detector requirements necessarily limit the chromatographic capabilities of the entire system. Fig. 7(A) shows an example of a model four-component mixture with three overlapping chromatographic components, and Figs. 7(B,C) are the IR and mass spectra taken from the largest peak. It is clear from both library searches and manual evaluation that the spectra are of mixtures rather than pure components. Figs. 8 and 9 show the second-stage separation of the mixture and the resulting spectra from the three large peaks, respectively. It is evident from the individual spectra in Fig. 9 that Figs. 7(B,C) are indeed the sum of these spectra.

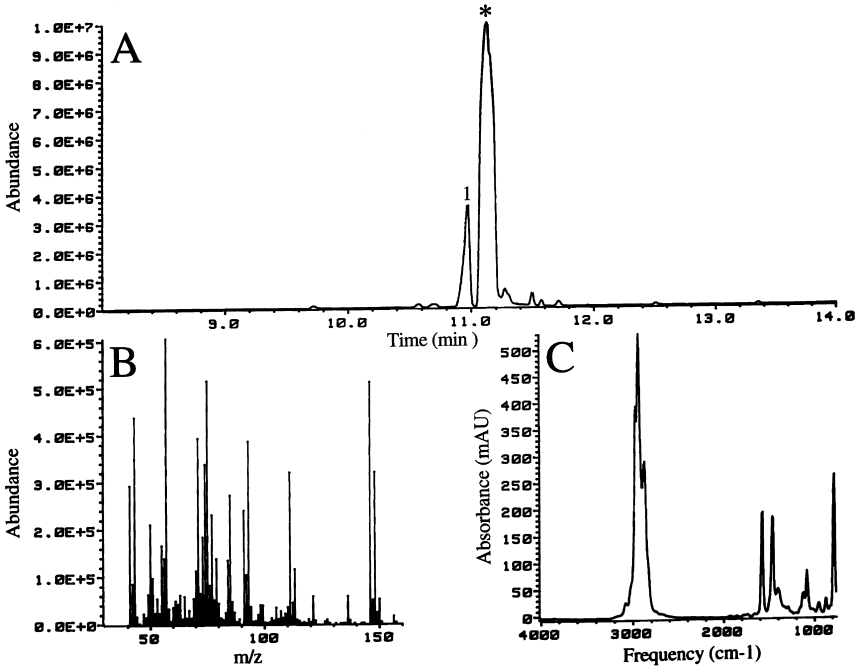

Fig. 7. Four-component model mixture primary separation. (A) Primary separation of the model mixture on an intermediate polarity column. Peak 1 is 1-isopropyl-4-methylbenzene. (B) Mass spectrum of peak indicated by * in (A). (C) IR spectrum of peak indicated by * in (A). (Used with permission from Ref. [51].)

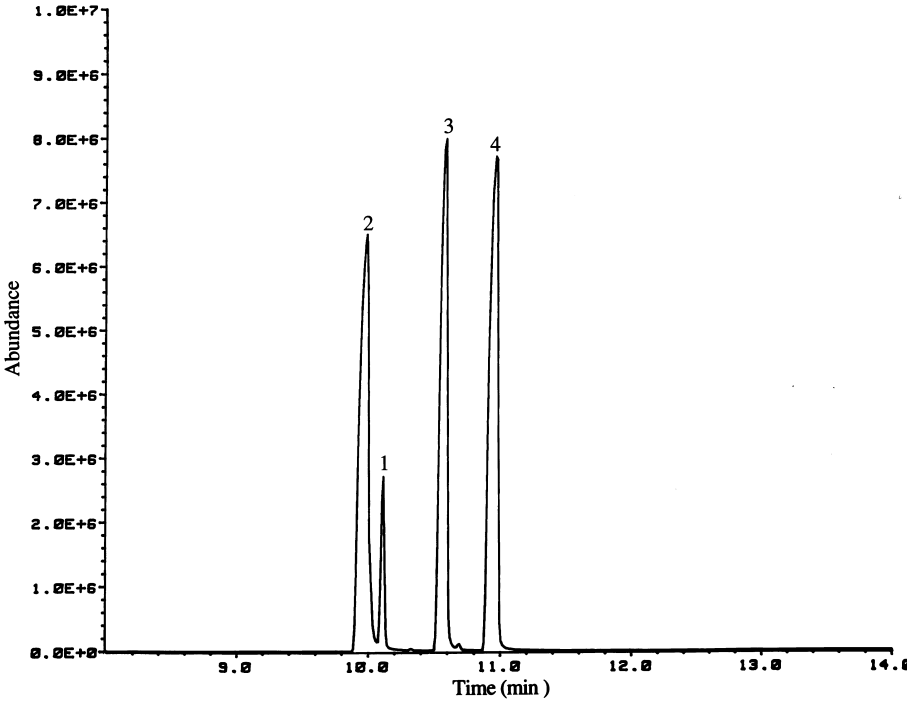

Fig. 8. Secondary separation of peak indicated by * in Fig. 7(A). Peaks: 1=1-isopropyl-4-methylbenzene; 2=1,3-dichlorobenzene; 3=γ-terpinene; and 4=undecane. Peak 1 is a residual from the heartcut. (Used with permission from Ref. [51].)

Several other articles in this journal cover the reasons why this is true, but it suffices to say that the qualitative information obtained about complex mixtures is of questionable quality in some cases. As mentioned in Section 2 of this review, one choice to overcome the limitations of a single separation dimension in a GC–FT-IR–MS system is to utilize multidimensional gas chromatography. Typical MDGC systems, such as the one diagrammed in Fig. 10, employ a single cryogenic trap interposed between a first-stage and a second-stage chromatographic column. Such a design provides a total qualitative analysis at the expense of a great increase in analysis time. In an effort to decrease analysis time and increase the chromatographic flexibility of the MDGC–FT-IR–MS system, a multiple parallel cryogenic trapping system schematically depicted in Fig. 11 was developed [46–51].

Qualitative analyses of gasoline [49,50] and essential oils such as eucalyptus [47] and cascarilla bark [48] have been carried out using MDGC–FT-IR–MS systems. Gasoline analysis was chosen to evaluate the system performance. It was shown that the retention time reproducibility was within ±3 s. The advantage of accurate retention times is the ability to detect component carry over between adjacent heartcuts without the use of any spectral detector. Additionally, in conjunction with spectral detectors, it provides a powerful means to correlate components in different adjacent cuts. Furthermore, the differences in chromatographic resolution as a function of heartcut times is illustrated in Fig. 12. Figs. 12(a,b) are second-stage separations of 72 s. heartcuts from an unleaded gasoline sample, and Fig. 12(c) is a series of second-stage separations of five 12 s. heartcuts from the same region of the chromatogram that Figs. 12(a,b) were taken. However, it is to be noted that increasing chromatographic resolution necessarily increases analysis time. Thus, there is a trade-off between time and information.

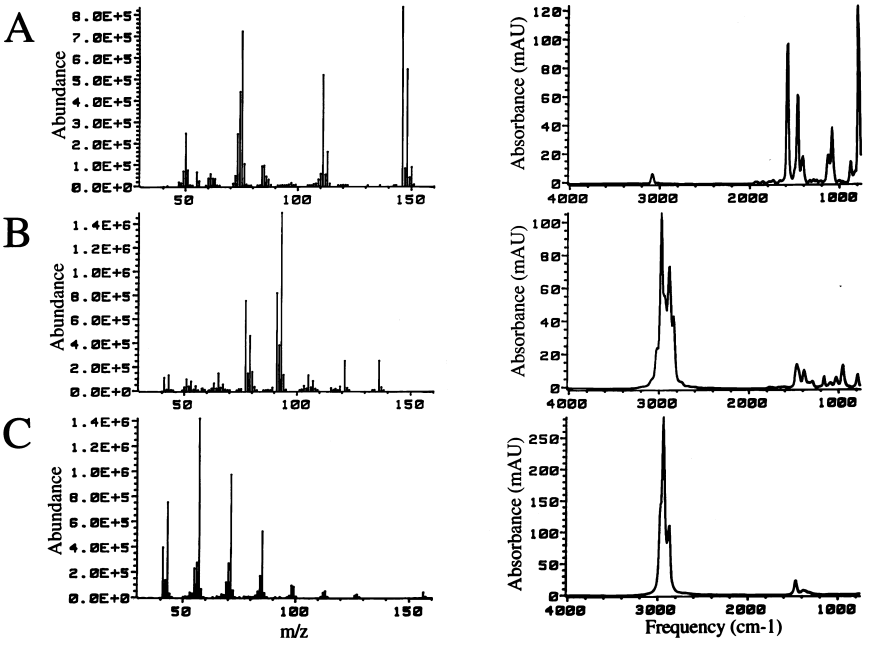

Fig. 9. Mass and IR spectra for peaks 2, 3 and 4 in Fig. 7. (A) Mass and IR spectra for peak 2 (1,3-dichlorobenzene). (B) Mass and IR spectra for peak 3 (γ-terpinene). (C) Mass and IR spectra for peak 4 (undecane). (Used with permission from Ref. [51].)

