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REVIEW

GAS CHROMATOGRAPHY WITH FOURIER TRANSFORM INFRARED SPECTROMETRY FOR BIOMEDICAL APPLICATIONS

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LIST OF ABBREVIATIONS

A/D	Analog-to-digital conversion
DRIFT	Diffuse reflectance infrared Fourier transform spectrometry
ECD	Electron-capture detection
EI	Electron impact
EPA	Environmental Protection Agency
FID	Flame ionization detection
FTIR	Fourier transform infrared spectrometry
GC	Gas chromatography
GC–FTIR	Coupled gas chromatography–Fourier transform infrared spectrometry
GC–MI–FTIR	Coupled gas chromatography–matrix isolation–Fourier transform infrared spectrometry
GC–MS	Coupled gas chromatography–mass spectrometry
He–Ne	Helium–neon
HPLC	High-performance liquid chromatography
HS–GC	Headspace gas chromatography
I D	Inner diameter
IR	Infrared spectrometry
ITD	Ion trap detector
MCT	Mercury–cadmium–tellurium detector
MIQ	Minimum identifiable quantity
MSD	Mass-selective detector
PCBs	Polychlorinated biphenyls
SCOT	Surface-coated open-tubular column
SFC	Supercritical fluid chromatography
S/N	Signal-to-noise ratio
TCDD	Tetrachlorodibenzodioxin
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
UV	Ultraviolet spectrometry

1 INTRODUCTION

The first attempts to use infrared spectrometry as a mode of detection in gas chromatography were made prior to the development of the Fourier transform

method. Indeed, the spectrum of molecular vibrations provides important information that can help the chemist to recognize molecular structures However, handicapped by the slowness and lack of sensitivity that characterize dispersive spectrometric techniques, the proposed approaches remained complicated to implement or produced only partial results Fourier transform infrared (FTIR) spectrometry overcame some of the difficulties and the development of rapid scanning interferometers and sophisticated data systems soon permitted the on-line identification of fractions separated using gas chromatography (GC).

The results of the first feasibility study of this technique were published by Low and Freeman [1] in 1967 Since then, there has been spectacular progress in instrumentation, with the result that FTIR detection performance in terms of speed and sensitivity now corresponds to that achieved with GC At the same time, progress was being made here too, thanks to the use of capillary columns These simultaneous advances in FTIR spectrometry and high-resolution GC have given analytical chemistry new tools to complement gas chromatography–mass spectrometry (GC–MS) In the wake of published work on technological improvements have come the applications There are a variety of areas of investigation and it should be noted that biomedical circles are showing signs of interest, although in this direction the applications are very recent and still few and far between.

In presenting the FTIR technique and describing the experimental setup, we shall establish the mutual constraints of FTIR and GC as a result of their coupling The software description will give an account of the information accessible via GC–FTIR. The work published in the biomedical area, extended to include environmental problems and natural molecules, will be analysed to highlight the potential of the technique, but also its limitations, with a view to presenting the solutions contemplated in methods currently being developed

2 THE GC-FTIR SYSTEM

2 1 FTIR spectrometry [2]

Most spectrometers on the market use a Michelson-type interferometer, whose theoretical principle provides a good basis for understanding the interferometry method Fig 1 shows a schematic diagram of the interferometer. Each monochromatic wavenumber component is modulated as a function of the difference in path δ [$\delta = 2(\mathrm{OM} - \mathrm{OF})$] according to

$$I_{\bar{\nu}}(\delta) = S(\bar{\nu})\cos 2\pi\bar{\nu}\delta$$

where $S(\bar{\nu})$ is the radiation intensity In practice, the signal detected represents the sum of the modulations of each elementary component

$$I(\delta)=\int_0^\infty I_{\bar{\nu}}(\delta)\mathrm{d}\bar{\nu}=\int_0^\infty S(\bar{\nu})\cos 2\pi\bar{\nu}\delta\mathrm{d}\bar{\nu}$$

By applying the inverse Fourier transform, we can calculate the spectrum $S(\bar{\nu})$ from the interferogram measured $I(\delta)$

$$S(\bar{\nu})=\int_0^\infty I(\delta)\cos 2\pi\bar{\nu}\delta\mathrm{d}\delta$$

In fact, for physical reasons, the interferogram is limited in space to a maximum path difference of $\Delta_{\max}$ Indeterminacy in wavenumber is thus equivalent to $\mathrm{d}\bar{\nu}=1/\Delta_{\max}$ when an apodization function is applied

Compared with dispersive spectrometry, FTIR is characterized by several advantages, as follows.

Fellgett's advantage The detector receives light throughout the period of measurement from all of the light components The signal-to-noise ratio (S/N) is multiplied by the square root of the number of spectral elements

$$M=\Delta\bar{\nu}/\mathrm{d}\bar{\nu}$$

where $\Delta\bar{\nu}$ is the spectral interval under study and $\mathrm{d}\bar{\nu}$ is the spectral resolution

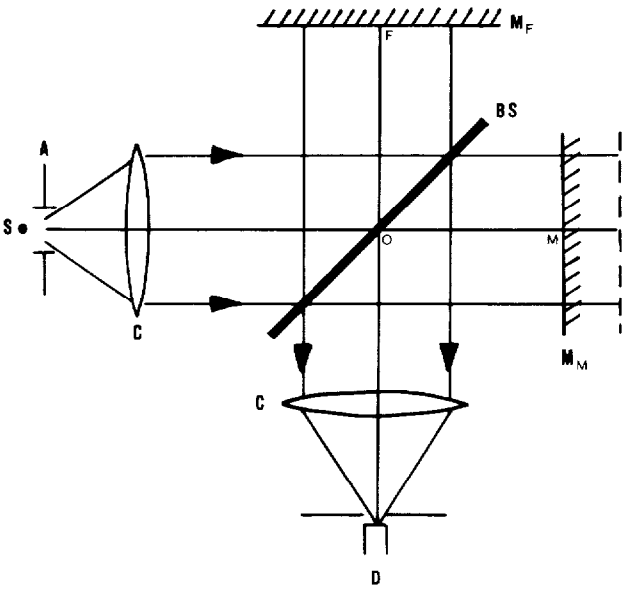

Fig 1 Schematic diagram of a Michelson interferometer S=Source, A=aperture, C, C′=collimators, BS=beamsplitter, M_F=fixed mirror, M_M=movable mirror, D=detector

During GC–FTIR analysis, when the resolution is often 8 cm^{-1} over a spectral range extending from 4000 to 800 cm^{-1}, the theoretical gain is equal to 20

Jacquinot's advantage Resolution of the spectrum is defined by the maximum path difference reached by the interferogram. It is not in contradiction with the optical throughput The light energy transmitted to the detector can be up to 100 times greater than that of a dispersive spectrometer, and this helps to improve the S/N. These two advantages will contribute to an improvement in the detection of weak signals, i e., increase the sensitivity

Connes' advantage The wavenumbers are calculated with very great precision This precision depends on a knowledge of the position of the moving mirror which is identified by means of the interferogram of an He–Ne laser

Speed of acquisition The interferometry method has made it possible to develop high-speed scanning systems. Several dozen interferograms can now be obtained per second using certain systems available on the market This possibility is exploited to increase the S/N by effecting numerous accumulations of signals on samples that do not evolve in time, or to monitor swiftly evolving phenomena such as elution in GC

