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COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY: A POWERFUL TOOL IN ANALYTICAL CHEMISTRY*

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1. INTRODUCTION

The combination of gas chromatography with mass spectrometry (GC–MS) is one of the most powerful tools available in analytical chemistry. The coupling of these two techniques enables the chemist to detect and identify very small amounts of organic compounds in complex mixtures. GC was introduced in 1952 but it was soon found that, like all analytical techniques, it had various shortcomings. It became clear that it was impossible to identify the hundreds of compounds present in fruit extracts, cigarette smoke, urine, etc., from GC data alone. The solution to this problem was obvious: a detection system was needed with a unique response to any component eluted from the GC column. Such a detector, the mass spectrometer, existed long before the chromatograph was introduced.

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Mass spectrometry has its origin in the mass spectrometers first designed by Aston[1] and Dempster[2]. It separates chemical elements into their isotopes and is based on the principal that ions accelerate to a certain kinetic energy by an electric field describe, in a subsequent magnetic field, paths that differ according to their mass-to-charge ratios. Nowadays mass spectrometers exist with mass analysers other than the magnetic type, such as quadrupole and time-of-flight mass spectrometers. In the ion source of the mass spectrometer the organic molecules are bombarded in a vacuum by energy-controlled electrons emitted from a heated filament. In the ionization process not only molecular ions are formed, but also a large number of different fragments.

The fragmentation pattern found is characteristic of the identity of the organic molecules ionized, and this was the basis for the application of mass spectrometry in the identification of organic compounds[3–6]. The application of mass spectrometry was satisfactory when applied to pure compounds, but failed when the compounds being analysed were not pure. As GC is an ideal separation tool and the mass spectrometer, as a very sensitive and characteristic detector, was available, it was obvious that these complementary techniques should be combined. The coupling of these two instruments, however, was not easy, owing to their incompatibility, the gas chromatograph operating at atmospheric pressure and the mass spectrometer at high vacuum.

In the early days of the application of the two techniques, each component was trapped as it emerged from the GC column and was transferred manually to the batch inlet system of the mass spectrometer. This procedure had the advantage that both instruments could operate without affecting each other's properties. This, however, was overshadowed by some major disadvantages. The trapping of, *e.g.*, 100 components as found in fruit extracts, for example, from a column is very time consuming and difficult. Even if they can be collected, many of the components will not be pure and there is the possibility that they will be hydrolysed, oxidized or decomposed by some other mechanism before they are analysed in the mass spectrometer. In order to overcome these problems, coupling of the two instruments was necessary. With the advent of combined GC–MS two different types of interfaces were developed: the frit separator introduced by Watson and Biemann[7], and the jet separator introduced by Becker[8] and Ryhage[9]. During the last 15 years, many interface devices have been developed[10,11].

2. REQUIREMENTS FOR OBTAINING AN OPTIMAL GC–MS COMBINATION

2.1. Chromatographic separation

In order to obtain useful information from the mass spectrum, it is necessary for the substance to be introduced into the mass spectrometer to be pure. This means that the separation power of the GC column should be optimal. If complex mixtures have to be analysed, high-resolution capillary columns should be used. Stationary phases are chosen, depending on the problems to be solved. Fractionation of complex mixtures is often also needed, as trace compounds have to be concentrated and separated from interfering main compounds.

However, when the characteristic masses of two compounds differ from each other, interpretation of the mixed spectra is possible, as will be shown later.

The stationary phase should have a low volatility and good thermal stability[12] in order to yield stable baselines and low bleed intensity so that the spectra will not be mixed up with background masses. Frequently excessive bleeding decreases the detection limit for the determination of trace compounds as their low-intensity masses interfere with those of the stationary phase. Continuous bleeding of, for instance, silicone vapours contaminate the ion source and the mass analyser. This may result in severe decreases in sensitivity, resolution and stability.

2.2. Requirements for the mass spectrometer

The requirements for the mass spectrometer in a GC-MS combination are more complicated. They have been described in the literature[10,13].

2.3. Vacuum system

The mass spectrometer must be kept under high vacuum for proper functioning, so that ion–molecule reactions and peak broadening can be avoided. Therefore, it has to be equipped with a differential pumping system, with one pump for the mass analyser and a second for the ion source. The latter is the most critical, as it has to pump off the entering carrier gas while maintaining an acceptable vacuum (10^{-5} Torr). Flow-rates accepted by existing commercial mass spectrometer vary from 0.5 to 10 ml/min of helium. Very good pumping capacities are achieved with turbomolecular pumps; nowadays these are being increasingly used in mass spectrometers. Pumping capacity-reducing cooling baffles and vacuum valve systems can be omitted.

2.4. Dynamic range

In order to cover the ranges of sample amounts used in GC, the dynamic range of the mass spectrometer should be about six orders of magnitude. Actually, the maximal ion current that can be recorded by the detector without saturation effects should be 10^6 times larger than the minimal ion current discernable above the noise level.

2.5. Ion source optics

The pressure linearity and stability of the ion source must be very good. This means that the intensity of the ion current has to vary linearly over a wide range with the amount of sample introduced. The quality of the ion source optics is also very important as it has to yield and maintain a well defined peak shape and resolution during the GC run, which is important in obtaining reliable results in quantitative trace analyses of complex mixtures with single or multiple ion detection.

2.6. Scanning (cycle) time

In order to obtain adequate information on the purity of a GC peak and to obtain representative spectra, the mass spectrometer should be able to record at least three mass spectra per GC peak (0.5–1 sec per mass decade is required for sharp peaks eluting from capillary columns).

2.7. Computer compatibility

For the efficient handling of the large amount of mass spectral information obtained in GC–MS analyses, connection with a computer system is indispensable.

The application of computers to mass spectrometers started with the processing of high-resolution data[14,15] and it soon became a great help for data acquisition, reduction and interpretation[16–19]. This computerization of a GC–MS system saves time and increases efficiency.

2.8. The GC–MS interface

The most critical part of the GC–MS system is the interface. Many different interface systems have been developed during the past 15 years and have been reviewed in the literature[10,11]. When combining GC with MS, there are some major problems: (a) the pressure drop between GC exit and the mass spectrometer; (b) the GC effluent contains only a small concentration of organic compounds, the remainder being carrier gas. In this context, two properties play an important role: the enrichment factor and the efficiency.

The enrichment factor is defined as the relative increase in concentration of the compound in the carrier gas after passing the interface. The efficiency is the percentage of the amount of the compound in the GC effluent entering the mass spectrometer.

The ideal properties of a GC–MS interface can be summarized as follows:

(1) the interface must not affect the properties of the gas chromatograph (separation) and the mass spectrometer (sensitivity, resolution);

(2) the whole of the sample but none of the carrier gas should be transferred to the mass spectrometer;

(3) no chemical changes in the sample should be caused by the interface;

(4) no discrimination against compounds with particular functional groups;

(5) no adsorption and memory effects;

(6) functioning independent of carrier gas flow-rate and temperature;

(7) the system is suitable for all types of columns, both capillary and packed.

It is obvious that none of these ideal conditions can be achieved by the existing interfaces. Moreover, they all have certain advantages and disadvantages. A brief survey of the most common coupling techniques is given in Fig. 1.

2.9. GC–MS interfacing without a molecular separator

In the early days of GC–MS, the gas chromatograph was connected with the mass spectrometer by means of a splitter system. In this way only a few percent of the GC effluent was admitted to the mass spectrometer. The maximal admissible amount was dictated by the performance of the vacuum system. The mass spectrometer was equipped with a needle valve or narrow inlet capillary so that only 0.1–4 ml of carrier gas (helium) was admitted[20–23] (Fig. 1A).

It is obvious that the efficiency was low (1–10%), whereas no enrichment was achieved. The great advantage of this method is that the GC conditions are not affected; moreover, it is suitable for all types of columns. It is a very simple and cheap device. However, for a long time this system was not very popular, owing to the low efficiency and easy blocking of the inlet capillary, correlated with the poor vacuum systems of the mass spectrometers.