Early papers did not utilize multiple analytical columns or carry out sample looping, but the addition of these two features have demonstrated the utility of such an approach [46].

The idea behind multidimensional GC–IR–MS is to provide the ability to correctly identify all or most of the components in complex mixtures. In this respect, it is necessary that the chromatographic separation be as complete as possible. In reality, linkage of various instruments, each with their particular sample requirements, introduces restrictions on each of the individual system components. Linkage of a lightpipe FT-IR to the parallel cryo-

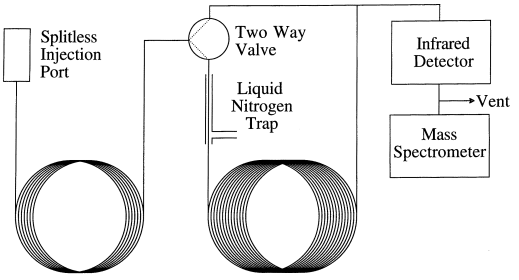

Fig. 10. Schematic diagram of the MDGC–IR–MS experiment. Both the preliminary and analytical columns were contained in one oven, and the liquid nitrogen trap and 2-way valve were also in the same oven. Detection was performed by using Hewlett-Packard IR and mass-selective detectors.

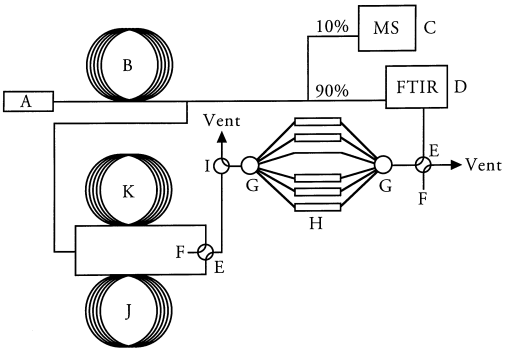

Fig. 11. Schematic diagram of the parallel cryogenic trap MDGC–FT-IR–MS system. A, splitless injection port; B, intermediate polarity first-stage column; C, HP 5970B MSD; D, HP 5965B IR′; E, four-port, two-way valve (300°C max. temp.); F, external auxiliary carrier gas; G, six-port selection valve (300°C max. temp.); H, stainless-steel cryogenic traps; I, three-port, two-way valve (300°C max. temp.); J, polar higher-stage column; K, non-polar higher-stage column. (Used with permission from Ref. [46].)

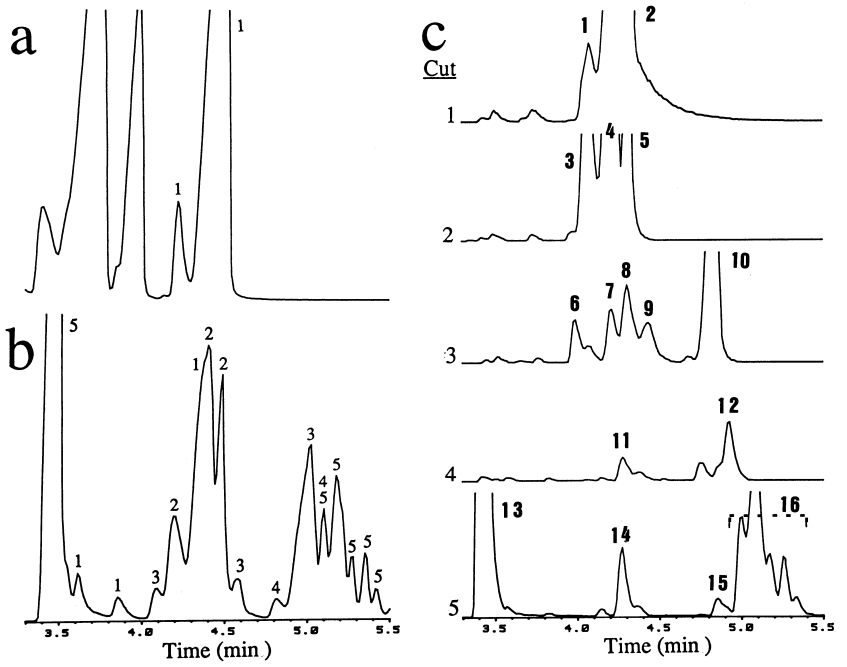

Fig. 12. Second-stage total ion chromatograms for unleaded gasoline. (a,b) A view of the total ion chromatograms of the 72 s first-stage heartcuts 1 (a) and 2 (b). The peak labels represent the cut number where that particular component appears in (c). (c) Second-stage total ion chromatograms of five 12 s heartcuts in the range 7.5–8.5 min after the first-stage separation. The peak labels in (c) correspond to the compound identification in Table 2. (Used with permission from Ref. [49].)

genic trapping system is useful and at the same time restrictive. The usefulness is the well-known non-destructive nature of the detector. On the other hand, its detection limits are of the order of tens of nanograms, and therefore, it requires a chromatographic column with a higher sample loading capacity, precluding the use of smaller I.D. columns. However, where analysis of complex mixtures is not performed at a trace level (1 ppm or less) or in cases where primary enrichment techniques could result in 10^{5-6} fold improvement, the lightpipe instrument provides significant advantages. Consider the eucalyptus [47] and cascarilla bark oil [48] analyses. The instrument used to analyze these mixtures is equipped with three gas chromatographic columns having different stationary phases with different retention properties. Effluents exiting the lightpipe either can be vented or cryogenically trapped. The advantage of this design is that data collection is achieved prior to trapping, and spectroscopic data for analysis is obtainable for all separation stages using any of the columns. Fig. 13 shows the initial demonstration of the feasibility of carrying out GC^3 by the method of double heartcutting, sample recycling and identifying components in the final heartcut from eucalyptus oil [47]. This technique allowed comparison of a known adulterated oil with a known natural oil. Additionally, sample recycling allows analysis of a complex mixture using three or more different columns in a single instrument for screening purposes. However, in order to carry out analysis in the third dimension, a priori knowledge of the second-stage chromatogram is necessary because only a limited number of traps are available for the second-stage effluents. This means that an extra run in the second dimension is required for choosing the region(s) to be analyzed in the third dimension. An alternative would be to analyze the complete second dimension heartcut in the third dimension [48]. This was demonstrated in the analysis of minor components in cascarilla bark oil. Three regions were chosen for enrichment by repeated injection of the sample and trapping the respective sections in three different cold traps, followed by two reinjec-