2 2 GC–FTIR coupling

Continuous measurement of the infrared absorbance of a gas cell and of its contents permits real-time monitoring of chromatographic elution The compounds separated by the column and supplied to the gas cell via a transfer line selectively absorb the infrared radiation emitted by the source (Fig 2) The Fourier transform of the interferogram measured establishes on a real-time basis the absorption spectrum of the eluted compound This spectrum can be used to identify the compound or to check, in quantitative applications, whether the chromatographic peak corresponds to a single compound whose spectrum does not change during elution

2 2 1 Description of the light-pipe

This is a tube made of glass, quartz or metal internally coated with gold to ensure that it is both chemically inert and a good reflector of infrared radiation Azarraga [3] in 1980, followed by Yang and co-workers [4,5], studied the problems linked with the design and configuration of gas cells with a view to optimizing the signal This tube is closed at each end by a window of a material that is IR-transparent There is a major problem regarding the dimensions required for the cell in order to maintain chromatographic resolution while, at the same time, preserving sufficient optical throughput It is agreed that the length/diameter ratio has to be high so as to obtain a laminar gas flow Griffiths et al [6] discussed the volume needed for the cell in relation to the type of column used. This volume must not exceed the volume of carrier gas between the half-height points of the narrowest peak in the chromatogram. This fact tends to

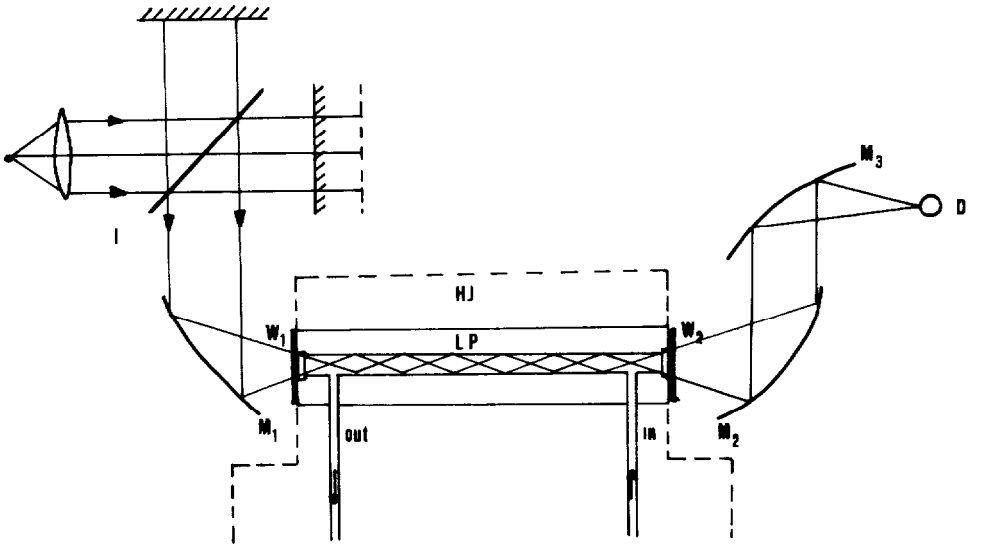

Fig 2 Schematic diagram of a typical GC–FTIR coupling I = interferometer (see Fig 1), M_1, M_2, M_3 = mirrors, W_1, W_2 = IR-transparent windows, in = inlet from gas chromatograph, out = outlet to gas chromatograph, LP = light-pipe, HJ = heated jacket, D = detector

confine use to capillary columns of I D 0.3–0 5 mm and with a film thickness of 0.5–1.5 μm in which a peak volume of 300 μl can be reached. With narrower columns, it may be advantageous to use a make-up gas to preserve resolution, but there is a risk of losing sensitivity. Most manufacturers have finally opted for an I.D of 1 mm and a length of 10–20 cm The problem relating to signal loss when the temperature of the light-pipe rises was solved in 1985 by Brown et al [7] They established that this defect was primarily due to the thermal emission of the cell. When this flux reaches the detector, the latter operates in a non-linear response region The S/N can be improved by installing a device to absorb this unmodulated heat at the output of the light-pipe. These technological advances since this work was published have led to commercial developments that make it possible to lower the detection limits to the 5–25-ng range for highly IR-absorbent samples [8]

2 2 2 The spectrometry viewpoint

Despite numerous attempts to improve the optical qualities of the light-pipe, it has to be admitted that the transmission rate remains in the order of 10% It is therefore important to use a sensitive detector with the experimental setup Most often, use will be made of a so-called "narrow-range" (4000–700 cm^{-1}) mercury–cadmium–tellurium detector (MCT) whose detectivity, D^*, is equivalent to $4\,6 \cdot 10^{10}$ cm Hz W^{-1} at 1 kHz [9] As D^* varies as a function of the modulation rate for the signal detected, allowances have to be made for the

scanning speed of the travelling mirror to optimize the recording conditions We know that, in an interferometer whose mirror travels at a speed V, the component of wavenumber $\bar{\nu}$ is modulated at a frequency given by

$$f_{\bar{\nu}} = 2V\bar{\nu}$$

This value has to be brought into line with the optimum level of detectivity The D^* versus $f_{\bar{\nu}}$ curves rise and end in a plateau, which shows that it is advisable to work at a fast speed (several interferograms per second) This is in accordance with the speed of chromatographic elution, but steps have to be taken to ensure compatibility with the A/D conversion rate, the speed of data transfer on to disk and, finally, the storage capacity, which is likely to be reached rapidly

2 2 3 Software for data collection

It is appropriate to differentiate between software packages permitting spectral data processing and those permitting the monitoring of chromatographic analysis

In the first instance, we use the conventional software of the FTIR spectrometer in which are chosen the experimental parameters that define optical configuration, mirror speed, optical resolution, etc The collection, storage and Fourier transform routines, on the other hand, will be called in the course of chromatographic analysis by the softwares proper to GC–FTIR which is to be discussed here

Two approaches are differentiated in real-time monitoring: one uses interferograms, whereas the other uses spectra The theoretical basis for the first method is the Gram–Schmidt orthogonalization algorithm [10] Briefly, each interferogram is considered as a vector The first interferograms collected before chromatographic injection form the reference vectors for constructing an orthonormed vector base After this stage, each interferogram obtained is considered as a sample vector that is projected in the reference base The variations in the modulus of the linearly independent component of each interferogram in relation to the vector base as a function of the retention time form the chromatogram in real time From the spectroscopic viewpoint, this means that we enter in the reference base all the events that will be common to the study, i e , the emission of the source, the efficiency of the separator and the transmission of the spectrometer and of the cell We measure to see if there exists on a sample interferogram a difference in relation to these common data This difference can then be ascribed to absorption by a compound present in the cell As indicated in the theoretical part (see Section 2 1), the interferogram contains simultaneous information on all the frequencies The chromatogram traced according to the interferograms will not be specific to a spectral region, and hence not specific to chemical functions characteristic of the eluted compounds This method is considered as a general one, leading to chromato-

graphic results close to those obtained using conventional non-specific detection techniques such as FID (Fig 3)

During mathematical processing, for the sake of speed, only a fraction of the interferogram measured constitutes the vector (reference or sample) The effect of each selected fraction (length, position, number of reference vectors) on the S/N of the reconstructed chromatogram was described in detail by Sparks et al [12] The choices made by different manufacturers are discussed but the final conclusion drawn is that there can be no general rule The quality of the results depends on the nature of the mixture to be analysed [13,14]. Hence the suggestions made by De Haseth and Isenhour [10] as early as 1977 are acknowledged as a good choice in most instances 100 interferogram data