With improvements in the MS vacuum systems and ion sources, open split coupling gained enormously in popularity, and at present it may be said that it is the most recommendable interfacing technique[24–28]. Another type of GC–MS coupling

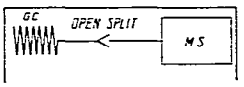
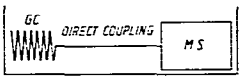
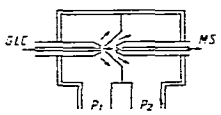
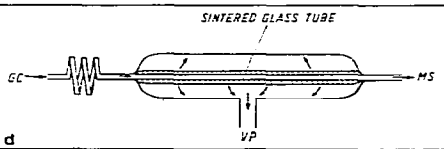
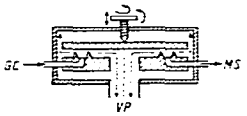
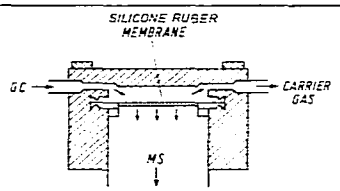
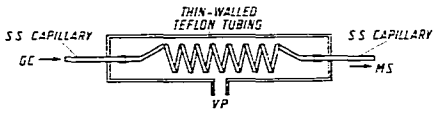
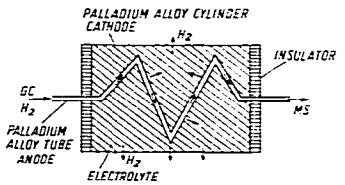
TYPE OF INTERFACE	FLOW-RATE RANGE ml/min *	EFFICIENCY ENRICHMENT **	OPERATING TEMPERATURE °C	DECOMPOSITION ABSORPTION EFFECT ***
a 	1 – 100	1–90 % —	< 400°	—
b 	≤ 10	100 % —	< 400°	—
c 	10 – 20	≈ 50 % ≈ 50	< 400°	+
d 	15 – 100	≈ 40 % 20 – 400	< 400°	+ +
e 	0 – 30	10 – 100 % 10 – 50	< 400°	+
f 	1 – 50	≈ 90 % 10^4	< 160 °C	+
g 	≤ 20	≈ 80 % 8	270 – 330 °C	+++
h 	≤ 5	≈ 100 % 10^6	≈ 300 °C	+++
i * Depends on type of column and on 1 or 2 stage version of separator ** Depends on flow-rate range and molecular weight *** – no effects + neglectable ++ small effects +++ pronounced effects				

Fig. 1. Schematic survey of the different interfacing techniques used in GC–MS. (A) Open split coupling; (B) vacuum coupling; (C) jet separator; (D) frit separator; (E) slit separator; (F) membrane separator; (G) Teflon separator; (H) electrolytic silver–palladium separator.

was accomplished by selecting columns for minimal carrier gas flow. The columns could then be connected directly to the ion source (Fig. 1B).

A narrow-bore capillary column (50 m × 0.25 mm I.D.) can be coupled directly to the mass spectrometer through a capillary restriction. The total GC effluent enters the mass spectrometer. This means that the efficiency is 100%. It is a disadvantage that the resolution properties of the column may be affected by the vacuum at the exit of the column. The system is simple and cheap, but is restricted to columns with flow-rates of 1–5 ml/min of helium, depending on the pumping capacity of the mass spectrometer. Vacuum-tight connections, especially at high temperatures, also cause problems, and it is not always easy to change the columns.

2.10. Molecular separators

The interfaces described above were not suitable for every GC–MS application. For the achievement of other coupling systems, enrichment devices, or so-called molecular separators, were constructed. The enrichment process can be divided into three categories:

(a) fractionation of gases in an expanding jet stream[8,9,29,30];

(b) selective effusion through fine pores or through a narrow slit[7,31–34];

(c) preferential diffusion of carrier gas or sample through a semi-permeable membrane (Teflon membrane separator, palladium–silver separator and silicone membrane separator)[35–42].

Today a variety of enrichment devices exist and are frequently applied.

2.10.1. Jet separator (Fig. 1C)

The jet separator, developed by Becker[8] and Ryhage[9], is very widely used. It consists of two- and one-stage designs made of glass and steel. The performance of this type of separator can be summarized as follows:

(1) good efficiency, 50% at a molecular weight of 200 and an enrichment factor of about 50;

(2) performance varies with flow-rate. Flow-rates from 10 to 80 ml/min of helium may be used;

(3) temperatures up to 400° can be applied;

(4) no adsorption or decomposition effects have been reported (hardly any active surface);

(5) separate heating and vacuum pumping are required;

(6) no discrimination against compounds with particular functional groups;

(7) no influence on the GC separation properties.

The single-stage version of this interface is suitable for use with capillary columns (low flow-rates) and the two-stage version for packed columns with flow-rates above 15 ml/min of helium.

2.10.2. Effusion-type separator

The effusion-type separator exists in many different versions. The most commonly used types are those in which the effusion takes place through the pores of a sintered glass frit, designed by Watson and Biemann[7], or through a variable slit between two sharp edges in the variable conductance separator designed by Brunnee

et al.[34]. In other investigations a stainless-steel sinter[43] or porous silver frits[32,33] were used.

Fig. 1D shows the Watson and Biemann separator, made out of glass and consisting of one- and two-stage designs[7,31]. It is widely used. The performance may be summarized as follows:

(a) yields of 50% at a molecular weight of 200 can be obtained as well as an enrichment factor varying from 50 to 400 for the one- and the two-stage version, respectively[31];

(b) for the two-stage version the performance is not affected at flow-rates between 15 and 80 ml/min of helium; at flow-rates less than 15 ml/min a single-stage separator is recommended[31];

(c) temperatures up to 400° can be applied;

(d) adsorption and decomposition effects have been reported[11,31]; these effects, observed for polar compounds, could mostly be eliminated by means of deactivation of the active surface of this type of separator by a silanization procedure[31];

(e) heating and pumping of the interface should be carried out separately;

(f) no discrimination against compounds with particular functional groups;

(g) loss of GC separation properties due to peak broadening has been observed with capillary columns[31].

The properties of the other frit separators with respect to adsorption, decomposition and discrimination effects are poor, which makes them unsuitable for application in organic chemistry.

The properties of the variable slit separator are similar to those of the Watson and Biemann separator. No adsorption or decomposition effects have been reported. However, they may possibly occur due to the metallic surface. The method can be optimized for a wide range of flow-rates from 1 to 100 ml/min of helium. Solvent or high concentrations of other compounds can be diverted from the ion source (Fig. 1E)[34].

2.10.3. Membrane separator

The silicone membrane separator is widely used. The carrier gas (helium) bypasses the membrane and the organic compounds diffuse through it and enter the mass spectrometer. The interface is easy to construct (Fig. 1F). One-[38] and two-stage versions[37] exist. The one-stage version, which was developed later, is the most commonly used because no separate pumping in the second stage is needed.

The performance of this type of interface may be summarized as follows:

(1) efficiencies of up to 95% can be reached and very high enrichment factors can be obtained (10^5 with the two-stage version);

(2) a wide range of carrier gas flow-rates (1–80 ml/min of helium) may be used;

(3) performance is temperature-dependent and limited to 200°; the operating temperature should be the same as the column temperature used for the compounds to be analysed;

(4) no chemical changes to organic compounds have so far been observed;

(5) no separated pumping is necessary (single-stage version); the separator can be built in the GC oven for heating; separate heating is recommended;

(6) no discrimination against compounds with particular functional groups is observed;

(7) GC separation properties are affected, owing to peak broadening[38,44];

(8) saturation and memory effects can be caused by working with large amounts of sample.

In the two other types of membrane separators, the carrier gas diffuses through the membrane instead of the organic compound. The Teflon separator, which was first introduced by Lipsky *et al.*[35], preferentially removes the helium through a very thin Teflon capillary mounted in a vacuum pumped chamber (Fig. 1G). The performance is as follows:

(a) yields up to 80% and enrichment factor 8[36];

(b) carrier gas flow-rate limited to 20 ml/min of helium;

(c) the interface can be operated only in a small temperature range (270–330°);

(d) decomposition of thermally unstable compounds, owing to the high operating temperature;

(e) separate pumping and heating are necessary;

(f) discrimination against functional groups[45];

(g) GC separation properties are affected by peak broadening[45].

As the performance of this interface is poor, it is no longer used in GC–MS systems.

2.10.4. Silver–palladium separator

This interesting interface was first proposed by Lucero and Haley[39], and developed by Simmonds and co-workers[40,41]. It is based on the unique property that a palladium–silver membrane is highly permeable to hydrogen but totally impermeable to other gases and organic compounds at 250°. In later versions it was designed as an electrolytic cell[46,47] and proposed for use in extraterrestrial GC–MS systems[42]. The electrolytic cell, filled with potassium hydroxide–lithium hydroxide, consists of two thin palladium–silver tubes. The inner tube transports the carrier gas and is the anode, and the outer tube is the cathode (Fig. 1H). The fundamental process was described by Lucero[47]. The hydrogen carrier gas is completely removed through the electrolytic cell, while the organic compounds and other gases continue into the mass spectrometer.

The performance of this interface is as follows:

(1) yields of 100%; enrichment factor infinite;

(2) flow-rate range strongly dependent on the interface design;

(3) operating temperature between 200° and 250°;

(4) chemical changes of organic compounds have been reported[40];

(5) pumping of the interface not necessary;

(6) no peak broadening and memory effects;

(7) only hydrogen can be used as the carrier gas;

(8) poisoning of the palladium–silver surface by sulphur and iodine compounds reduces the hydrogen removal.

Owing to the catalytic effects that may occur with unknown organic compounds, which are the most commonly analysed substances in GC–MS applications, this type of separator is not very popular in organic analytical laboratories.

From the many publications on GC–MS applications, it can be concluded that the open split, direct coupling, jet separator, glass frit, slit separator and silicone membrane separator are the most commonly used interfaces. An ideal interface

system which meets all requirements does not exist; for each investigation the best type has to be chosen to solve the particular problems involved.

3. METHODS AND APPLICATION IN GC–MS

In GC–MS applications, two kinds of problems may be distinguished: (a) the investigator wants to know what compounds are present in a sample or extract, and (b) the investigator wants to know if a certain compound or group of compounds is present in an extract. The latter mostly involves trace analysis of complex mixtures.