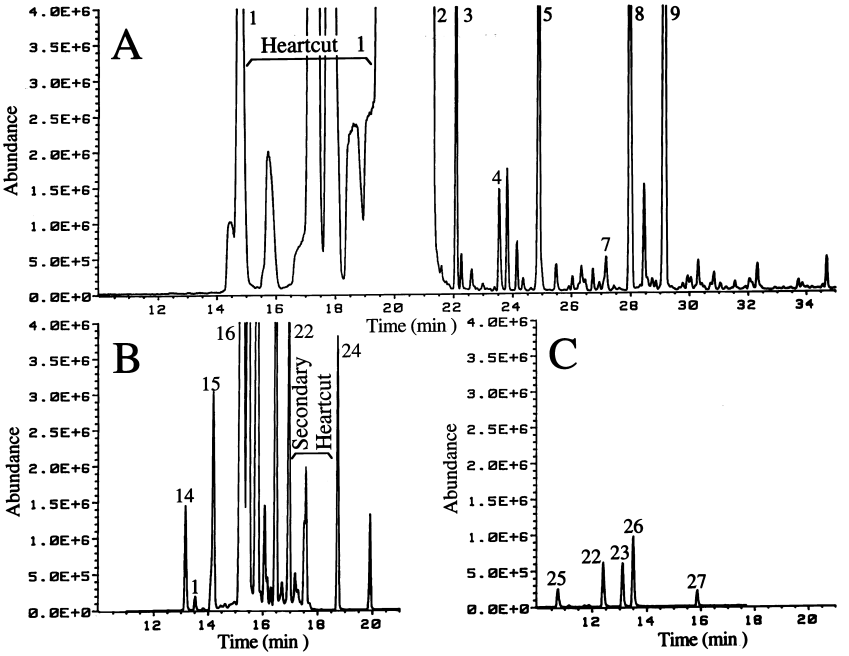

Fig. 13. TIC's of the known adulterated sample. (A) Precolumn chromatogram; (B) chromatogram resulting from secondary separation of heartcut 1 [same as Fig. 7(C)]; (C) chromatogram resulting from tertiary separation of the heartcut from the secondary separation (17.0–18.5 min). Peak numbers refer to component identifications in Table 3. (Used with permission from Ref. [47].)

tions and cold trapping for analysis on the two secondary dimensions. This analysis showed that enrichment of several chosen sections is possible *simultaneously*. Furthermore, once enriched, analyses could be performed in more than two dimensions. The end result of these methods is the possibility of separation and identification of components in several sections of a complex mixture and an example of the second and third-stage separations of one of these regions is shown in Fig. 14. The peaks labeled in Fig. 14(b) correspond to the identifications in Table 4.

In addition to complex mixture analysis, valve adsorption and possible reactivity was studied [46]. Here, sample recycling and looping capabilities of the valve-based system were investigated. Standard samples included Grob, hazardous substance, phenol, base neutral, and chlorinated hydrocarbon mixtures. Approximately 500 ng of each mix was injected onto the primary column and cryogenically trapped. This step was followed by reinjecting the trapped segment onto a second-stage column and repeatedly trapping the effluents exiting the lightpipe. The process was stopped when the detection limit was reached in either the IR or mass spectrometer. It was found that 35 of the 40 total components examined could be recycled for more than six times, and for several chlorinated hydrocarbons it was shown that a 23-stage recycling is possible. During each recycle, 10% is lost due to the MS and 10% or more due the presence of valves (5 total). Fig. 15 shows these results. The limitation on some of the components that showed relatively low cycling efficiency was ascribed to temperature control of the valves in the trap oven and the compound basicity. Thus, the investigations carried out using the valve-based system verified that some of the existing deficiencies in the GC–GC heartcutting technique can be corrected by means of a system of parallel cryogenic traps (Table 5).

Krock and Wilkins investigated the application of multidimensional gas chromatography to contaminated environmental extracts, in the first such application of this approach which utilized a combined [163] multidimensional GC–FT-IR–MS system for qualitative analysis of mixture components.

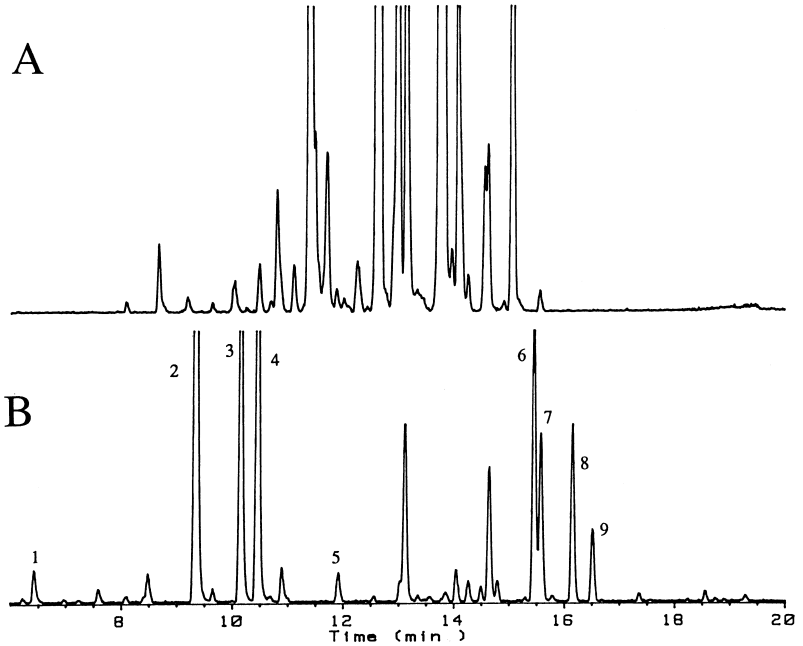

Fig. 14. Total ion chromatograms for the second and third-stage separations of an enriched heartcut taken from a cascarilla bark oil sample. (a) Secondary separation performed on a non-polar column. (b) Tertiary separation performed on a polar column. Peak numbers refer to component identifications by IR and mass spectra in Table 4. (Used with permission from Ref. [48].)

Recently, Tomlinson and Wilkins studied the presence of irritants in a variety of soaps utilizing multidimensional GC–FT-IR–MS [164]. With their lightpipe GC–IR–MS system, they analyzed the fragrance content, in order to determine whether or not six different irritants which can cause contact dermatitis were present. In such analyses, many of the components responsible for fragrances are chiral. Thus one enantiomer, or a specific ratio of enantiomers, may be responsible for characteristic smell. Many components which cause contact dermatitis are chiral as well, with one optical isomer sometimes being an irritant, while the other is not. In this study, several components in the soaps investigated, including many enantiomers, were successfully separated. Mass and IR spectra, as well as GC retention times were used to make identifications.

Table 4
Component identifications for Fig. 14 supported by both IR and mass spectra (adapted with permission from Ref. [48])

Peak no.	Peak identification
1	Octanoic acid, 2-methyl ester
2	Nonanone
3	Alcohol
4	Methyl formate
5	α-Thujone
6	β-Thijone
7	Aldehyde
8	Nonan-2-ol
9	Alcohol

5.3. Summary

It has been amply demonstrated that linked GC–FT-IR–MS systems dramatically increase capabilities for complex mixture analysis. With the addition of multidimensional gas chromatographic techniques linked to the detection systems, the improved chromatographic resolution has subsequently provided pure IR and mass spectra to use in the qualitative understanding of complex mixtures. The possibility of comprehensively performing qualitative analysis on a complex mixture using both IR and mass spectral techniques has been demonstrated. Of course, one consequence of such linked systems is