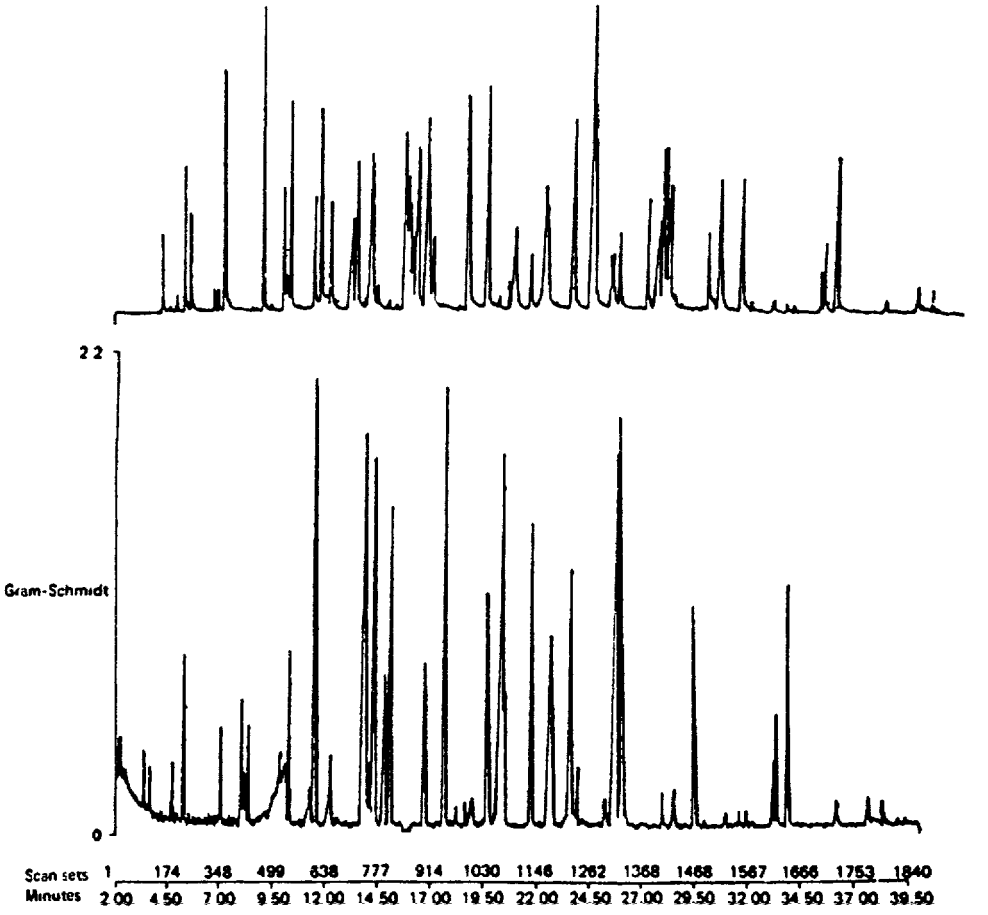

Fig 3 Comparison between Gram-Schmidt (bottom) and flame ionization detection (top) chromatograms of a perfume mixture From ref 11, with permission

points are selected 60 points after the centerburst, only the recommended number of reference vectors (60) is often reduced to 20 or even 10

The other method of monitoring chromatographic analysis is to measure the absorbance of the light-pipe as a function of time In this instance, each interferogram is transformed into a spectrum The single-beam spectrum is first related to a reference spectrum obtained before the analysis, then calculated in terms of absorbance Integration in one or more spectral windows provides information on chromatographic elution This time, the plot (Fig 4) forms a chromatogram specific to the chemical functions of the compounds of the mixture, known as a "chemigram" [15,16] A user who does not specialize in spectroscopy may find it difficult to make the choice expected of him with regard to the spectral regions analysed in real time position and width We have developed a methodology [17] permitting general detection on a routine basis during organic analyses Here again, real-time monitoring imposes speed constraints The Fourier transform will thus be calculated in this stage on the basis of the initial part of the interferogram obtained, generally the first quarter This leads to a very poor resolution spectrum (32 cm^{-1}), on the basis of which

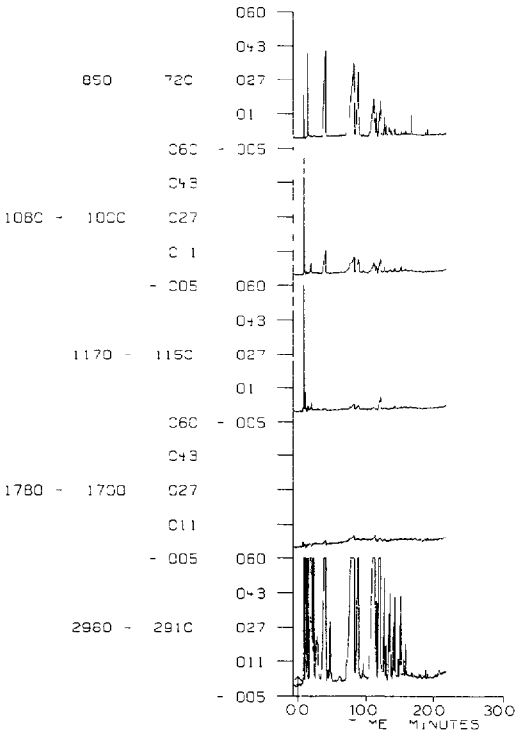

Fig 4 Chemigram™ data from a capillary analysis of petrol fraction IR absorbance is measured in five spectral windows 850–720, 1080–1000, 1170–1150, 1780–1700 and 2960–2910 cm^{-1} For each plot, the y-axis is in absorbance units from − 005 to 060

integrated absorbance is calculated However, the complete interferograms are stored to permit fuller spectroscopic use in off-line operation. This method is open to criticism, first because it does not enable a general chromatogram to be obtained, and second because more mathematical operations are required than with the Gram–Schmidt method [18]. Nonetheless, we think that it can permit an improvement in sensitivity through the choice of a spectral region well suited to the compound under study

2 2 4 Software for reconstruction

As in conventional GC or HPLC practice, the chromatogram can be reconstructed at the end of the analysis on the basis of the experimental data obtained. Certain parameters such as vertical amplitude or the time slot can then be adapted according to requirements The two types of software previously described can be used and the result can be optimized if they are used off-line

With the Gram–Schmidt method (Fig. 5), interferograms obtained in the course of analysis can be added as reference vectors, this makes it possible to incorporate in the reference base any changes occurring during the study. Reconstructions of the integrated absorbance type (Fig 6) can be improved in terms of presentation by redefining the spectral regions characteristic of compounds which would only have been poorly detected in real-time operation

It is possible to apply to the reconstructed chromatograms the software con-

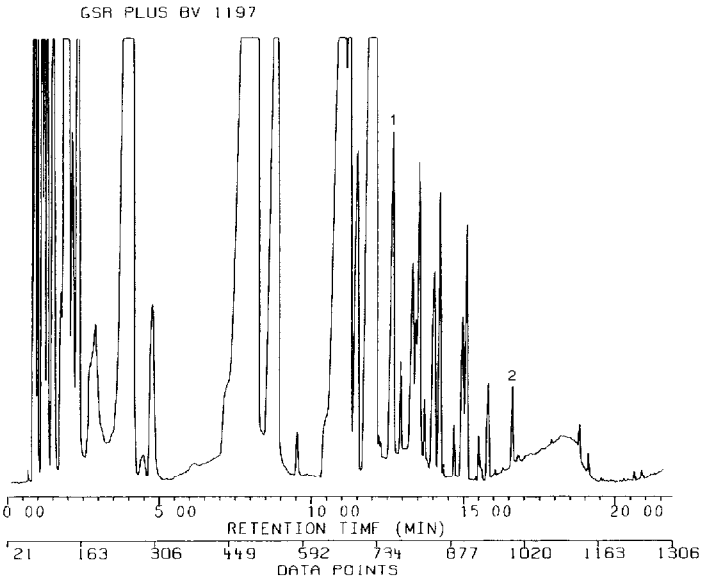

Fig 5 Gram–Schmidt reconstruction of the analysis in Fig 4 Interferogram 1197 (RT = 20 min) has been included as a reference vector in the original basis set