In the first instance the mass spectroscopist may be overwhelmed by a tremendous amount of information. In the second instance, GC retention times and mass spectra of the compound to be determined are known and the search can be a direct one, as in general it will be sufficient to confirm only the presence of certain masses, characteristic of the compounds at their particular retention times. A common problem which causes difficulties in both instances is that the peaks emerging from the column are not pure, but contain more than one compound. This leaves the spectroscopist with the difficult task of interpreting complex mass spectra and solving interference problems in single or multiple ion detection. To establish whether a GC peak contains more than one compound, it is necessary to record more spectra from every GC peak and then study these with changes in the fragmentation pattern.

Fig. 2 shows a capillary chromatogram with mass spectra taken at the two flanks and the top of the GC peak. The first three spectra are identical, the next three spectra are not identical and there is a change in fragmentation pattern. In the first spectrum the masses of ethylbenzene are dominant, then the masses of amyl alcohol appear and in the last spectrum the masses 106 and 81 of ethylbenzene have decreased. This example is not complicated, but it illustrates well what may happen and the necessity for taking more than one spectrum of a GC peak, in order to obtain information on its purity.

It is clear that many problems exist when a complex chromatogram must be analysed. Such an analysis, resulting in several hundred spectra that have to be calibrated and examined, is an enormous task. The only way of solving the problem of handling such large amounts of data is to connect a computer to the GC–MS system.

Fig. 3 shows schematically a computerized GC–MS combination. The MS yields two signals, one representing the total ion current and the other the MS signal. Both signals are digitized and processed by the computer. The calibrated mass spectral information is stored on magnetic tape or disc. The mass spectrometer can operate in a cyclic scan mode or with the scan controlled by the computer, which means that, for example, every second a mass spectrum is recorded from the GC effluent, so that the GC effluent is sampled mass spectrometrically every second and the complete MS information is available to the investigator after having completed the analysis. This can be of great value, especially when the analyses cannot be repeated. The benefits of such a system are that it will produce the total ion current chromatogram, any individual mass spectrum at any retention time of interest and the mass fragmentogram for any chosen m/e value that is of interest.

The so-called computer mass fragmentography is a very important technique, which enables the investigator to localize any selected masses characteristic of the

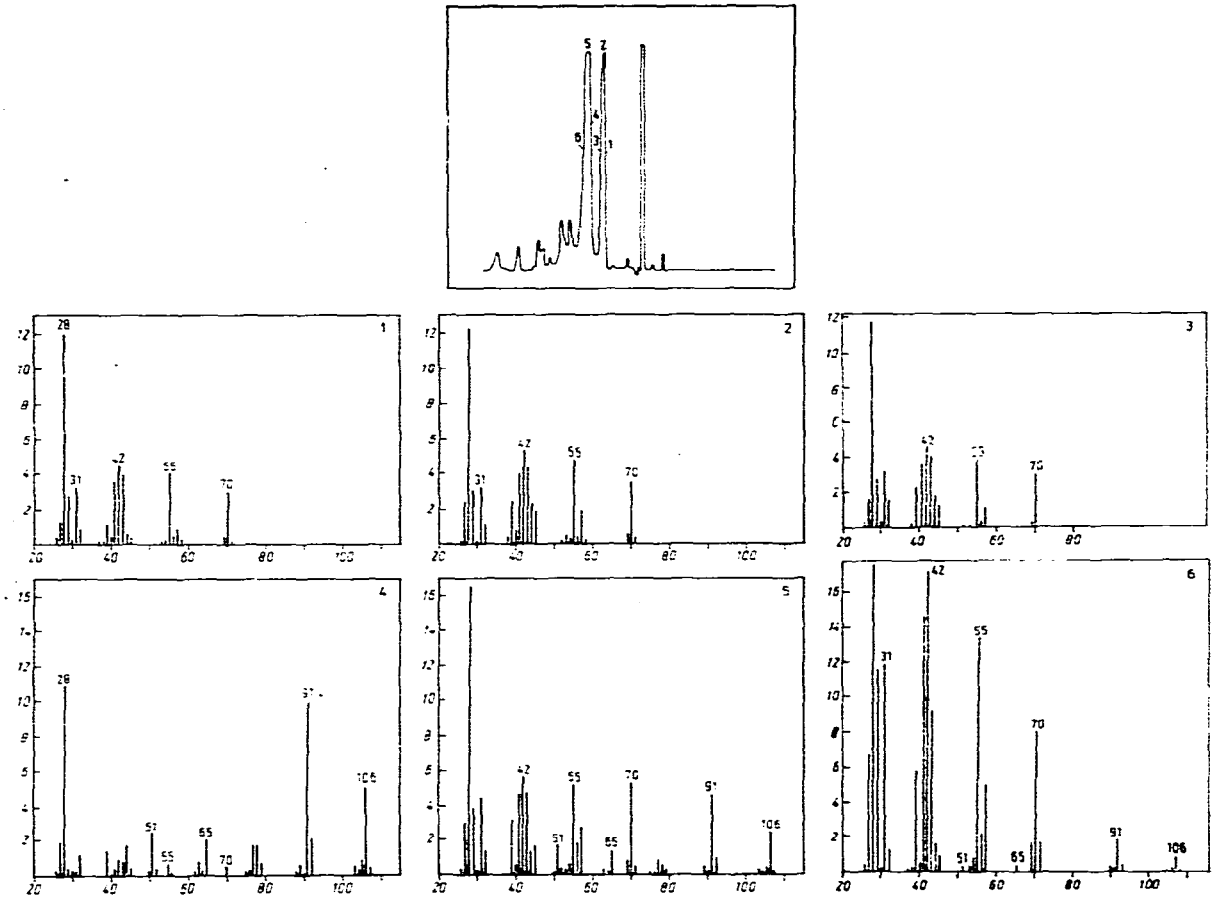


Fig. 2. Capillary gas chromatogram and mass spectrum of a mixture of *sec.*-butanol, isoamyl alcohol, ethylbenzene and *n*-amyl alcohol

compounds or group of compounds under investigation. This means that it is very useful for the evaluation of GC peaks that contain more than one compound. Further, spectrum refining can be performed by subtracting mass spectra from each other in order to eliminate interfering masses from neighbouring or overlapping GC peaks

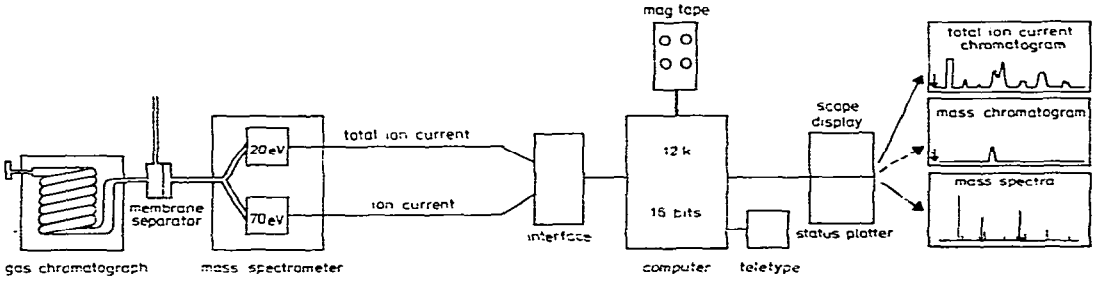


Fig. 3. Schematic diagram of a computerized GC–MS system.

and column bleeding. Library searches of spectra in a reference library and filing of GC–MS analyses are other useful features.

Secondly, knowing the characteristics (retention time and masses) of the compound to be determined, it is not necessary to have all mass spectrometric information available.

This type of analysis can be performed by single or multiple ion detection so that the mass spectrometer is degraded to a very selective and expensive GC detector. The mass spectrometer is equipped with a so-called peak selector, which instructs the mass spectrometer to measure only pre-selected masses. This method is much more sensitive than mass fragmentography performed with a computer and mass spectrometer in the cyclic scan mode. However, this needs careful handling so as to prevent misinterpretations while performing trace analyses in a complex matrix, especially when quantitative results have to be obtained.