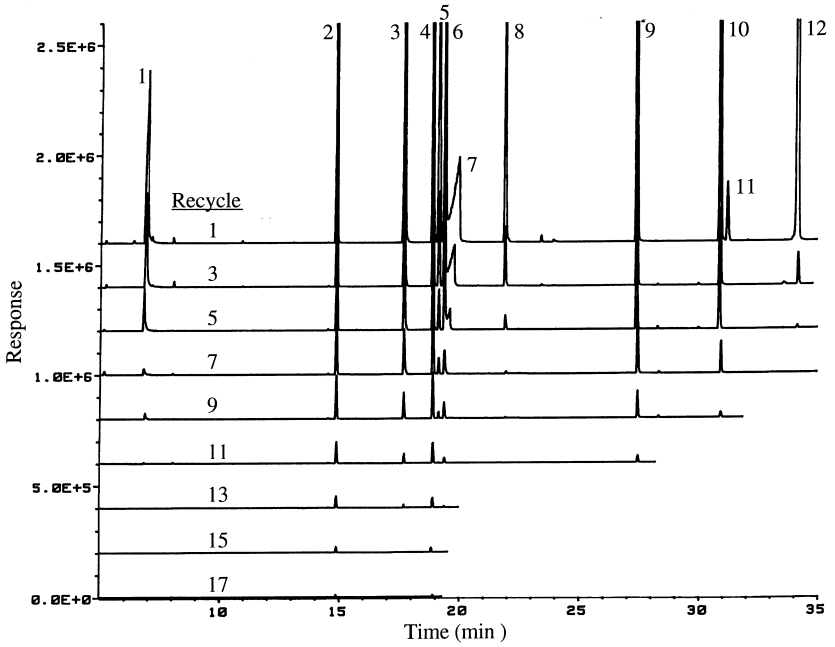

Fig. 15. MS total ion chromatograms of the Grob test mixture recycling experiments. In order to improve clarity, only every other separation is shown. Peak labels correspond to components listed in Table 5. (Used with permission from Ref. [46].)

the enormous quantity of spectral data that are produced during a single chromatographic analysis. With the addition of chromatographic techniques such as sample recycling, heartcutting, and GC^n, the amount of data is further increased, and it becomes increasingly difficult to turn the data into useful information. To this end, advances in computer data analysis provide a method with which the raw spectral and chromatographic data can become accurate and useful qualitative information.

6. Data analysis methods

Gas chromatography combined with IR spectroscopy and/or mass spectrometry produces an abundance of data, but until recently, little could be found in various instrument manufacturer's software that went beyond library search and target analysis. Sophisticated information extraction and pattern recognition methods were performed with additional software packages, or programmed by the analysts themselves. Now, it seems that a critical mass has been reached in chemometric research, and new methods and applications are reported much more frequently and are moving into the mainstream of data analysis for GC–IR and GC–MS. This section deals with articles focusing either directly on new data treatment methods for GC–IR and GC–MS, or chemometric methods developed for IR and mass spectra and then applied to GC–IR and GC–MS problems. In addition, methods are reviewed that attempt to deconvolute IR or mass spectra of mixtures, even if they were not published in the context of separation science. An excellent recent review article [169] observes that ''Most of the articles cited, in this review period (1996–1998) involved applications of chemometric methods.'' Thus, with the continued developments of coupled separations and spectroscopy and computer advances, the emphasis is shifting toward applications, as analysts are confronted with the need to evaluate the very high volumes of information coupled techniques provide.

6.1. Databases and library search

The most popular method of spectral interpretation and identification remains the comparison of sample

Table 5
Recycling study mixture components and result summary[a]

Mixture	Peak no.	Component	Quantity injected (ng)	Maximum no. of recycles		R^2 for linear regression of 10 recycles		Slope-based recycle (%)	
				MS	IR	MS	IR	MS	IR
Grob mixture	1	2,3-Butanediol	530	11	12	0.991	0.950	64	66
	2	Decane	280	18	18	0.999	0.999	75	77
	3	1-Octanol	360	13	16	0.997	0.999	69	73
	4	Undecane	290	18	18	0.999	1.00	74	76
	5	Nonanal	400	10	12	0.991	0.996	60	66
	6	2,6-Dimethylphenol	320	13	12	0.993	0.999	65	69
	7	2-Ethylhexanoic acid	380	6	6	0.957	0.990	45	60
	8	2,6-Dimethylaniline	320	8	6	0.992	0.996	46	50
	9	C_{10} acid methyl ester	420	12	11	0.991	0.993	64	65
	10	C_{11} acid methyl ester	420	9	9	0.943	0.971	48	55
	11	Dicyclohexylamine	310	1	1	N/A	N/A	N/A	N/A
	12	C_{12} acid methyl ester	410	6	5	0.993	0.993	32	37
Hazardous substances mixture	13	Aniline	500	10	10	0.989	0.989	60	58
	14	Benzyl alcohol	500	11	10	0.990	0.991	61	62
	15	*p*-Chloroaniline	500	6	8	0.993	0.970	45	50
	16	2-Methyl-naphthalene	500	11	8	0.971	0.972	59	68
	17	*m*-Nitroaniline	500	2	6	1.00	0.959	17	45
	18	Dibenzofuran	500	4	2	0.956	1.00	25	46
Phenol mixture	19	2-Chlorophenol	500	16	16	0.991	0.983	73	73
	20	2-Methylphenol	500	13	9	0.981	0.988	70	71
	21	4-Methylphenol	500	13	12	0.972	0.992	64	66
	22	2,4-Dimethylphenol	500	13	12	0.972	0.992	66	68
	23	2,6-Dichlorophenol	500	11	10	0.938	0.976	57	61
	24	2,4,5-Trichlorophenol	500	5	5	0.990	0.981	33	40
	25	2,3,4,6-Tetrachlorophenol	500	2	3	1.00	0.981	49	22
Base–neutrals mixture	26	*N*-Nitrosodimethylamine	500	12	11	0.993	0.989	70	69
	27	2-Chloroethyl ether	500	14	14	0.998	0.988	78	79
	28	2-Chloroisopropyl ether	500	14	14	0.970	0.999	80	80
	29	*N*-Nitrosodipropylamine	500	12	12	0.988	0.998	62	65
	30	2-Chloroethoxymethane	500	14	13	0.999	0.999	72	72
	31	Dimethyl phthalate	500	10	10	0.987	0.996	52	56
	32	Diethyl phthalate	500	9	6	0.909	0.986	46	32
	33	4-Chlorophenyl phenyl ether	500	6	6	0.969	0.982	30	30
Chlorinated hydrocarbon mixture	34	1,3-Dichlorobenzene	500	23	20	0.998	0.999	78	77
	35	1,4-Dichlorobenzene	500	23	20	0.999	0.998	76	75
	36	1,2-Dichlorobenzene	500	23	19	0.998	0.999	79	78
	37	Hexachloroethane	500	15	22	0.996	0.997	75	75
	38	1 2,4-Trichlorobenzene	500	23	14	0.997	0.999	71	71
	39	Hexachlorobutadiene	500	15	16	0.996	0.999	80	79
	40	2-Chloronaphthalene	500	12	4	0.987	0.962	35	36

[a] Adapted with permission from Ref. [46]; N/A, not applicable.

spectra with collections of reference spectra. Recent reviews cover a wide range of databases and search algorithms [170–172], and an in-depth comparison of mass spectral identity search algorithms applies five different methods to the same set of GC–MS problems [173]. Furthermore, the problems concerning spectroscopic data handling, quality control, and exchange between vastly differing computer systems have been illuminated [174]. A somewhat older review discusses the automated interpretation of vibrational spectra with library search, pattern recognition and knowledge-based systems [175].

Macro programming for increased productivity with GC–MS software and higher laboratory throughput has been the topic of several publications [176–181]. Pesticide screening in ground water was conducted with electron-capture (ECD), nitrogen–phosphorus (NPD) and mass-selective detection (MSD). In order to reduce library search time and increase the hit quality, a dedicated library only containing 360 pesticides was used. Using retention time and mass spectral information, adequate results were obtained for 1 ng injected material even when closely eluting matrix compounds exhibited a 30 times higher signal than the analyte of interest [176]. Road safety was the topic of a paper on screening 120 medicines and social drugs in blood plasma samples with GC–MS. The presence of a target drug was established if two characteristic mass spectral peak appeared within a given retention window, and if the area ratios from the peaks of their ion chromatograms matched a predefined value range. This was accomplished with a GC–MS software macro and worked for full scan as well as selected ion monitoring mode (SIM) [177]. The macro program AUTARG for target compound recognition in environmental samples uses a similar approach. Spectral searches are conducted in dedicated pollutant libraries, and for target compounds with excellent retention time correlation, but poor library search results, a deconvolution is carried out: Shifts in the reconstructed ion trace for characteristic ions from the target and unknown compounds reveal matrix overlaps, which are corrected [178]. A subsequent application of AUTARG to trace pollutant analysis in groundwater demonstrates the usefulness of this macro [179]. The analysis of fire debris samples has been automated with a macro, which generates ion profiles for characteristic target compound ions, and uses mass spectral library search as well as retention information for compound identification [180]. A complete listing of this macro for the Unix- and Pascal-based Hewlett-Packard GC–MS instruments has been published [181]. An analogous program for Hewlett-Packard instruments employs mass spectra and retention indices for compound identification [182] and has been applied to essential oil analysis [183].