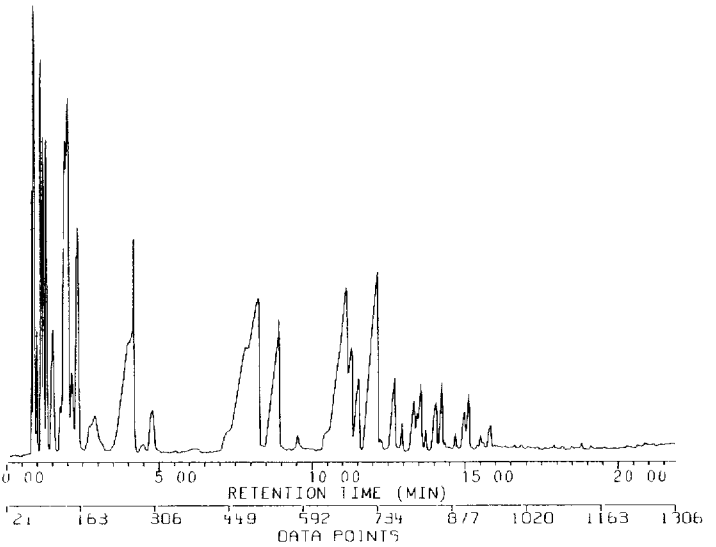

Fig 6 Reconstructed chromatogram performed using integrated absorbance calculation in the 2981–2908 cm^{-1} spectral region

ventionally used in chromatography, i e., peak-picker, integration of areas beneath the peak with a view to quantitative applications [19]

2 2 5 Software for identification

The infrared spectra of the eluted compounds can be calculated by using the interferograms obtained at the apices of the chromatographic peaks The resolution is generally 8 cm^{-1} when the Fourier transform of the complete interferogram is calculated To obtain the transmission spectrum, the reference can be taken either at the beginning of analysis, prior to elution, or from the baseline of the chromatogram at the foot of the peak to be identified The spectrum can be interpreted by the user on the basis of his or her skills in vibration spectroscopy or by automatic interpreters according to the design concepts introduced by Woodruff and Smith [20] Finally, and more frequently, the spectrum is compared with a library of infrared spectra in the vapour phase in order to find the name of the compound that most closely resembles the unknown constituent The first library available was that developed by Azarraga of the Environmental Protection Agency (EPA) [21] It was developed commercially by Sadtler Research Laboratories within the framework of a library that currently contains 1000 spectra An exclusive joint service provided by Nicolet (Madison, WI, U S A) and Aldrich (Milwaukee, WI, U S A) offers a bank containing 5000 FTIR spectra in the vapour phase

From the proposed search software packages, we have chosen a method based on the complete spectra as described by Lowry and Huppler [22] The stan-

dardized absorbance values s_i and r_i for each resolution element (8 cm^{-1}) in the sample and reference spectra are compared by calculating one of the following four indices:

$$I_{AB} = \sum_{700}^{4000} |s_i - r_i|$$

$$I_{SQ} = \sum_{700}^{4000} (s_i - r_i)^2$$

$$I_{AD} = \sum_{700}^{4000} |\Delta s_i - \Delta r_i|$$

$$I_{SD} = \sum_{700}^{4000} (\Delta s_i - \Delta r_i)^2$$

where $\Delta s_i = s_i - s_{i-1}$ and $\Delta r_i = r_i - r_{i-1}$. The differential modes AD and SD are adapted to the spectra for which the S/N is poor, which is often the case when the amounts of product become small

The names of the 1 to 32 products for which the calculated indices are lowest are listed and the spectra can be visually displayed with different presentations (Figs 7 and 8)

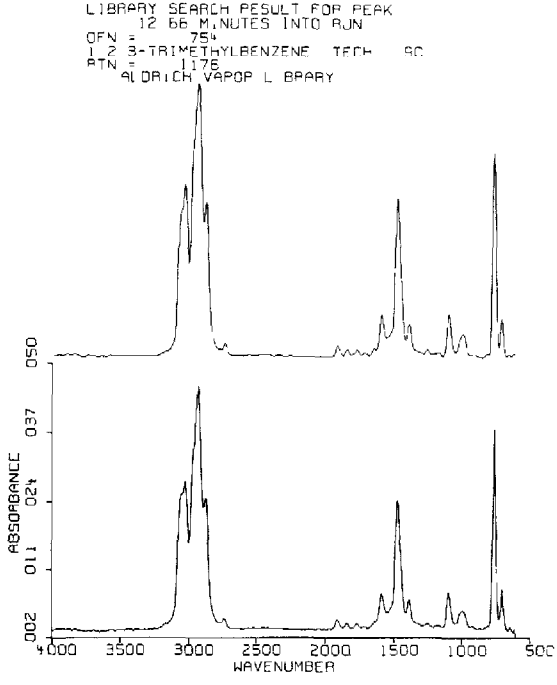

Fig 7 Automatic search result for compound 1 in Fig 5 (retention time = 12 7 min)

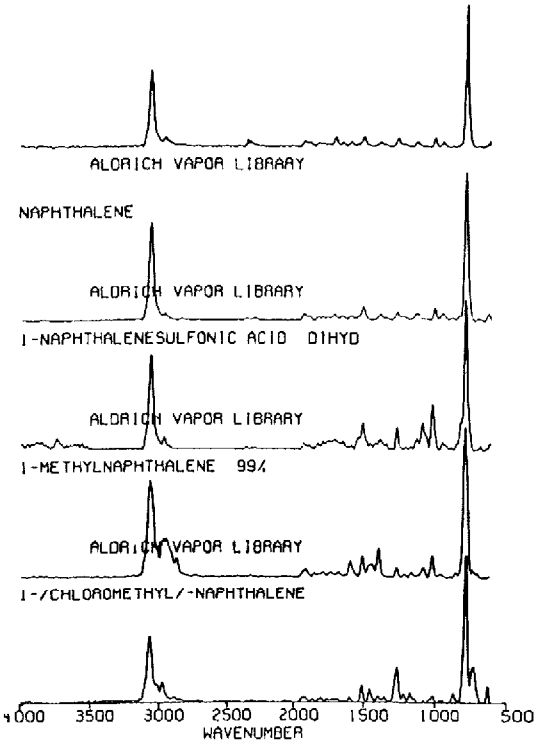

Fig 8 GC–FTIR absorbance spectrum of compound 2 in Fig 5 (retention time = 16 6 min) and the four closest library matches

It is important to note that this search always leads to a diagnosis, even if the desired spectrum is not to be found in the library The role of the user is thus of prime importance in deciding the quality of the result Reproducibility for two or three spectra selected in a peak or in the course of repeated analyses seems to give good reasons for confidence However, even an erroneous result can give useful information on the structure of a compound; for instance, in order to identify the linalyl acetate peak during a lavender analysis [17], we obtained a fairly reproducible diagnosis of 3,7-dimethyl-1,6-octadiene, which represents the structure of the alcohol part of the ester under study

Even in this unfavourable case, in which the spectrum is not to be found in the library, infrared spectrometry provides important information that can be used in conjunction with that obtained from chromatography itself or other coupled techniques such as GC–MS, finally, it should be pointed out that the user can create and complete his or her own data bank within his or her standard applications