The situation is simple when the masses to be searched for are relatively characteristic of those of the matrix in which the trace compounds are hidden. If not, interference of the characteristic masses with the same nominal masses from other compounds occurs. To decrease the chances of interference, increasing the resolving power of the mass spectrometer is a great help because then one can tune the instrument not only to the nominal mass of interest but even to the elemental composition of the *m/e* value one is searching for. For example, if there is a nominal mass 114 characteristic of a compound with the elemental composition $C_5H_{10}N_2O$, there can be interference with the molecular ion of octane, which has the elemental composition

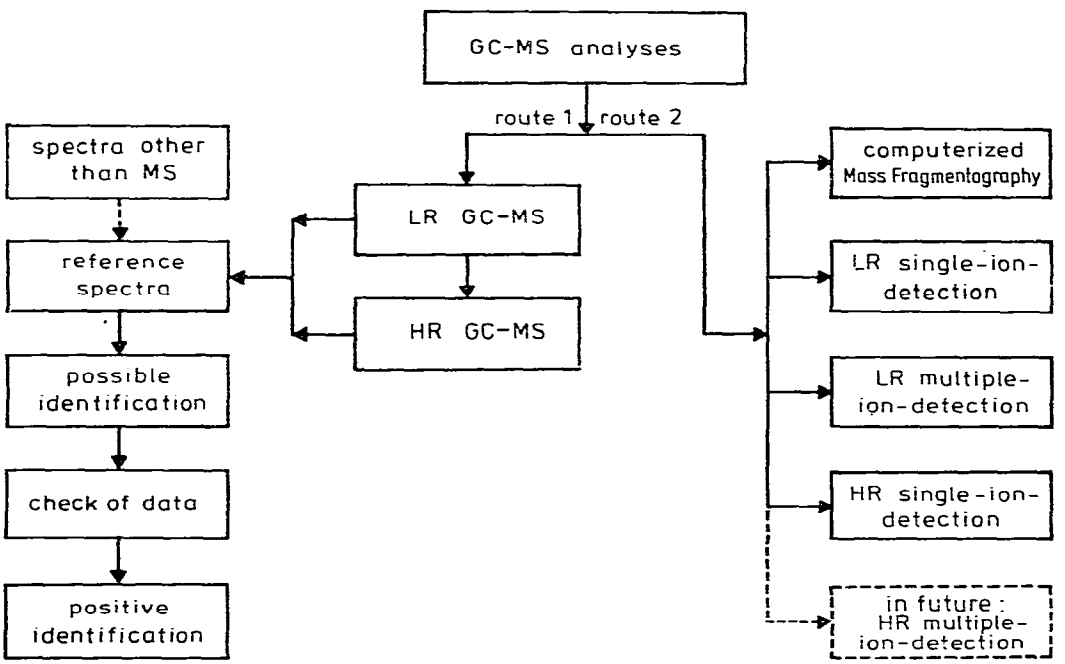


Fig. 4. Flow chart of the different procedures applied in GC–MS analyses. Route 1, multi-component analysis of complex mixtures; route 2, qualitative and quantitative trace analysis of complex mixtures.

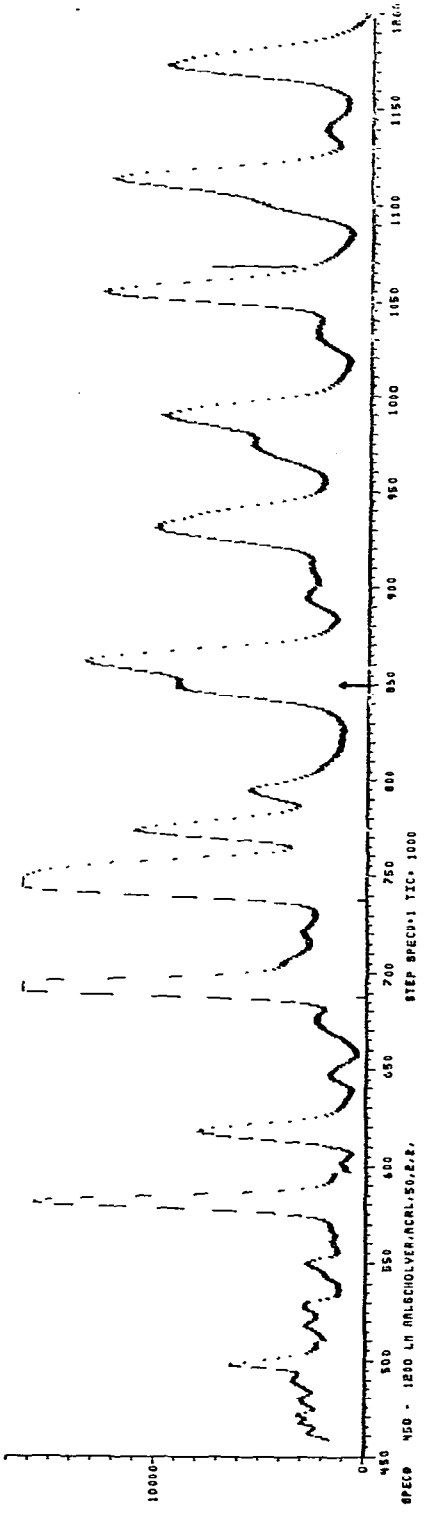


Fig. 5. Total ion current chromatogram of 0.2 μl of cormorant extract, starting at spectrum number 460 and ending at number 1270[19].

C_8H_{18}. The difference is only 0.06 mass units, so that a resolution of 2000 would be sufficient to eliminate this interference. Resolution is obtained with an ideal peak shape that is triangular.

Fig. 4 summarizes the different procedures which can be followed in GC–MS applications[48].

To illustrate route 1, the following example has been chosen. Extracts of total body homogenates of cormorants, found dead in the field, were analysed by GC–MS in order to identify the many different compounds present in these extracts[49]. Fig. 5 shows the total ion current chromatogram obtained from a computerized GC–MS combination operating in the cyclic scan mode, each number on the *x*-axis corresponding to a mass spectrum. Most of the compounds in the extract were identified as polychlorobiphenyls (PCBs). However, at the location marked with an arrow, spectrum number 850, a mass spectrum is found that contains chloro isotope clusters at *m/e* 376, 341 and 306 belonging to an (at that time) unknown compound, and at *m/e* 290 and 220 characteristic of a polychlorobiphenyl (Fig. 6).

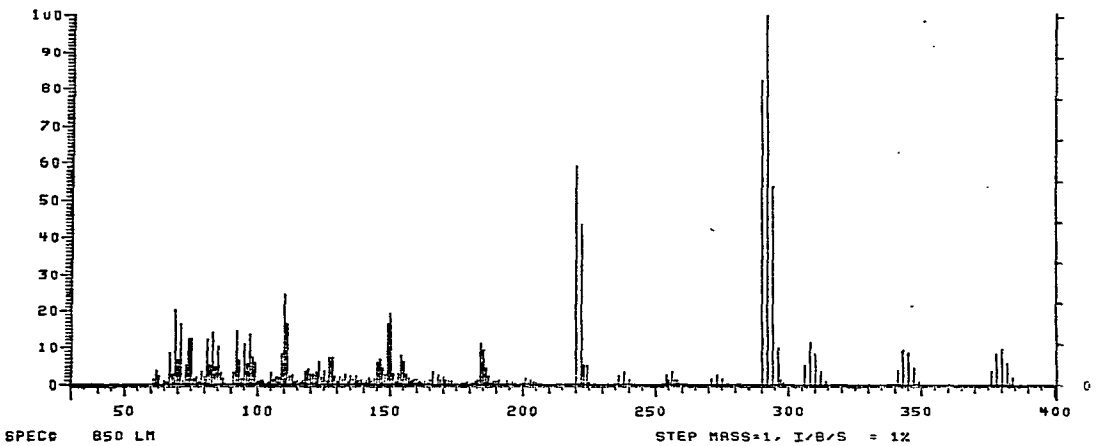


Fig. 6. Uncorrected mass spectrum at location 850 in the total ion current chromatogram.

To find the exact location in the chromatogram and the spectrum that could be subtracted in order to eliminate the PCB masses, the computer was instructed to produce a mass fragmentogram of the masses 376, 341 and 306, as is shown in Fig. 7. The three selected masses coincide fairly well, which justifies the conclusion that they belong to one compound.

To obtain a mass spectrum corrected for the interference with the PCB compound of molecular weight 290, the neighbouring spectrum which does not contain the masses of the unknown compound is subtracted from this main spectrum, shown in Fig. 6. Fig. 8 shows the result of this subtraction procedure and indicates that the PCB masses are eliminated. The compound was identified as octachlorostyrene.

After identification of this compound, the question arose of whether heptachlorostyrenes were present in the extract. The characteristic masses for these compounds were easy to predict, and the computer was ordered to search for their presence. At two locations in the chromatogram, spectrum number 690 and 738, these

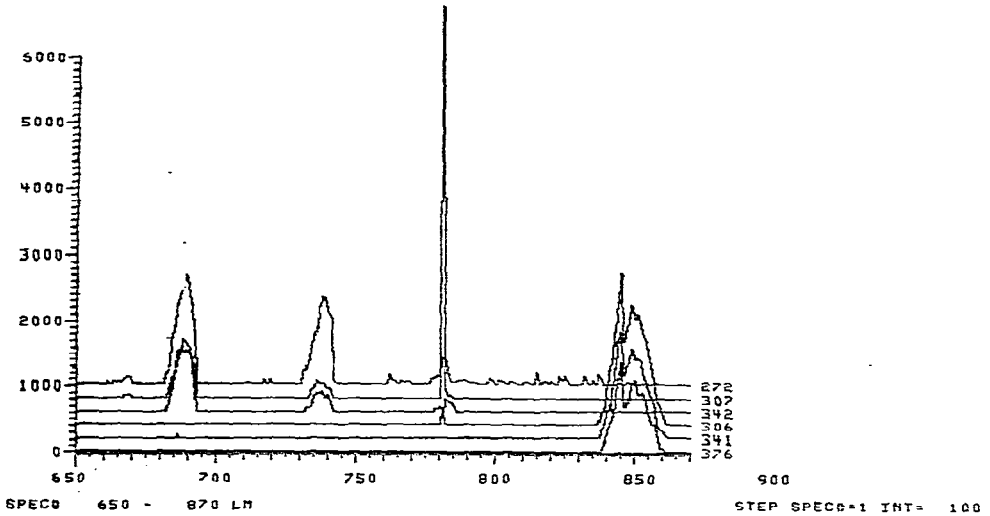


Fig. 7. Selected mass plot section of spectrum 670–870 for the masses *m/e* 376, 341, 306, 342, 307 and 272[49].

masses appeared. They proved to be correct for the isomers of heptachlorostyrene (Fig. 7). These two compounds were completely overlooked during the evaluation of the different mass spectra, as these masses were overshadowed by those of the PCBs. As all MS information was still available on magnetic tape, it was not necessary to prepare a new sample or to repeat the analyses. This example illustrates the benefit of a computerised GC–MS combination in a multi-component analysis of a complex mixture.