Most of the research on library search for IR spectra in conjunction with separation has been conducted for identifying spectra of mixtures. A method (‘mix-match search’) based on principal component regression (PCR) first finds the greatest similarity between the unknown mixture and a library spectrum. For subsequent identification of the remaining compounds of the mixture, all similarities of the first target compound are removed from the unknown spectrum and a selected subgroup of the library spectra, and principal component regression is applied again. This procedure is repeated until the residual similarity of the remaining mixture spectrum with a library spectrum falls below a predetermined threshold. All constituents of five two- and three-component mixtures were identified correctly with two small libraries of 103 and 256 IR spectra, respectively [184]. In larger libraries, spectra are no longer completely independent from each other, and too many similar spectra would be retrieved as candidates for a mixture constituent. Therefore, PCR is used to prefilter a 3169-compound library to obtain a subset of spectra, before the dot product metric selects a target compound. This mix-match search for mixture identification was also compared with four other similarity metrics and found to be superior for mixture identification from IR spectra [185]. A different approach to mixture identification via library search employs peak table comparisons. With subsequent least-squares regression, two- and three-component mixtures of carbohydrates were not only identified from the IR mixture spectrum, but also successfully quantified [186].

6.2. Retention index

Running a GC–MS instrument and simply using

the gas chromatograph as a convenient separating sample feeder for a mass spectrometer makes sense only with simple mixtures; the same is true when the mass spectrometer is seen as just an elaborate GC detector. Therefore, the synergistic use of both retention and mass spectral information continues to be researched. Special attention goes to the calculation and prediction of retention indices, which unfortunately still remains largely in the empirical domain.

The problem of poor retention index reproducibility between different laboratories and separation columns was addressed with the compilation of a specialized database for GC–MS trace analysis. Most retention indices in that database were averaged from a multitude of sources. Thus, a standard deviation and a defined degree of uncertainty were obtained for the retention time. Together with a dedicated mass spectral library, which was reduced to about eight peaks per spectrum, satisfactory results for target analysis of air pollutants were reported [187]. The often sporadic retention information in mass spectral databases hampers GC–MS analyses to a certain degree, and retention index calculation from first principles has had very limited success. Instead of using a large number of linear parameters, an equation based on the logarithmic treatment of the boiling point, and linear additive factors such as molecular mass and number of carbons has been proposed. Equation coefficients for 42 different compound classes are reported for nonpolar and polar stationary phases, yielding satisfactory retention index results [188]. This work has been extended to illuminate the informational value of physico-chemical constants for retention index calculation, especially from partition coefficients [189]. A prediction scheme for retention indices and temperature gradients of 94 C_9–C_{12} alkylbenzenes has been proposed, helping to overcome the sometimes imperceptible differences between their mass spectra. It uses the molecular mass as a basic parameter, augmented by ten tuning factors for all benzene substitution positions, and finds an excellent agreement between experimental and calculated values [190]. Combined use of spectral and retention information has received much less attention for GC–IR instruments, and only one paper has been found over the time period covered by this review. This paper describes a two-dimensional library search routine for IR spectra/Kovats index [191].

6.3. Pattern recognition, expert systems and artificial intelligence

An area of intense research activity appears to encompass various chemometric methods, which attempt to go beyond mere library comparison for spectral interpretation and identification. However, all these methods derive the chemometric relationship between spectra and structures from spectral collections and can be viewed as ‘indirect’ library usage. A review of such spectral interpretation algorithms for mass, IR and NMR spectra appeared recently [171]. An older survey compiles automated interpretation attempts of vibrational spectra with library search, pattern recognition and knowledge-based systems [175].

An interesting paper describes the design and application of an expert system for GC–MS arson analysis. Based on the presence and absence of a numerous compounds, compound classes, mass spectral peak intensities and ratios, a chromatogram may be classified into one of five fire accelerant categories, taking into account various degrees of evaporation. For a final decision, similar chromatograms of the same accelerant class can be compared. The expert system fared well under varying experimental conditions and only failed occasionally when faced with overwhelming matrix interferences [192]. Locating the double bond in monounsaturated C_{14}-acetates from GC–MS data is a problem frequently encountered in pheromone analysis. Because mass spectra for all 13 different positional isomers are very similar, a fuzzy decision technique has been developed. Five intensity ratios of several neighboring mass spectral fragments are calculated for each of the 13 isomers, and unknown spectra could be identified by calculating a similarity index between the intensity ratios of the unknown and the reference spectra. It was found that this method worked well using a quadrupole or a single-focusing mass spectrometer as a mass selective detector [193]. An attempt to identify the sources of hydrocarbon spills from their GC–MS data has been made using pattern recognition techniques. For this purpose, several different jet fuel classes were analyzed and

their chromatograms separated into 63 retention windows. From principal component scores of this data, statistical isolinear multicategory analysis (SIMCA) classifiers proved to be a 100% reliable tool for neat fuel classification, when factors associated with instrumental drift had been blocked out. A simulation of actual spills involved the analysis of water contaminated with the jet fuels. After elimination of descriptors with low discriminatory or modelling power, SIMCA was able to classify 97% of the samples correctly into four jet fuel classes [194]. Noise reduction for GC–MS data with principal component analysis (PCA) was the theme of a paper dealing with postprocessing of mass spectra. This 'NIPALS' algorithm uses the first six to eight PCA scores to increase the signal-to-noise ratio (S/N) in mass spectra obtained from components eluting from a GC column. Compared with a 10-point Savitsky-Golay smoothing, NIPALS yields somewhat better S/N improvement for lower analyte concentrations, ranging between a factor of 2 and 5. For a high raw S/N of 2300, NIPALS polished this number by a factor of 120. Not surprisingly, precision, linearity and PBM library search quality improved as well, although only marginally. Unfortunately, for these figures of merit, no comparison with traditional smoothing algorithms was carried out [195]. A quite unusual study was presented in a paper on flavor classification and quantification of GC–MS profiles from orange aroma. Several different aqueous orange aromas were presented to an eight-member sensory panel for olfactory classification ('grassy-green', 'floral-woody', etc.), quantification within each of the flavor classes ('non-detectable' to 'extremely strong'), and overall quality impression ('strong reject' to 'excellent'). Various pattern recognition techniques were used to correlate the subjective olfactory analysis with GC–MS data from the orange aromas, and SIMCA returned the best training set results, compared with PCA, non-linear mapping, and *k*-nearest neighbor methods. In order to achieve a better model for varying olfactory sensitivity to different aroma classes, the data was treated with variance and Fisher weighting, with the latter yielding somewhat better aroma predictions. A GC–MS analysis of a test aroma was classified to belong to some of the olfactory classes and even allowed quantitative assignment of some specific compounds to these classes [196]. Some questions commonly associated with the interpretation of pattern recognition results ask about the reliability of algorithm answers and the value of discriminants and distinguishing features. A new algorithm for discriminant analysis (IFRAC, individual feature reliability approach to classification) similar to the Fisher discriminant analysis has been developed, trying to address the question of classification ability of individual features or 'chemical markers'. Comparative analysis with cross-validation and training/test sets of GC or GC–MS datasets was performed (carbohydrates, pyrolyzed sugars and cells), showing a better performance with fewer features of IFRAC than the Fisher discriminant analysis in most cases [197]. This new algorithm was put to work in a study which used pattern recognition to enhance the fingerprinting of pyrolysis–GC–MS data from nucleosides. Prior to applying distance measures between an unknown and a reference chromatogram, all significant chemical markers or peaks were extracted from chromatograms and mass spectra to reduce the dimensionality of the problem. The Euclidean and Mahalanobis distance metrics subsequently identified unknown chromatograms, with mass spectra used for cross confirmation [198]. 'Chemometric detectors' are specialized computer procedures, set up to extract the presence or absence of a structural feature from spectral information. Several of these detectors have been developed for mass spectra in conjunction with GC. Their application for selective group chromatograms was demonstrated with chlorophenol, nitrophenol and aliphatic hydrocarbon detectors [199]. A comparison of automated GC–MS analysis methods was presented in a report on the identification of chlorinated phenols. A chemometric detector, capable of determining the number of chlorine atoms from a mass spectrum, seemed to produce the most reliable results, followed by classical library search. In addition, a pattern recognition procedure, which could categorize eight chlorinated phenol classes, had been trained with characteristic ions of these classes and clustered them into groups. The classes were recognized by calculating the distance between an unknown spectrum and the class clusters. However, this method turned out to be the least reliable method for automated spectral analysis, because less than half of the chlorinated phenols were recognized correctly in a model mixture [200]. Much work has revolved