The object of this first part was to provide a simple description of the GC–FTIR technique for use by non-specialists in IR spectrometry The theoretical

concepts introduced have served to establish both the potential and the limitations of the method, a fuller description of which is given in the second part, which is devoted to specific applications

3 GC–FTIR APPLICATIONS

The number of papers devoted to applications proper is still limited Indeed, most of the 300 or so articles covered in Chemical Abstracts report on technological developments illustrated by applications the purpose of which is to underline the importance of the innovation presented. The technical description given in the preceding section was the result of analysing this body of work We shall confine ourselves here to examining the results obtained using systems just as they are, isolating the contribution made by FTIR in relation to the other methods of detection in GC

The problems of industrial analysis account for a large number of these applications We shall only discuss these briefly Our attention will be chiefly focused, in fact, on substances of biological origin or importance

3 1 Industrial applications

GC–FTIR has been used in an original way in the field of polymers and plastics, the gas phase for analysis being obtained by heating the sample We can differentiate between those methods of heating which lead to decomposition of the polymer and those which are confined to degassing

The pyrolysis technique enables the structure of the polymer to be specified from identification of the fragments Hummel et al [23] have used this method to analyse certain acrylic resins and have established that GC–FTIR offered the advantage of directly identifying the chemical nature of the different fragments by comparison with mass spectrometry with field ionization Zhou et al [24] have studied the pyrolysis of polybutadiene and differentiated between eleven $-CH=CH_2$ monosubstituted compounds on the basis of characteristic vibrations in the 910–1000 cm^{-1} range Elastomers in automobile construction [25,26] and epoxy, phenolic or epoxy–phenolic resins for packaging lacquers [27] have been studied in the same way

In another field, Smith and Durig [28] proposed a system that makes it possible to study the thermal decomposition of low-molecular-mass phosphorated volatile compounds Analysis of cigarette smoke [29] revealed, out of seventeen components identified, the presence of eleven aldehydes or ketones, while three octanone isomers were distinguished on the basis of their lower frequency vibrations Using moderate heating methods, we confine ourselves to characterizing desorption or degassing products Sensitivity at the micrograms per gram level was obtained by Jansen and Haas [30] for the detection of trioxal in polyacetal, the mass of the samples being 100 mg

GC–FTIR also makes it possible to monitor reactions such as polymerization [31] and catalytic oxidation [32] This technique has been used more traditionally to identify jet fuels [33] and coal-tar wash oils [34] and to detect traces in organic analysis in difficult areas of polymer characterization [35]

Many other industrial products (aromatic polycyclics, pesticides, etc.) pose major problems of isomer differentiation, some of which can be solved using FTIR The study of such compounds will be discussed in a later section with reference to their toxic nature

3 2 Analysis of natural products of vegetable origin

Essential oils are typical examples of complex mixtures for which the use of conventional techniques, retention index and GC–MS, etc., has limited scope for identification The need for additional structural information such as the infrared spectrum has justified the use of GC–FTIR The example of the *Anthemis nobilis* L. analysis [36] demonstrates the contribution made by IR spectrometry in distinguishing geometric isomers This mixture is, in fact, constituted by terpenoids and saturated and unsaturated C_4 and C_5 acids esterified by saturated or unsaturated C_3–C_6 alcohols It is impossible to distinguish between the angelates and tiglates of unsaturated alcohols by GC–MS with the EI method in the absence of a characteristic high peak These geometric isomers do, on the other hand, reveal absorption values differing by several dozen cm^{-1}, which has made it possible to characterize natural products such as angelates The same approach has been used to distinguish between crotonates and methacrylates and between linear and branched esters

For this class of products, Herres [37] gave a compilation of 73 GC–FTIR spectra of terpenes and associated molecules These original data were acquired on-the-fly at a spectral resolution of 8 cm^{-1}. Among the results, the author pointed to a number of examples for which the differences observed are large, whereas the structures are very similar (Fig 9)

Another interesting example was quoted by Kalasinsky and McDonald [38]: γ-terpinene and α-pinene, although chemically different, give fragmentations that vary only with regard to the size of the peaks (Fig. 10) On the other hand, the IR spectra are sufficiently specific They pointed out that no two terpenoids involved in this study have identical vapour-phase IR spectra

Limits are indicated, however They originate primarily from the great difference in concentration that exists between the components of these natural mixtures [39] Even if we are not restricted by the amount of substance available, it is sometimes difficult to identify a small peak occurring among large peaks This can be taken as a problem of sensitivity essentially due to resolution [17] Indeed, we have to bear in mind the already mentioned constraints imposed by the coupling on the choice of column Le Quéré et al [40] discussed this problem and concluded that the choice between capillary and wide-bore

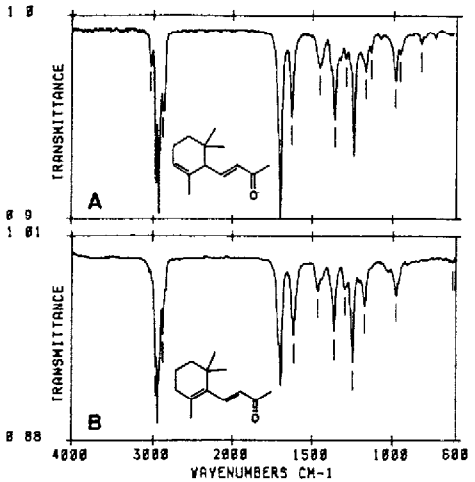

Fig 9 Vapour-phase IR spectra of the two isomers (A) α-ionone and (B) β-ionone From ref 37, with permission

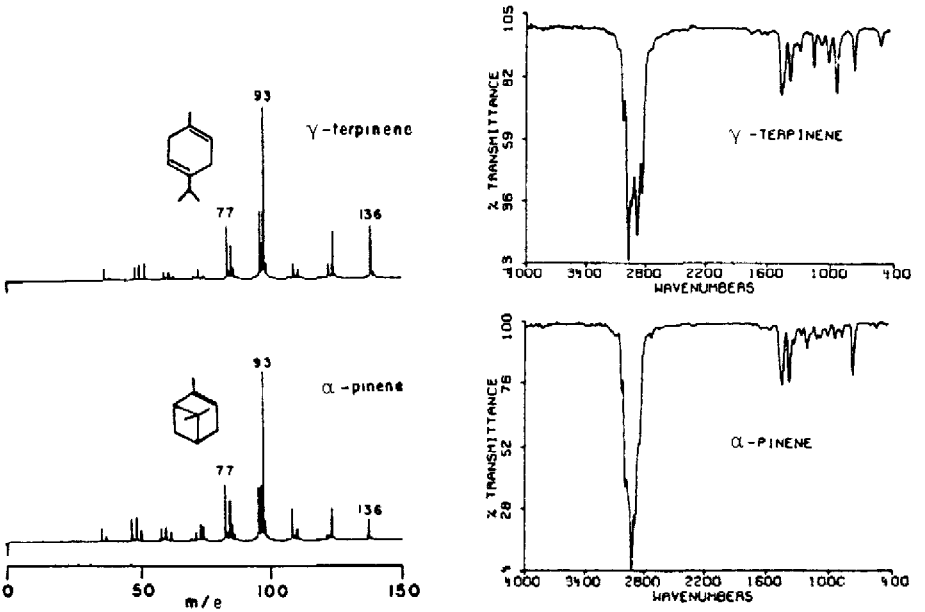

Fig 10 Mass spectra and vapour-phase IR spectra of γ-terpinene and α-pinene From ref 38, with permission

column depends on the ratio of chromatographic resolution to column capacity that we wish to obtain

The mathematical processing habitually applied in IR spectrometry (specific reconstruction, spectrum subtraction, etc) can contribute to an artificial improvement in the quality of chromatographic separation [41,42] Finally, several authors [40,43] have regretted the current inadequacy of libraries of vapour-phase IR-spectra

3 3 Identification of toxic substances in environmental analysis

As we have already said in connection with the previous two types of application, the object of acquiring information on the structure of an industrial product is to optimize its quality and efficiency This also applies to pesticides (insecticides, fungicides, nematocides, etc). Biologically, the toxicity of these products has made it necessary to monitor their evolution in the food chain, soil and water in order to specify their effects on the environment and the human organism.