Identification from low-resolution mass spectra only is not always successful. High-resolution GC–MS data which also yield the elemental compositions of the masses can be most helpful in identifying completely unknown compounds. The following example[50] demonstrates that it is possible to obtain useful elemental com-

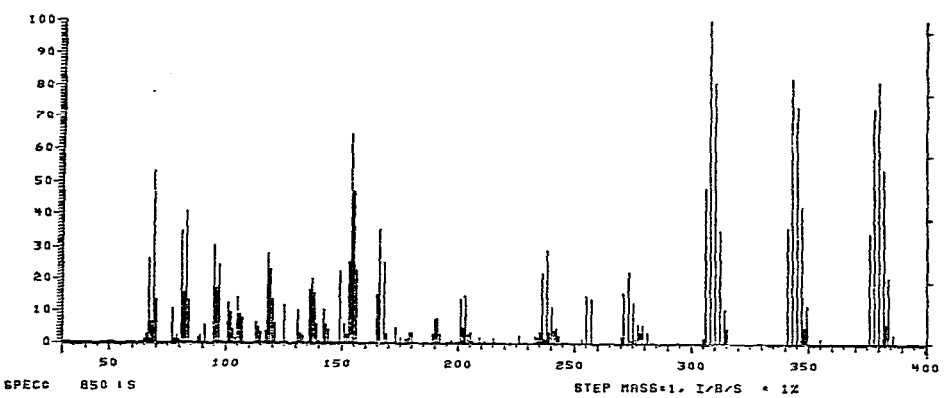


Fig. 8. Corrected mass spectrum number 850 LS, obtained by subtracting spectrum number 860 from 850[49].

positions of compounds eluting from a capillary column connected to a medium-resolution double-focusing mass spectrometer operating at a resolution of 3300 and scanning at 2 sec per mass decade.

Fig. 9 shows a computer-reconstructed gas chromatogram of a mixture of anthracene, pyrene, *p*-terphenyl and triphenylene. Fig. 10 shows the elemental composition obtained from the last peak. Here the molecular ion 228 is measured with a 0.0015 mass unit deviation from the theoretical value ($C_{18}H_{12}$). These data were obtained from only 40 ng of triphenylene.

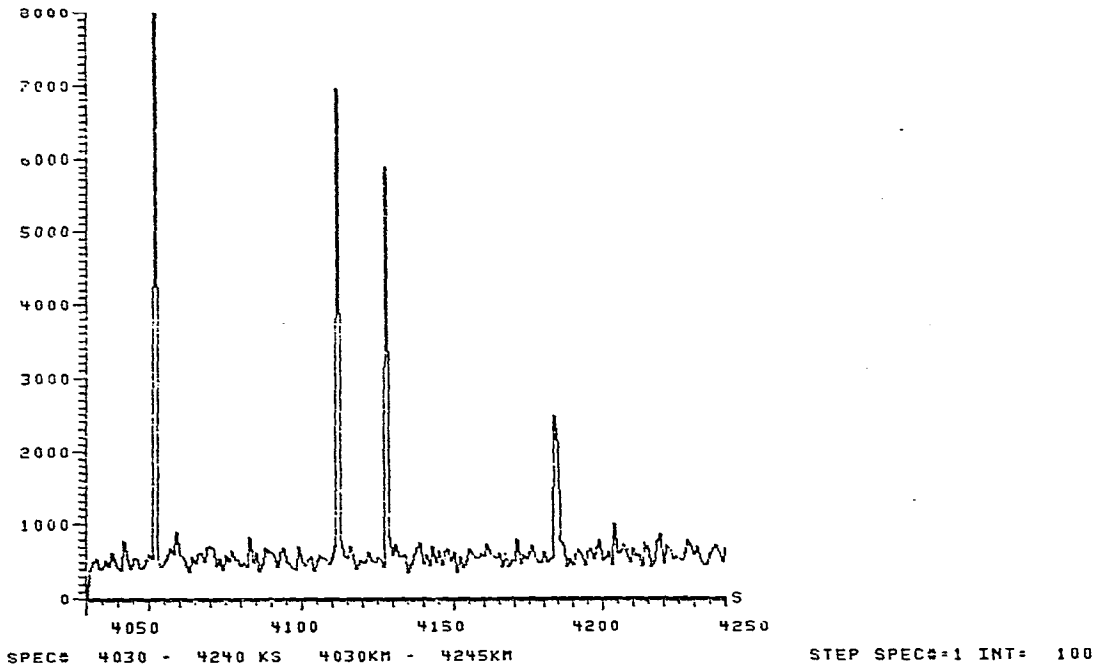
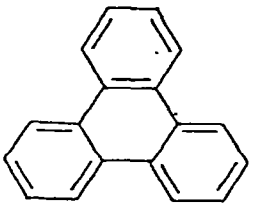


Fig. 9. Total ion current chromatogram of a mixture of anthracene, pyrene, *p*-terphenyl and triphenylene[50].

The following examples deal with problems that can be solved by means of route 2 (Fig. 4). The application of mass spectrometry in the quantitative trace analysis of complex mixtures is increasing, especially in the fields of clinical chemistry, toxicology, forensic medicine and off-flavour research.

Fig. 11 shows a total ion current chromatogram together with some mass fragmentograms of a concentrate of the volatile compounds of white beans. These beans, which are used in the confectionary industry as raw material, had a musty taint which, from experience with previous off-flavour problems, could be caused by chloroanisoles[51,52]. A direct search for these compounds was performed, in this instance at masses 210 and 212 for trichloroanisole and 244 and 246 for tetrachloroanisole. Only one location was found with a retention time corresponding to 2,4,6-trichloroanisole and none for the tetrachloroanisoles.

For this application it is not necessary to have all mass spectrometric infor-



>>DATE<</SPEC# 4194/GM/

BASE PEAK 7019 / MASS 228

PEAK	I/BASE	MASS		DIFF	C/C'	H
					12/13	1
5	2.36 %	41.0355	*	-3.6	3/0	5
				0.9	2/1	4
9	3.21 %	56.0679		5.3	4/0	8
16	1.75 %	75.0267		3.2	6/0	3
17	1.93 %	76.0271		-4.1	6/0	4
				0.3	5/1	3
22	1.33 %	87.0262		2.7	7/0	3
23	4.55 %	88.0266		-4.6	7/0	4
				-0.2	6/1	3
34	11.76 %	113.0313		-7.8	9/0	5
				-3.3	8/1	4
36	8.09 %	114.0527		5.7	9/0	6
62	1.86 %	188.0545		-3.6	14/1	7
78	6.16 %	205.0657		-4.7	15/0	9
79	26.49 %	226.0781		-0.1	18/0	10
				4.3	17/1	9
80	24.10 %	227.0872		1.1	18/0	11
				5.6	17/1	10
81	100.00 %	228.0924		-1.5	18/0	12
				3.0	17/1	11
83	16.69 %	229.0935		-3.6	17/1	12

Fig. 10. Computer-printed elemental composition of triphenylene[50].

mation available, so one can consider the use of a cheaper multiple ion selector. The method is more sensitive and may reveal the presence of the other chloroanisoles. The result obtained with a four-channel ion selector from the same extract is shown in Fig. 12. The upper trace represents the total ion current and the lower four traces are the selected masses. By applying this method it was possible to detect trace amounts of one other trichloroanisole and two tetrachloroanisoles at the correct retention times.

In the above examples, chlorinated compounds that possess masses very different from those of the compounds in the matrix had to be detected; there were not many chances of misinterpretation. As mentioned before, difficulties will arise when the masses of the compounds to be searched for are not so characteristic. An example is the presence of nitrosamines in cigarette smoke and meat products. Nitrosamines are very toxic and carcinogenic compounds and should not be present in food.

Fig. 13 shows the result of an analysis obtained with a computerized GC–MS system. Cigarette smoke (3 ml) was injected on to a 150-m capillary column coated with Ucon B[43,53]. The upper trace represents the total ion current and the other five the characteristic masses of nitrosopiperidine (30, 42, 55, 56 and 114). None of them are very characteristic ions. For instance, mass 30 can also be the isotope peak of a large mass 29 fragment.

In the retention time range of nitrosopiperidine, all five masses are present and coincide. However, to draw the conclusion from this information that nitrosopiperidine is present is very risky and may lead to misinterpretations.