around the development of pattern recognition and expert systems for molecular mass estimators from GC–MS data [201–206]. An empirical rule-based system estimated the lower limit for molecular masses from the highest mass with an intensity of at least 5% of the base peak (MAXMASS). It uses ternary intensity encoded mass spectra: In the range 0–4.99% intensity of the base peak, 0.0 will be taken as the lowest ternary intensity; peaks in the range 5–49.99% are 0.5, and the rest are assigned 1.0. For a test set of 400 NIST spectra, MAXMASS was higher by two or more mass units for only 4.8% of all spectra. This rule was incorporated into an existing expert system. For a better estimate of the true molecular mass, MAXMASS was adjusted with empirical constants for six compound classes identified by the expert system. Compared with the mass spectral interpretation program STIRS [207], the expert system yielded worse accuracy with library reference spectra. However, for actual field GC–MS data, the average absolute deviation from the true mass was better than STIRS, and a pronounced speed advantage was observed for the expert system [202]. Frequently, the molecular ion is of low intensity or absent altogether. Therefore, the previously developed MAXMASS rule was extended to incorporate further 'fudge factors' within the given compound classes of the expert system. As a result, the median absolute deviation from the true mass improved somewhat compared with the previous work [201]. For a large-scale evaluation, the expert system has been tested with 31 378 mass spectra from the NIST collection, and mass spectra of 400 pharmaceutical compounds [204]. A subsequent rule addition to the expert system set a higher limit to molecular weight estimates (HIMAX1), so that the true molecular weight was bracketed between the lower limit MAXMASS and the higher limit HIMAX1, which had been defined as the highest mass peak with at least 1% intensity of the base peak. This cut the true mass calculation error to values below the ones obtained with STIRS from library reference spectra [203]. These rules were extended with a misclassification filter, and tested with pharmaceutical and field GC–MS data [206]. A more detailed description of the misclassification filters has been published as well [205].

Much less activity for combined GC–IR pattern recognition approaches has been reported in the literature, although IR spectroscopy remains a popular research topic in chemometrics. The fairly direct relationship of band positions to functional groups simplifies the development of chemometric detectors. PCA scores were used to feed a sigmoidal discriminator, determining the presence or absence of functional groups during a GC–IR run from IR spectra. For every GC time interval, the result from the sigmoidal discriminator was multiplied with the reconstructed chromatogram intensity obtained from Gram–Schmidt orthogonalization. These functional group specific chromatograms were plotted for aldehydes, ketones, carboxylic acids, esters and alcohols, showing good discrimination capabilities between these substructures. Thus, they are more specific than selected wavelength chromatograms, which can be set to detect, e.g. carbonyls, but are unable to distinguish between ketones, aldehydes, carboxylic acids and esters [208]. A simpler approach to overcome the shortcomings of selective wavelength scanning for functional group detection from GC–IR was demonstrated with the development of a chemometric detector for aromatic compounds. A set of 30 reference IR spectra containing the aromatic functionality was converted into peak lists and scanned for commonly appearing peaks, basically compiling the group frequencies for aromatic compounds automatically. Although three different spectral regions provided a somewhat reliable measure to detect the occurrence of aromatic compounds, the separation between present and absent functionalities was unsatisfactory due to a large number of false positives [209].

6.4. Quantitative determination

The topic of quantitative analysis using GC with various detectors is discussed in more depth by other papers in this issue. This section only describes unusual mathematic procedures or novel applications for quantitation purposes with IR and mass spectra. Neural networks were employed to determine the amount of casamino acid mixtures in glycogen from their pyrolysis mass spectra, which had been scanned between 51 and 200 u. Each nominal mass constituted one input node. With eight hidden and one output node, the neural network was trained with the backpropagation algorithm to minimize the output error to approximately 0.005. It was found that the

network possessed good interpolation capabilities when trained with equally spaced concentration values, but yielded less satisfactory results for extrapolating to concentration values outside its training range [210]. An attempt had been made to determine the number of Cl, Br and S atoms in molecules from their mass spectra. It employed heuristic algorithms for isotope cluster analysis, worked for up 10 chlorine, six bromine and six sulfur atoms, and could be used as a chemometric detector to obtain atom-specific reconstructed chromatograms. Its accuracy level for chlorine- and/or bromine-containing compounds ranged mostly between 90 and 100%, but rarely exceeded 50% for sulfur [211]. With a similar result in mind, isotope ratios of chlorine were determined with SIM as a chemometric tool, scanning the $(M+2n)^+$ ions ($n=$ 0, 1, 2, ...). Intensity errors between two adjacent ions were taken into account as well, which were caused by changing analyte concentration during elution from the GC column, and it was possible to characterize a PCB mixture and determine between two and six chlorine atoms correctly even for coeluting compounds [212]. Detection limits of GC–MS instruments vary considerably with mass spectral acquisition mode. A detailed study for the detection of organotin compounds was carried out, comparing full-scan total ion chromatograms, selected ion chromatograms from full scans (post-acquisition data processing), and selected ion chromatograms from SIM (pre-acquisition data processing). Limits of detection were approximately 10 ng (full-scan TIC), 2 ng (selected ion chromatograms), and 0.03 ng (SIM) injected organotin compounds [213]. Another quantitation study for GC–MS went through theoretical considerations of 'decision limits' (present/absent) and 'detection limits' (how much when present). Detection limits of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were determined in three different ways, using SIM responses from m/z 322 alone, combining m/z 320+322, and calculating the first score of the principal component analysis of three SIM ions m/z 257, 320 and 322. Observing just m/z 322 was found to be as sensitive as the first PCA score of three SIM traces, although the differences between all three ways was not very pronounced [214]. Full-scan and peak top SIM mass spectral acquisition modes have their advantages for compound identification and determination, but also exhibit some distinct shortcomings. As a compromise for high-resolution double-focusing mass spectrometers, mass profiles (MP's) can be monitored which use the entire peak curve of a predetermined m/z value instead of its apex alone. A much higher certainty in accurate mass measurement was achieved in the MP mode, despite scanning at lower resolving power to compensate for the loss of sensitivity compared with peak top SIM. It was demonstrated that the MP mode could detect coeluting interferences from centroid shifts when several mass profiles were observed simultaneously, and instrumental stability was much less critical than for peak top SIM mode [215].