Conventional analytical and monitoring procedures initially consisted in using GC [44] to search for a limited number of target compounds However, shortcomings were soon noted when it came to identifying other residues that were suspect but not defined by pre-established lists Coupled methods (GC–MS, then GC–FTIR) have made it possible to generalize the procedure thanks to the spectroscopic information provided [45]

Generally, the problems created by this type of analysis are of three kinds a considerable number of products are involved; it is important to differentiate between isomers in order to obtain clear characterization of biological efficiency; and degradation of the pesticide leads to the creation of new molecules that are sometimes similar and whose structures have to be defined so that their toxicity towards man can be studied GC–FTIR provides a solution that is highly suitable for tackling these three types of problem.

In view of the number of products to be tested and identified, Gurka and coworkers [9,45–47] undertook a thorough study for the EPA and determined the minimum identifiable quantities (MIQ) of 52 typical environmental contaminants in soil and water The improvements that they made to the interface enabled them to detect and identify components present at concentrations of 10–60 ppb in water and 200 ppb–1 ppm in soil The MIQ also range from 20 to 120 ng The use of capillary columns enabled them to reduce 2–8-fold the MIQ previously established with the help of packed columns [48] The construction of databases intended to characterize products according to their retention time and their group frequencies has been undertaken [9] with a view to developing automatic detection protocols [49]

A complementary paper by Shafer et al [50] showed that a compound can be identified with greater precision using GC–FTIR than GC–MS Most often,

the latter technique only enables the compound type to be defined This can be ascribed to the existence of numerous positional isomers of the substituted benzenes studied in this work

This result brings us to the second point mentioned earlier differentiation between isomers This problem has been satisfactorily solved for sixteen chlorinated phenols by Malissa et al [51] and for twenty polycyclic aromatic compounds by Chiu et al. [52] Here again, the data from GC–FTIR analysis makes it possible to differentiate between the isomers without any ambiguity, whereas GC–MS enables precise information to be obtained only on molecular masses

Fig 11 [52] shows a typical example, concerning 2- and 1-methylnaphthalenes Considerable differences are apparent in the IR spectra a closely spaced doublet is observed at 770 and 790 cm^{-1} (1-methylnaphthalene) instead of three bands at 740, 810 and 830 cm^{-1} (2-methylnaphthalene) These vibrational modes are interpreted as out-of-plane deformations of the aromatic C–H

Grainger and co-workers [53,54] accomplished a very important piece of work in establishing the vapour-phase IR spectra of the 22 isomers of tetrachlorodibenzodioxin (TCDD) at low microgram concentrations By correlating these spectroscopic results with the molecular structures, they established that each TCDD isomer can be individualized by absorption in the C–O–C asymmetric and symmetric stretching region These bands, situated at about 1200–1300 cm^{-1}, are indicative of the partial double bond character of the

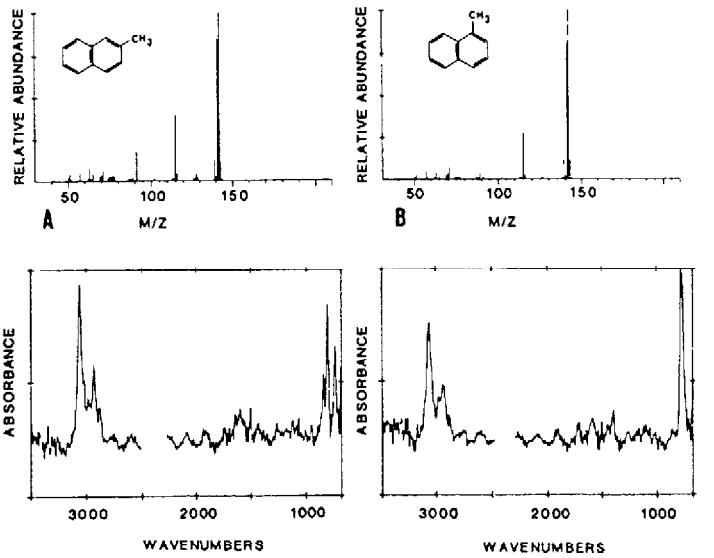

Fig 11 Mass spectra and vapour-phase IR spectra of (A) 2-methylnaphthalene and (B) 1-methylnaphthalene From ref 52, with permission

ether linkage Despite the value and coherence of these results, we must remember that the spectra were obtained in the vapour phase Caution in interpretation is therefore advisable, as demonstrated by Warthen and McGovern [55] in connection with analyses of isomers of the insect attractant trimedlure In this instance, differences observed both in wavenumbers and in intensities for the vibration $\nu(C=O)$ of halocarbonyl compounds are not confirmed on the spectra in the condensed phase at ambient temperature They suggested the occurrence of changes in molecular structure due to the temperature in the light-pipe

Finally, GC–FTIR makes it possible to identify pesticide degradation products. A study has been conducted on mirex (hexachloropentadiene dimer, Merck Index 6073) by Kalasinsky [56] After experimental photodecomposition, she identified the degradation products of the bait formulation as mono- or dihydrogenated derivatives of mirex However, the MIQ are of the order of 5 μg as the IR absorption coefficients are low. This also poses the problem of using capillary GC in GC–FTIR as the minimum IR detection levels are more than the maximum capillary column capacity The use of the high load capillary GC–FTIR system appears to be essential for the purpose of studying low-volatility pesticides and their residues in biological, animal or vegetable samples

3 4 Biomedical applications

Owing to its nearly absolute ability to identify a compound, GC–FTIR can be used in the biomedical field to reveal the presence of medicaments, toxic compounds, narcotics and their metabolites in biological samples (blood, urine, bile, etc.)

Biochemical applications in the strict sense of the word are not as yet extensively developed In one particular case involving a study of amino acids and esterified fatty acids, Tajima et al [57] use the data obtained to identify different components of the human skin

GC–FTIR also permits the precise qualitative analysis of illicitly manufactured and used chemical products such as drugs of abuse whose impurities and degradation residues are little known

For analytical purposes, it is essential to obtain clean extracts Here, the classical methods of liquid–liquid extraction [58] and even solid-phase extraction are perfectly suitable for the preparation of samples prior to analysis using FTIR [59] However, the matrix effects can remain important [37] (Fig 12a)

3 4 1 Identification of medicaments in biological fluids

Certain medicaments are used illicitly for doping sportsmen or racehorses, e g , amfepramone (diethyl propion, Merck Index 3113) This molecule, while primarily anexoric, does have an indirect psychotonic action Maylin et al. [60] have shown that hordinine, the main metabolite of amfepramone, could