In order to investigate the value of the information obtained, it is necessary to

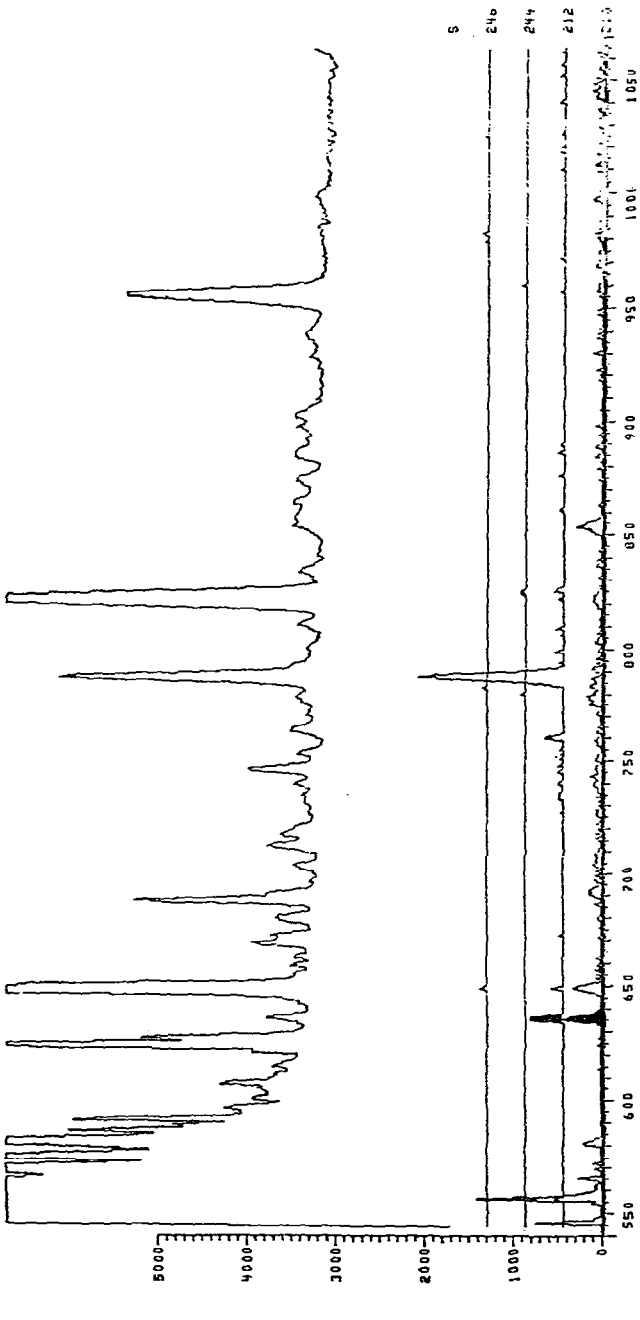


Fig. 11. Total ion current chromatogram of an extract of white beans and mass fragmentograms at *m/e* 210, 212, 244 and 246[18].

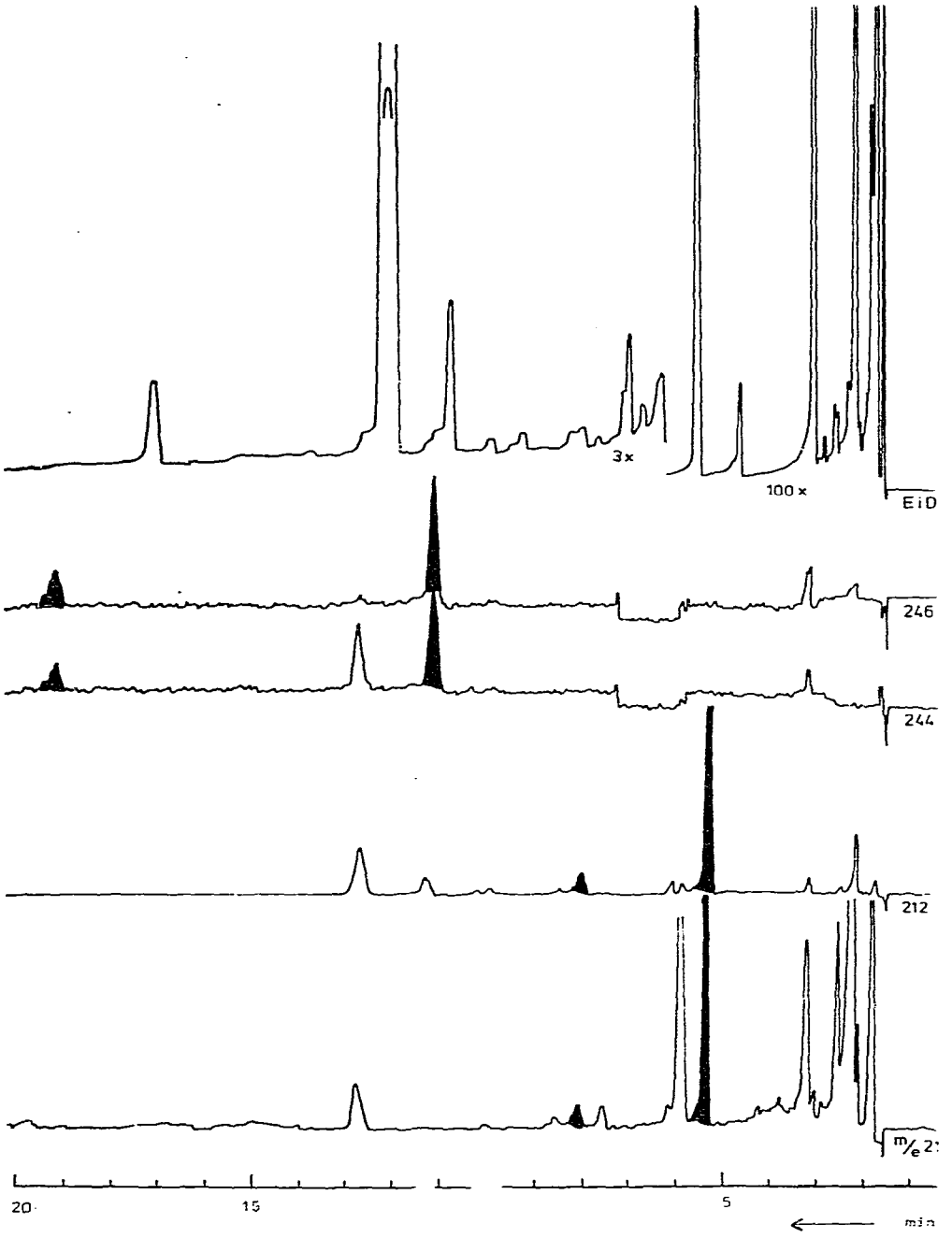


Fig. 12. Total ion current chromatogram and mass fragmentograms of an extract of white beans obtained from a Varian-MAT 112S double-focusing mass spectrometer equipped with a four-channel ion selector.

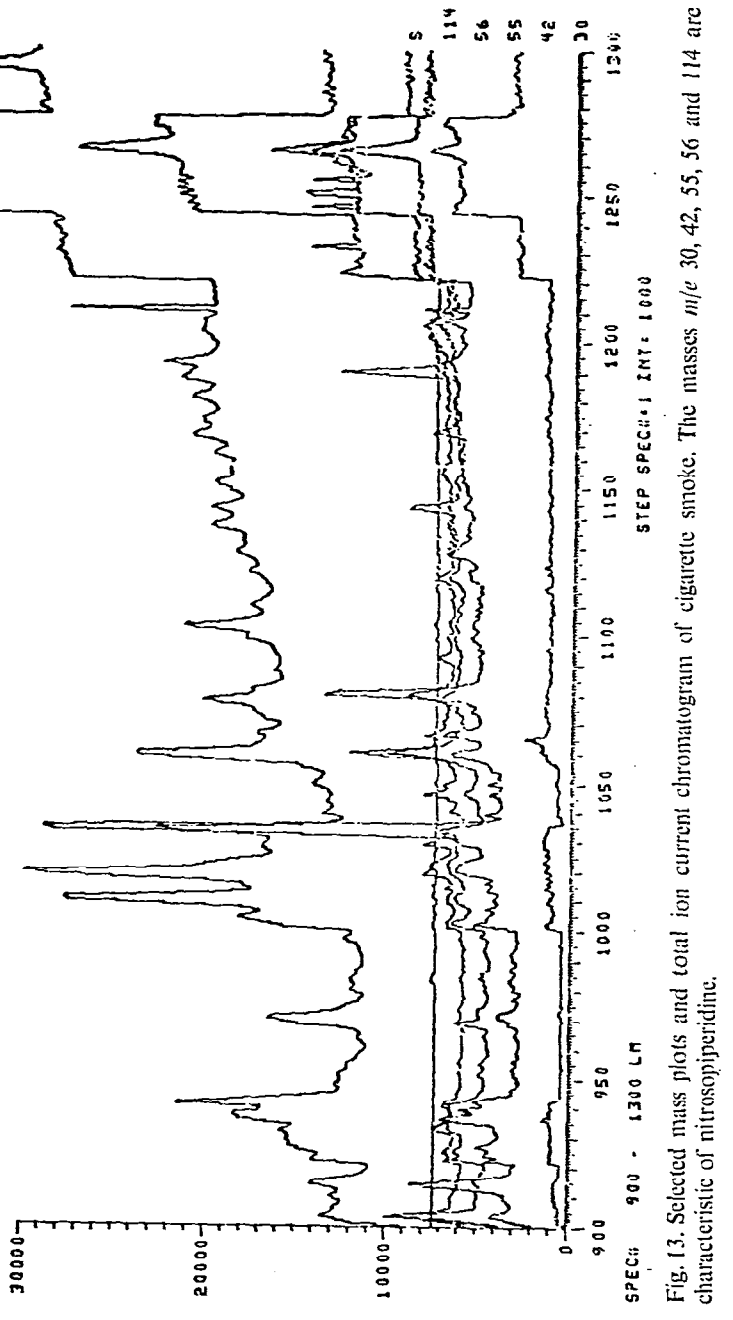


Fig. 13. Selected mass plots and total ion current chromatogram of cigarette smoke. The masses *m/e* 30, 42, 55, 56 and 114 are characteristic of nitrosopiperidine.