6.5. Deconvolution of gas chromatographic peaks

Coeluting species are a common sight in complex chromatograms. The possibility of deconvoluting chromatographic peaks representing two or more compounds is one of the distinct advantages of specific GC detectors such as IR and mass spectrometers. They provide the additional dimensionality of wavelength or mass-to-charge ratio. It has been mentioned in earlier subsections of the data analysis part of this review, that one may follow the traces of specific ions in order to distinguish between coeluting compounds [179,180,216]. A simple, but very powerful method was introduced to deconvolute a quite astonishing number of compounds per total ion chromatogram peak, fully utilizing the two dimensions of time and mass-to-charge. After acquisition of an entire GC–MS run, mass chromatograms were reconstructed for every nominal mass in the scan bandwidth, and time centroids were calculated for each chromatographic peak in every mass chromatogram. A 'deconvoluted total ion chromatogram' (DTIC) was reconstructed from these centroids with a resolution of 0.1 scans, summing up all centroids of at least 1% of the base peak within the same 0.1 scan retention window, yielding a standard deviation of 0.04 scans. This improved the reconstructed chromatographic resolution by at least an order of magnitude. Using the DTIC trace, a deconvoluted mass spectrum was composed by picking all mass centroids belonging to the same retention window. Fig. 16 demonstrates the validity of this approach

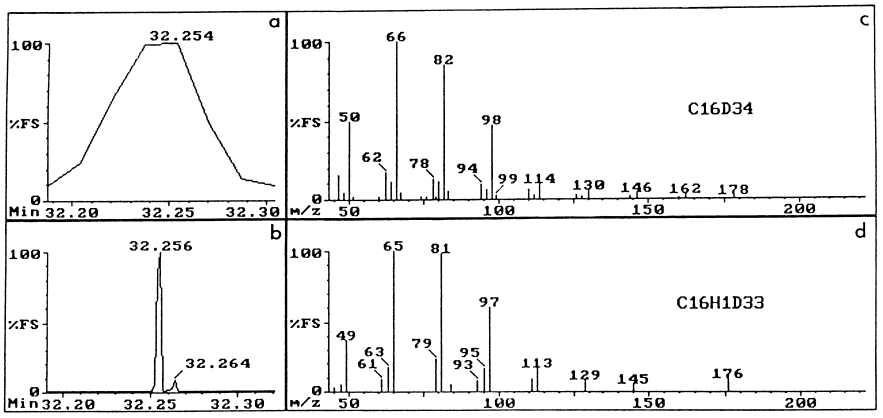

Fig. 16. Chromatograms and mass spectra of [$^{2}H_{34}$]hexadecane. (a) TIC trace; (b) DTIC trace; (c) deconvoluted mass spectrum of [$^{2}H_{34}$]hexadecane; (d) deconvoluted mass spectrum of [$^{2}H_{33}$]hexadecane. These data were acquired with a 1.0 s scan cycle time. (Used with permission from Ref. [216].)

with the deconvolution of [$^{2}H_{33}$]- and [$^{2}H_{34}$]hexadecane spectra, originally only 0.48 scans apart, but baseline resolved as chromatographic peaks in the DTIC [216]. Restoring accuracy of overlapping, but still recognizable peaks in GC–combustion–isotope ratio mass spectrometry was the goal of a comparative study of several chromatographic curve-fitting equations. The Haarhoff–VanderLinde function (HVL) appeared to yield the best results, with an exponentially modified Gaussian function (EMG) coming in second. The combination HVL/EMG for the first/second overlapping peak yielded the best accuracy for calculating the $^{13}C/^{12}C$ isotope ratio [217]. Unresolved complex hydrocarbon mixtures appear frequently in oil and gasoline analysis and complicate the source identification, when spills of these products are investigated with fingerprinting GC–MS methods. Instead of refining chromatographic or chemometric deconvolution methods, these hydrocarbons were oxidatively degraded, leading to a more separable mixture. Using a Euclidean distance metric and cluster analysis, a successful correlation was achieved between hydrocarbon oxidation products and their original sources [218]. Evolving factor analysis was applied for the first time to the deconvolution of mass spectra obtained from a GC separation. Taking into consideration the changing analyte concentration over a mass spectrum scan time, the procedure resolved several good quality mass spectra out of a single GC peak envelope, but no quantitative assessment was given on the minimum chromatographic resolution required, or the improvement achieved [219].

GC–IR deconvolution methods are relatively time-consuming, considering the much larger number of datapoints produced for an IR spectrum, as compared to a mass spectrum. Still, a few method applications to GC–IR deconvolution could be found in the literature. The condition index evolving profile performed a singular value decomposition between each unknown IR spectrum of a GC trace and every library spectrum, obtaining a match indicator for all library spectra. The best match was chosen, its contribution calculated at every point of the elution profile, and subtracted from all unknown spectra. This procedure was repeated until no more compounds could be identified from the elution profile. With this method, two- to four-component mixtures were successfully resolved and identified [220]. Nonlinear Gaussian and Lorentzian curve fitting was carried out on GC–IR elution profiles for extracting coeluting compounds and on IR spectra for quantitative determination with IR bands. This was accomplished with ‘eigenstructure tracking analysis’ (ETA), an algorithm similar to PCA, but a good estimate of the number of peaks/bands contributing to the resulting composite was required [221]. This restriction was removed by applying local PCA to a

retention window, determining the number of analytes at every retention time and/or wavelength [222].

6.6. Analysis of mixture spectra

Somewhat related to hyphenated techniques is the analysis and decomposition of mixture spectra which were not recorded from compounds introduced into a spectrometer via separation techniques. These spectra cannot be analyzed with methods which depend on spectral variables (wavenumber, mass-to-charge ratio) changing over time within a GC peak. However, there are unfavorable cases when compounds coelute at nearly the same retention time, making it necessary to treat the composite spectra as mixture spectra. Only one paper on mass spectral mixture deconvolution was found up until December of 1994: it analyzed spectra from biomass pyrolysis with the 'pure variable' concept, assuming that at least a few of the peaks in a mass spectrum originate from only a single compound. The method was called 'simple-to-use interactive mixture analysis' (SIMPLISMA) and measured the purity of a variable (e.g. a certain m/z) with the ratio of standard deviation to mean. When all pure variables were found, the contribution of the compounds to the mixture spectrum was calculated by solving multiple equations with multiple variables. At least five mass spectra could be extracted from the pyrolysis mass spectrum, although considering the number of compounds usually generated by pyrolysis, this value appears to be quite low [223].

Judging by the number of papers found on the analysis of IR mixture spectra, it seems to be much easier to approach that topic. This makes sense because it is easier to determine the noise level in continuous signals such as IR spectra, than to estimate noise in discrete signals like stick plot mass spectra. In the subsection on library search, an approach to this problem with PCA has already been introduced [184,185], and peak table comparisons between mixture and library spectra seemed to be similar to the pure variable concept [186]. The SIMPLISMA approach (see above) was also applied to near-IR data, using the second derivative of the spectrum instead of the original data to get rid of severe baseline fluctuations [224]. In FT-IR microscopy, SIMPLISMA was applied to analyze a multilayer laminate, with layers ranging between 2 and 175 μm thickness. The thinnest layer could not be resolved with conventional IR microscopy techniques, but spatial variance in the collected IR spectra was used in conjunction with SIMPLISMA to identify the composition of that layer [225]. An expert system guided the deconvolution of vapor-phase IR spectra and compound identification, comparing found IR spectral bands to stored band information (intensity, position, width) of previously encountered spectra. The expert system reported the likelihood of presence or absence of a reference compound in the mixture, but was never able to analyze mixtures 100% correctly within any concentration range [226]. A 'soft' decomposition algorithm was proposed using iterative spectral exhaustion. It compared the condensed-phase IR spectrum of a mixture of three carbohydrates to nine reference spectra, selecting the most likely constituent. A small fraction of this compound was subtracted from the mixture spectrum, and its accumulated concentration increased by that fraction. Then, the next iteration was started, again finding the most likely compound from the nine reference spectra. Within ~150 steps, the original mixture spectrum reached very low intensity values, the procedure was stopped, and the concentration values of the original mixture constituents (0.33 each) were found to be in good agreement with the numbers obtained by iterative spectral exhaustion (0.30, 0.30, 0.39). This method worked well for a very limited number of target compounds, but for a reasonable IR database size, the computing time would be prohibitive [227].

6.7. Miscellaneous

Two reports deal with the design and development of data systems for GC–MS systems. The scope of these papers is rather narrow, so they will be mentioned only briefly here. An old MX-1321 GC–MS system out of Soviet production was retrofitted for modern mass spectral data analysis, and its outdated computer with 64 kB RAM replaced using an IBM-compatible personal computer [228]. The capabilities and operation of the commercial software package 'Target Compound Analysis Software' (TACO) for Finnigan/MAT GC–MS systems was

described in a publication. Given its contents and the fact that it does not contain a single reference, it may rather be viewed as a software tutorial than a research publication [229].