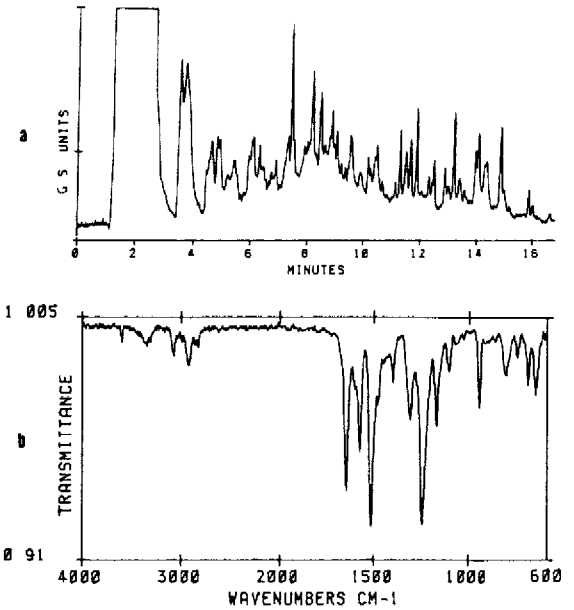

Fig 12 (a) Matrix effects during GC–FTIR analysis of human urine (Gram–Schmidt reconstruction) (b) GC–FTIR spectrum of the plotted peak in (a) 5-chloro-2-methylaminobenzophenone, a metabolite from diazepam From ref 37, with permission

be identified in equine urine They determined an MIQ of 50 ng. The advantage of GC–FTIR is again apparent in identifying the diastereoisomers of other psychogenic amines such as amphetamine and 2-ethylaminopropiophenone. Miyata et al [61] used GC–FTIR to isolate and identify ephedrine derivatives, they observed that *l*- and *d*-pseudoephedrine have different IR spectra, whereas *l*- and *dl*-methylephedrine have identical spectra.

Other biological matrices, such as bile [62], have been used for studying the metabolisms of central nervous stimulants After enzymatic hydrolysis and purification on amberlite XAD-2 resin, the extracts are analysed using GC–FTIR after derivatization by flash methylation. The main metabolite of 1,3-diphenyl-2-aminopropane has been identified as 1-(4′-hydroxyphenyl)-3-phenyl-2-aminopropane

3 4 2 Study of metabolites

The metabolism of benzodiazepines is complex and not all their metabolites have been identified Herres [37] succeeded in revealing the presence in urine of 5-chloro-2-methylaminobenzene, a metabolite of diazepam (Fig. 12b).

Other metabolism studies have been carried out using GC–FTIR, as follows.

Polycyclic aromatic hydrocarbons The carcinogenic risks of benzo[*a*]pyrene have been clearly demonstrated and the metabolites 7- and 6-methylbenzo[*a*]pyrene have been identified in the rat [63] It should be noted that,

in order to differentiate between the 6- and 7-isomers, it was necessary to use the argon matrix isolation technique described later (Section 4 1)

Trifluoromethylethyl benzhydrol This is an effective hepatic enzyme inducer Many metabolites are polyhydroxy compounds for which good chromatographic separations are possible only by preparing trimethylsilyl (TMS) derivatives The use of this derivatization is very uncommon in GC–FTIR as there is a risk of the TMS substituents severely disturbing the IR spectrum by their own absorptions. Jalsovszky et al [64] considered that the effects of trimethylsilylation are limited, however, there are no libraries of spectra of derivatized products, which tends to confirm that automatic identification is hindered by the disturbances affecting the spectrum

3 4 3 Applications in toxicology

One of the areas on which the interest of our group has focused is toxicology in forensic medecine. In this field, it is very important to identify with certainty the product that is responsible for fatal poisoning or the narcotic seized The main applications that we have developed are the identification of the herbicide paraquat [65,66] and the diagnosis of fatal poisoning due to the vapours of petrol [67] and volatile solvents [66,68,69]

The herbicide paraquat Initially, we established the spectrum of the pure product in the vapour phase. Reduction by sodium borohydride to diene-paraquat makes it possible to obtain the chromatogram and the IR spectrum of this derivative, the form in which the paraquat is extracted from the biological matrix Then post-mortem blood from the suspected fatal poisoning was analysed and we established the presence of paraquat by comparison with the reference spectrum

Volatile toxic substances The inhalation of fuel or solvent vapours is an addictive practice that developed in the 1970s Unfortunately, during "sniffing", complications can frequently occur, and these can be fatal It was thus essential to be able to identify the fuel or solvent concerned in the biological fluids or in the organs for clinical or forensic purposes

In the case of fuel vapours [67], packed-column separation enables only eleven compounds to be identified, whereas the amount injected remains substantial (several micrograms)

The other volatile solvents used by young drug addicts are simple products such as acetone, diethyl ether, trichlorethylene, methyl ethyl ketone, chloroform, carbon tetrachloride, benzene, hexane and toluene A separation method was developed using a Supelco Carbopack B–3% SP-1500 column [66]. Initially, extraction of the toxic solvent from biological samples was carried out using an extraction solvent such as diethyl ether or chloroform The solution obtained, which could not be concentrated, was injected in a large volume (several microlitres) Although it permitted the identification of acetone and trichloroethylene in the blood or lungs, this method could not be considered sat-

isfactory as there was a risk of the peak due to the elution of the extraction solvent masking the toxic substances sought for Consequently, we studied the capability of GC–FTIR after headspace sampling (HS-GC–FTIR) The detection limits were established for five solvents acetone, 8 ppm, 2-butanone, 6 ppm, diethyl ether, 5 ppm; toluene, 30 ppm; and trichlorethylene, 20 ppm A salting-out effect study showed that potassium carbonate produces the greatest effect This study was carried out at a time when, to our knowledge, no papers on the use of headspace injection in GC–FTIR had been published In the same period, two papers [70,71] were published that showed the feasibility of this mode of injection, hitherto very conventional in the study of volatile compounds using GC–FID and GC–ECD The increase in sensitivity has made it possible to detect and identify an acetone metabolite, isopropanol, in rats [69,72] and a metabolite of 2-butanone, 2-butanol, in the blood, liver, brain and lungs [69] Recently, Schmidt et al. [73] developed an HS-GC–FTIR analysis using a dynamic headspace

Analysis of narcotics and drugs of abuse Narcotics and drugs of abuse such as heroin, cannabis, LSD, cocaine, amphetamines, meperidine, barbiturates or phencyclidine can be analysed using classical colour test techniques, TLC, UV and IR spectrometry, HPLC or GC–MS The last method is both sensitive and specific, but GC–FTIR appears to be essential in order to identify the numerous isomers Kempfert [74] identified a number of drugs of abuse, he easily distinguished amphetamine from methamphetamine and from phentermine, and also cocaine from its diastereoisomer, pseudo-cocaine A rarer example was given· GC–FTIR clearly distinguished two closely related compounds, LSD and lysergic acid methylpropylamide (LAMPA) (Fig 13) Where the IR spectra are very similar to each other, e g , for pentobarbital and butabarbital, we can use the techniques of interactive spectral subtraction and overlaying of C–H stretching regions

A chemical study of narcotics during their storage reveals decomposition products liable to be encountered again subsequently in biological samples from addicts Beckstead and Neville [70] suggest using a GC–FTIR technique to identify the ethyl acetate (1 1) complex of O^6-acetylmorphine by headspace analysis

By identifying impurities, it is also possible to establish a "fingerprint" of the drug seized and thus to make and check hypotheses as to the geographical origins of the product Such results have been established in our laboratory in connection with hashish [75] Extraction under rigorously controlled conditions can prevent any decomposition of the cannabinoids Cannabinol (CBN), cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) are clearly separated An area-calculating program can be used to obtain the ratios $A = \text{THC}/\text{CBD}$ and $B = (\text{THC} + \text{CBN})/\text{CBD}$ The values of A and B vary according to the geographical origin of the plant.