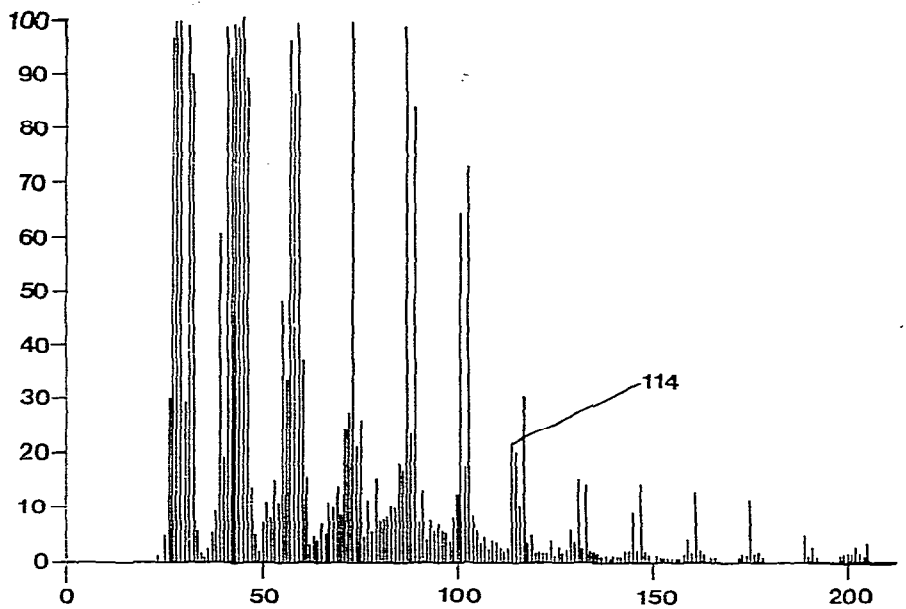


Fig. 14. Uncorrected spectrum at location 1265 LM.

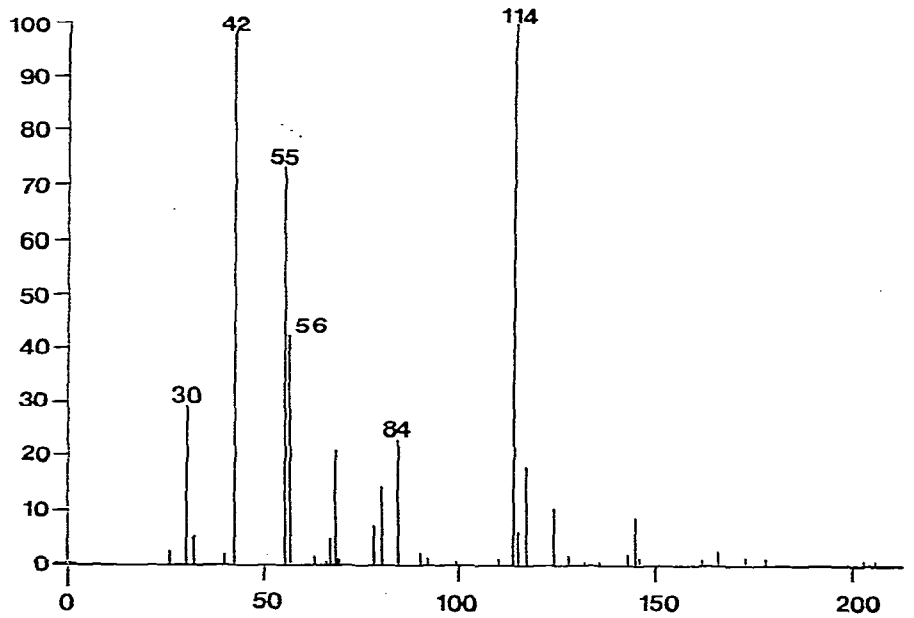


Fig. 15. Mass spectrum of nitrosopiperidine in cigarette smoke, obtained from spectrum 1265 LM by subtraction of a neighbouring background spectrum.

examine the mass spectrum location 1264 derived from the mass fragmentogram. This mass spectrum (see Fig. 14) looks like a forest from which no significant information can be retrieved; even the molecular ion 114 of nitrosopiperidine is completely hidden between other fragment ions. However, a subtraction procedure applied to this spectrum produces a clearer picture, as shown in Fig. 15. This spectrum corresponds well with the reference spectrum of nitrosopiperidine. Only for these particular compounds it was possible to obtain any significant information; for other nitrosamines the interference with the other masses could not be eliminated and no useful information could be obtained.

In order to obtain reasonably quantitative results, any contribution of an interfering mass to the selected mass on which calculations are performed results in

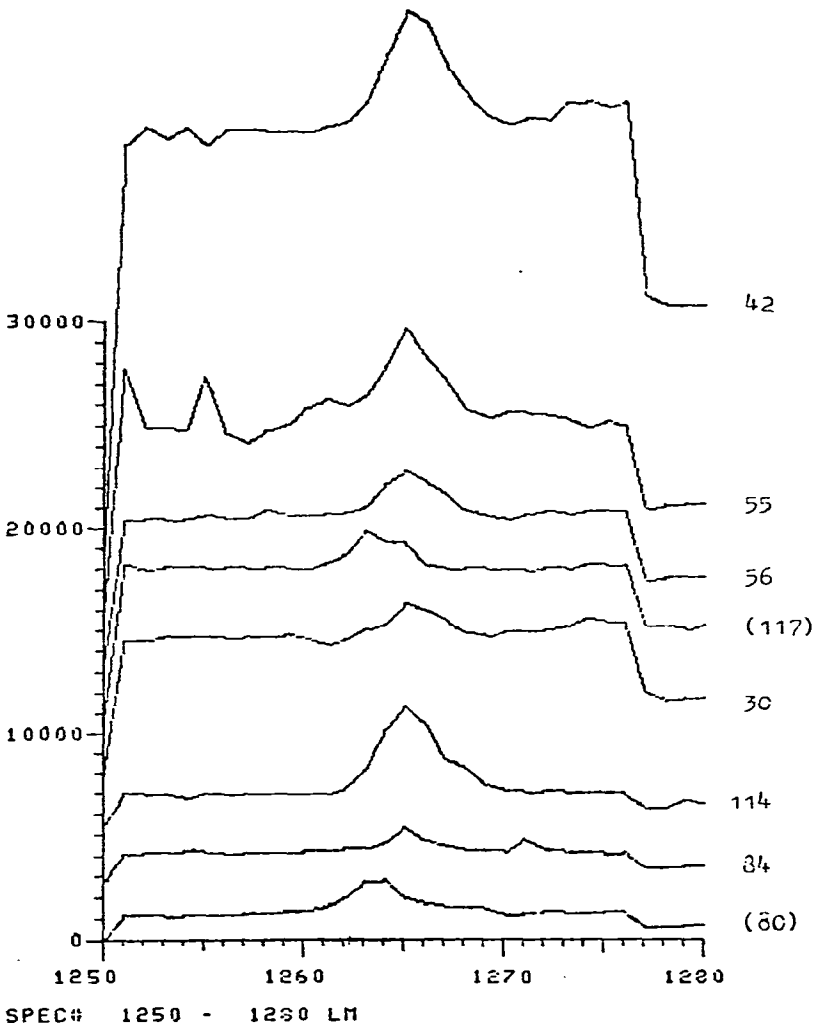


Fig. 16. Selected mass plots of masses *m/e* 30, 42, 55, 56 and 114, characteristic of nitrosopiperidine. and *m/e* 117, originating from an interfering compound.