In a comprehensive approach, the simulation of GC–MS data was reported. Working from stored reference mass spectra of known compounds, it simulated the signal generation and acquisition of mass spectral peaks from GC eluents. Combining numerous equations of theoretical and heuristic origin, 33 parameters could be adjusted for the simulation, e.g. chromatographic peak shape with fronting/tailing, type of column and column bleed, electronic noise, chemical noise from minor components, etc. Compared with real GC–MS data, a high degree of similarity was found [230]. Total analysis of an unknown complex mixture remains a task fraught with uncertainties and unsatisfactory results. Spelling out several rules, a classification system was proposed for four identification levels of organics from GC–MS analysis. *Confirmed* identification encompasses high similarity of mass spectra and retention time when compared with pure reference data. A discrepancy in retention time, but a good library match for mass spectra constitutes a *provisional* identification. If no reference spectra can be found in a library, sufficient partial information leads to *compound class* assignment, otherwise it must be labeled *unidentified*. The application of this classification system was demonstrated with a GC–MS sample run of water pollutants [231]. Spectral resolution for the acquisition of GC–lightpipe FT-IR spectra is generally limited to 8–16 cm^{-1} resolution to accommodate the collection of several spectra while a GC eluent flows through the lightpipe. Unfortunately, many spectral details are lost this way, which are needed in some cases for unambiguous identification or in-depth spectral interpretation. Fourier self-deconvolution was applied as a tool to determine vapor band contours from these low-resolution IR spectra, obtaining a good agreement with high-resolution literature spectra [232]. In GC–IR analysis, it is common practice to increase the analyte signal intensity by coadding several IR spectra collected around a GC peak apex, instead of using a single spectrum from the peak top. However, the actual S/N gain depends on the number of background scans. In a study on S/N improvement for GC–IR analysis, it was shown that a maximum S/N gain can be calculated for any number of background scans. For four background scans, coaddition of IR spectra below 80% of the maximum peak height would actually start to degrade the S/N, and if spectra were coadded to below 5% of the maximum peak height, the result would be worse than the single spectrum at the apex. The authors also proposed the application of classical least-squares fit (CLS) to all considered spectra within a GC peak in order to improve the S/N by up to a factor of 2. As Fig. 17 shows, they calculated that CLS improved the S/N—albeit slowly—with the inclusion of more spectra from a GC peak for any

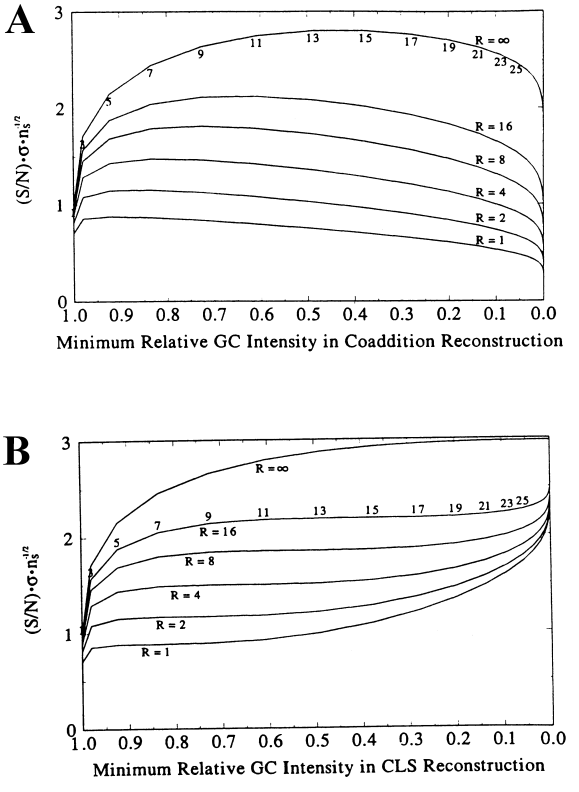

Fig. 17. Scaled S/N for composite spectra generated from the Gaussian GC band. Each curve relates to a single value of R (=number of background scans/number of sample scans). The abscissa indicates the threshold minimum relative intensity of the GC peak (peak apex 1.0). The number of spectra used to form the composite spectrum for a given threshold is indicated below the curve for $R=\infty$: (a) coaddition reconstruction; (b) classical least-squares (CLS) reconstruction. (Used with permission from Ref. [233].)

number of background scans, as long as any signal could be found in the analyte spectra. It was also proven mathematically that $(S/N)_{CLS} \geq (S/N)_{coadd}$ is true for all conditions [233].

6.8. Summary

A general trend is to develop and apply more sophisticated methods for qualitative and quantitative data analysis used with hyphenated techniques. This can be attributed to the general availability of fast personal computers, which are able to handle the enormous datasets generated by spectroscopic detectors. Equipped with enough RAM and disk space, emerging chemometric program packages and spreadsheet capabilities can perform large matrix operations, and computationally intensive calculations move within reach for most chromatographers and spectroscopists. Although an increasing number of chemometric publications and journals touch all aspects of analytical chemistry, chemometrics has only started to move into the analytical chemist's formal education. As as a result, available data analysis methods still tend to be limited to the choices provided by commercial instrument manufacturers.

7. Outlook for the future

It is clear, in the light of this review, that the recent developments in multispectral detection systems for gas chromatographic effluents have provided unrivalled analytical capabilities to analytical chemists. The impressive developments in both GC–MS and GC–FT-IR promise to yield improved analytical power for combined GC–FT-IR–MS in the near future. With the development of fast GC, it would appear that the evolution of a fast GC system coupled to a compact time-of-flight mass spectrometer and a variable-speed deposition-type IR spectrometer is imminent. Because a system like this would provide the fastest and most sensitive IR and MS data acquisition possible, it could conceivably be coupled to a comprehensive two-dimensional gas chromatographic system, and this system would provide a true two-dimensional separation, in addition to complete sets of IR and mass spectral data for each chromatographic peak. However, this is only one possible method to achieve highly accurate total qualitative and quantitative analyses of complex mixtures.

Another possible solution may be the adaptation of the sample recycling technique to a 'fully flexible' MDGC–FT-IR–MS system which would allow selection of the separation characteristics at every stage of analysis. This capability would provide the maximum flexibility in a separation and detection system, and with this flexibility comes powerful qualitative and quantitative possibilities for both target and general analysis. Coincident with improvement in the separation and detection computer systems, there will undoubtedly be impressive advances in the software which will be required to handle the tremendous quantity of data generated by these systems. At some time in the near future, computers may be able to process spectral data on-the-fly and decide, based on rapid spectral searches or neural network analysis, whether or not a separation may be complete. Depending upon the results of this rapid analysis, the computer could control the instrument to, for example, recycle the incompletely separated effluent, trap the effluent in a cryogenic trap and subsequently perform a second-, third-, etc. stage of separation. Additionally, each of the separation-stage selectivity characteristics could be chosen by the computer based on the retention time and spectra of the previous-stage separation.

While these types of linked systems may not benefit some of the more specific target GC–MS, GC–FT-IR or GC–FT-IR–MS applications, there will assuredly be technological crossovers, such as hardware and software, which will benefit the entire analytical community. However, even though applications for these advanced MDGC–FT-IR–MS systems appear primarily qualitative in nature, fields such as flavor and fragrance, environmental, forensic and clinical/medical analysis are increasingly requiring both highly accurate qualitative and quantitative information on several or all components in very complex mixtures. To this end, these types of general analysis systems may become more important, depending on the future demands of these and other fields. True computer-assisted chemical analysis by multidimensional gas chromatography with IR and mass spectral detection appears plausible in the near

future. It also seems to be a practical solution to the problem of total complex mixture analysis, and with its advent, analytical chemists can more easily concentrate their energy on turning computer-generated data into useful chemical information.

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