A THC dosing method was then developed [19] The THC concentration is

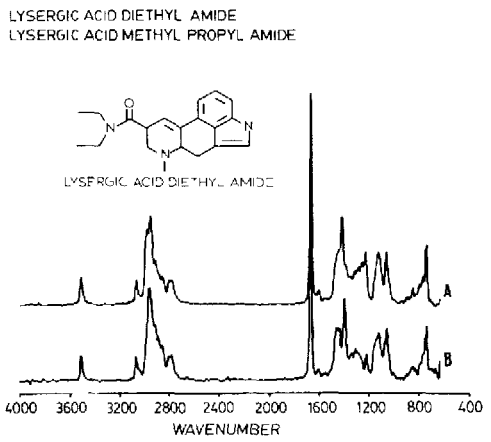

Fig 13 Vapour-phase spectra of (A) LSD and (B) LAMPA From ref 74, with permission

also characteristic of each region of production In recently produced hashish, no degradation products have been found

4 TECHNOLOGICAL IMPROVEMENTS

The still exceptional use of GC–FTIR in biology highlights the existence of problems to which solutions are currently being sought through the development of new methods.

4 1 Matrix isolation [76]

The technique using matrix isolation has been developed with a view to improving sensitivity, the limitations of which remain the major drawback of GC–FTIR At the output of the chromatographic column, the gaseous effluents are crystallized on a cylindrical mirror whose temperature is maintained at about 12–13 K by a helium cryostat The vector gas (helium or hydrogen) contains 1 5% of argon, whose crystallization on the cryogenic surface creates for the compound under study an environment in the form of an IR-transparent inert matrix. The excess vector gas is pumped out The mixture of argon and eluted products is continuously crystallized on the cylindrical mirror whose rotational–translational movement permits the storage of up to 5 h of chromatogram A small portion of the gas flow sent to a flame ionization detector creates a chromatographic signal permitting control of the cylindrical mirror The frozen deposit is analysed spectroscopically off-line The retention time given by the FID signal enables the infrared beam to be positioned on the cylindrical mirror to record the transmission–reflection spectrum. This a posteriori treatment has the advantage of optimizing the spectrum recording pa-

rameters and, above all, of carrying out signal accumulations The abundance of the argon, the amount of which can be as much as 200 times that of the sample, results in each molecule of the sample being crystallized in isolation Consequently, the bands are very fine as the vibrational modes are free from any crystalline effect These spectra obtained in the solid phase resemble those of the vapour phase without the components due to rotation This is reflected by an increase in the intensity of the bands, and hence in sensitivity, and the libraries of spectra in the vapour phase are also still utilizable. Detection limits below 10 ng are currently being indicated [77], with the best performances moving in the direction of the picogram range [8,78].

The other advantage of this technique is that it maintains the excellent resolution given by narrow-bore (0.25–0 1 mm I D) capillary columns, which are now usable by reason of the very small amounts injected Two products separated by a difference in retention time of 6 s have been identified [76] Application to the analysis of complex mixtures such as PCBs [79] and dioxins [80,81] reveals the value of GC–MI–FTIR in environmental studies

4 2 Supercritical fluid chromatography

To overcome the limitations of GC with regard to thermodegradable compounds, and given HPLC–IR coupling difficulties, supercritical fluid chromatography (SFC) has been associated with IR detection since 1983 [82] Carbon dioxide maintained above its critical pressure, at ambient temperature, is used as a vector fluid in capillary columns. Chromatographic selectivity is obtained by programming the pressure of the fluid. The relative infrared transparency of supercritical carbon dioxide, except in the 3600 and 2400 cm^{-1} regions, makes it possible to detect the absorption spectrum of the compounds analysed for identification purposes [83]

The performance of this technique, when adapted for the analysis of compounds of high molecular mass, remains limited in terms of sensitivity when a flow-through cell is used for detection [84] Various spectrometric modes of detection have therefore been contemplated, e.g , DRIFT [85] and matrix isolation [86]

4 3 Combined GC–FTIR–MS

In order to maximize the reliability of the diagnosis in identifying a given compound, which is the ultimate objective in using a coupling method, it soon became clear that it was advantageous to have mass and infrared spectra available simultaneously Given the difficulties entailed, this objective, which dates back 20 years, has evolved with the passage of time, as recently described in a detailed account by Wilkins [87] From the instrumental viewpoint, the problems deriving from differences in flow-rate, pressure and sensitivity between

MS and FTIR have resulted in choices as contradictory as parallel or series mounting [88] Initially, the use of packed columns led to the association in parallel of the two spectrometric techniques The gas stream is split into two components of variable intensity at the output of the chromatograph by means of a splitter The low flow-rate of the capillary columns has removed this possibility by favouring series mounting, which exploits the non-destructive nature of the IR technique As there is a risk of this type of set-up reducing the chromatographic resolution, the use of SCOT columns by Crawford et al [89] was a good compromise that no longer necessitated series mounting

For the interpretation of the results, a simultaneous knowledge of the two spectra permits the development of software performing cross-correlation of spectral library search results Cooper et al [90] described the application of software packages using the Chemical Abstracts Registry Numbers as a cross-correlation tool to mixtures of 14 and 23 compounds

Here again, differences are apparent between MS and FTIR as to the size of the spectral libraries (MS, 30 000, IR, 3000) This has led certain authors [88] to recommend the use of the IR spectrum as a functional group prefilter Nonetheless, we must not disregard the information supplied by the vibrational spectrum which is, most often, able to solve problems of isomerism, as we have already seen

GC–FTIR–MS is still at present a laboratory tool, the applications of which are few and far between The only two works published to date concern analyses of environmental samples [47] and of flavours [91]

Despite the still evolutionary nature of double coupling, limitations can already be seen They relate primarily to a lack of sensitivity, as the MIQ does not go below ca 100 ng This appears to be ascribable to FTIR detection, and the addition of the method using matrix isolation should give an improvement However, this package of sophisticated techniques is likely to result in a cost that is detrimental to commercial development According to current thinking, the way to reduce the cost of the whole system would be to simplify the mass spectrometer part by using an MSD or ion trap detector [92]

5 CONCLUSION

The applications of GC–FTIR in the biomedical field are still few and far between If we analyse the results obtained for industrial products, vegetable extracts and pesticides, we can find an explanation for this GC–FTIR still appears to have limitations at this stage, especially in terms of sensitivity, but also of resolution Thus, when sufficient amounts of the sample are available, very interesting results are obtained, particularly as regards the differentiation of isomers, in which GC–FTIR very efficiently complements GC–MS However, when the sample is extracted in small amounts from a biological material, the use of GC–FTIR remains a delicate matter The problem created when

using the classical derivatization techniques merely aggravate this difficult situation Under these circumstances, several groups of researchers have tried to push back the limits The matrix isolation method has increased sensitivity by several orders of magnitude, establishing MIQ levels comparable to those of GC–MS Finally, in biology, certain problems of chromatographic origin can be solved using SFC, which is better suited than HPLC to IR detection

6 SUMMARY

The basic concepts relating to Fourier transform infrared spectroscopy (FTIR) have been set out to enable chromatographers to make use of gas chromatography (GC)–FTIR coupling To describe the current position regarding applications, studies are briefly presented that are representative of the field of industrial products, natural vegetable extracts and pesticides. Biomedical applications are described in detail The analyses of medicaments, metabolism studies and toxicological investigations are reviewed Given the limited amount of published work in this field, it is possible to survey the limitations of GC–FTIR The solutions provided by technological developments currently in progress are described

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