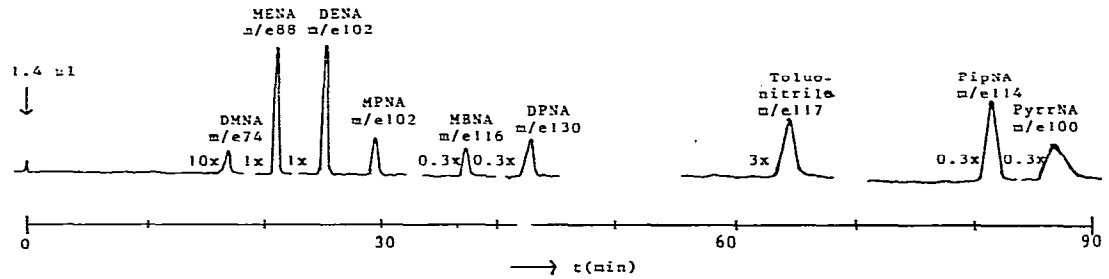


Fig. 17. Mass chromatogram of the molecular ion masses of DMNA (*m/e* 74.05), MENA (88.06), DENA (102.08), MPNA (102.08), MBNA (116.10), DPNA (130.11), PipNA (114.08) and PyrrNA (100.06) of a 10 ppm nitrosamine standard solution at resolution 4000. DMNA = dimethylnitrosamine; MENA = methylethylnitrosamine; DENA = diethylnitrosamine; MPNA = methylpropylnitrosamine; MBNA = methylbutylnitrosamine; DPNA = dipropylnitrosamine; PipNA = nitrosopiperidine; PyrrNA = nitrosopyrrolidine.

higher values of the concentration of the trace compounds that have to be determined. In the above spectrum masses other than those of nitrosopiperidine are present, *e.g.*, mass 117. To find out how this mass is located in relation to those of the nitrosopiperidine, a mass fragmentogram is produced (Fig. 16) by the computer, showing that *m/e* 117 is shifted slightly from those of the nitrosamine. However, it is obvious that it cannot be eliminated by means of a subtraction procedure.

As has already been stated, there was no possibility of obtaining any reliable results for other nitrosamines, owing to the heavy interference from other masses. Increasing the MS resolution might solve the problem. The benefit of increasing the resolution of the mass spectrometer is demonstrated below for the analysis of eight nitrosamines in smoked horse meat by GC–MS medium-resolution single-ion detection[43].

A 150-m wide-bore capillary was connected to a high-resolution mass spectrometer, which was tuned to a resolution of 4000 with a trapezium-shaped peak. This means that the mass spectrometer was first tuned to a resolution of about 8000, then the exit slit was opened in order to obtain a trapezium-shaped peak to perform the measurements under more stable conditions. Perfluorokerosene was led in continuously to generate reference masses in order to calibrate and control the exact mass tuning of the selected mass corresponding to a particular elemental composition. After the

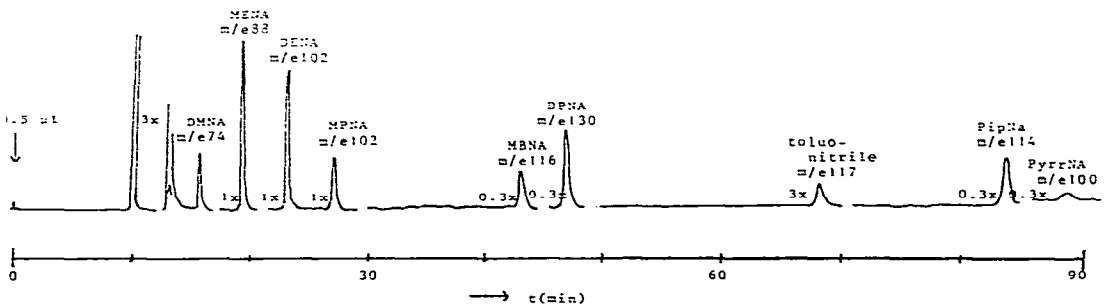


Fig. 18. Mass chromatogram of the molecular ion masses of DMNA, MENA, DENA, MPNA, MBNA, DPNA, PipNA and PyrrNA of an extract of spiked horse meat (cured and smoked) at resolution 4000.

retention time of each nitrosamine, the exact mass was tuned to the exact mass of the next nitrosamine. Switching from mass to mass takes about 1 min, whereas the intervals between peaks are 3 min or more.

Fig. 17 shows the the fragmentograms of a 10 ppm standard solution of the eight nitrosamines (14 ng per peak). *o*-Toluonitrile was added as an internal standard. The efficiency of the isolation and concentration procedures was determined by analysing smoked horse meat spiked with appropriate amounts of nitrosamine standard solution so as to obtain concentrations in the product of 25, 50 and 100 μg/kg.

The fragmentograms of such a recovery test are shown in Fig. 18; the number of non-nitrosamine peaks is very small. When the same analysis is performed with a resolution of 800, a complex and overloaded chromatogram is obtained, from which no reliable conclusion can be drawn as to the presence of nitrosamines at the micrograms per kilogram level.

Fig. 19 gives the results of an analysis of unspiked horse meat. In this product 3 μg/kg of DMNA and 90 μg/kg of DENA were detected and none of the other nitrosamines (no interference from other compounds occurs). Under unfavourable GC–MS conditions, higher concentrations might be suggested, owing to interference with the isotopic masses from the trimethylsilyl ion at *m/e* 73 belonging to some silicone compounds in the extract[43,54,55]. To distinguish the molecular mass 74.048 of DMNA from the two isotope masses of silicon (29) and carbon (13) from the trimethylsilyl ion at *m/e* 73, which differ by 0.0011 and 0.0027 mass units, respectively, a resolution of about 60,000 is required.

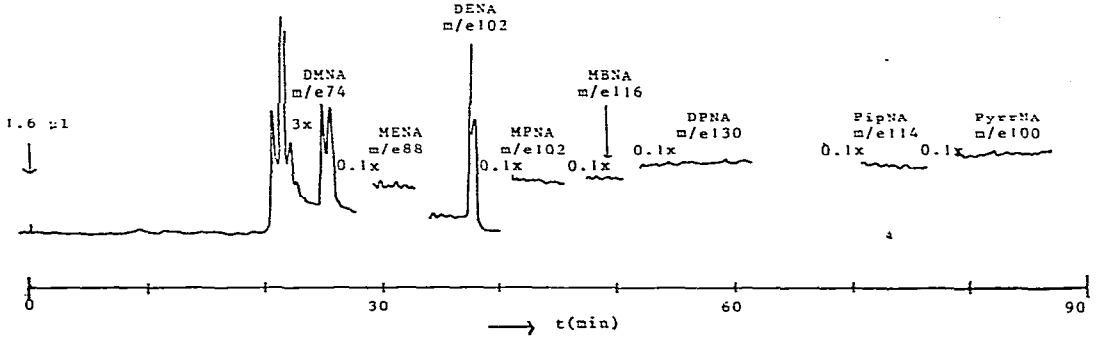


Fig. 19. Mass chromatogram of the molecular ion masses of DMNA, MENA, DENA, MPNA, MBNA, DPNA, PipNA and PyrrNA of an extract of unspiked horse meat (cured and smoked) at resolution 4000.

Fig. 20 shows a high-resolution plot obtained from the peak match display at a resolution of 70,000 at *m/e* 74. Peak 1 belongs to a perfluorokerosene ion, peak 2 represents a silicon impurity and belongs to the silicon isotope of the trimethylsilyl ion at *m/e* 74.0469, $C_3^{12}H_9^1Si^{29}$. Peak 3 belongs to the molecular ion at *m/e* 74.048 of DMNA with elemental composition $C_5H_{10}N_2O$. Peak 4 represents the carbon-13 isotope of the trimethylsilyl ion at *m/e* 74.0507, $C_2^{12}C^{13}H_9^1Si^{28}$. This high-resolution measurement confirmed the presence of DMNA in smoked horse meat. The example above illustrates well how complicated the situation can be and how careful the investigation should be so as not to draw wrong conclusions, especially in quantitative

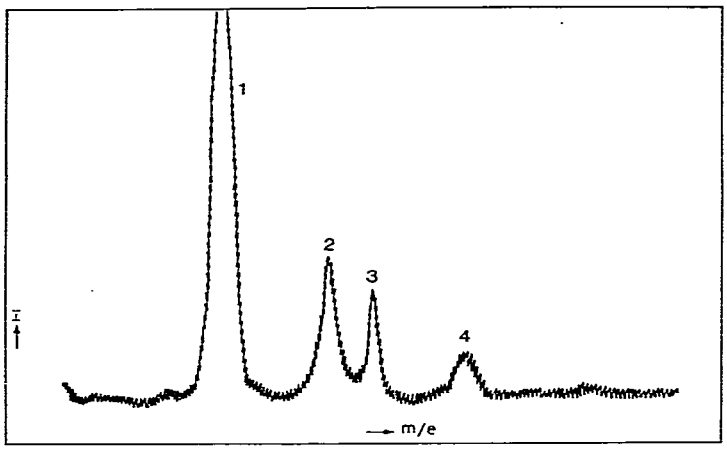


Fig. 20. High-resolution display at a resolution of 70,000 at *m/e* 74 obtained from smoked horse meat. Peaks: 1 = perfluorokerosene; 2 = $C_3^{12}H_9^1Si^{29}$ (74.0469); 3 = $C_5H_{10}N_2O$ (74.048); 4 = $C_2^{12}C^{13}H_9^1Si^{28}$ (74.0507).

analyses, where interference with other masses may lead to a higher concentration value for a certain compound than is actually present in the extract.

The application of chemical ionization is an other promising technique for decreasing interferences in trace analysis. By means of this technique the fragmentation pattern of the spectrum is considerably reduced and new intense pseudo-molecular ions are created, which can be seen as some kind of derivatization of organic compounds in the ion source of the mass spectrometer. With this technique interference problems can also be reduced and the selectivity increased.

Fig. 21 illustrates the electron-impact mass spectrum of caryophyllene oxide,

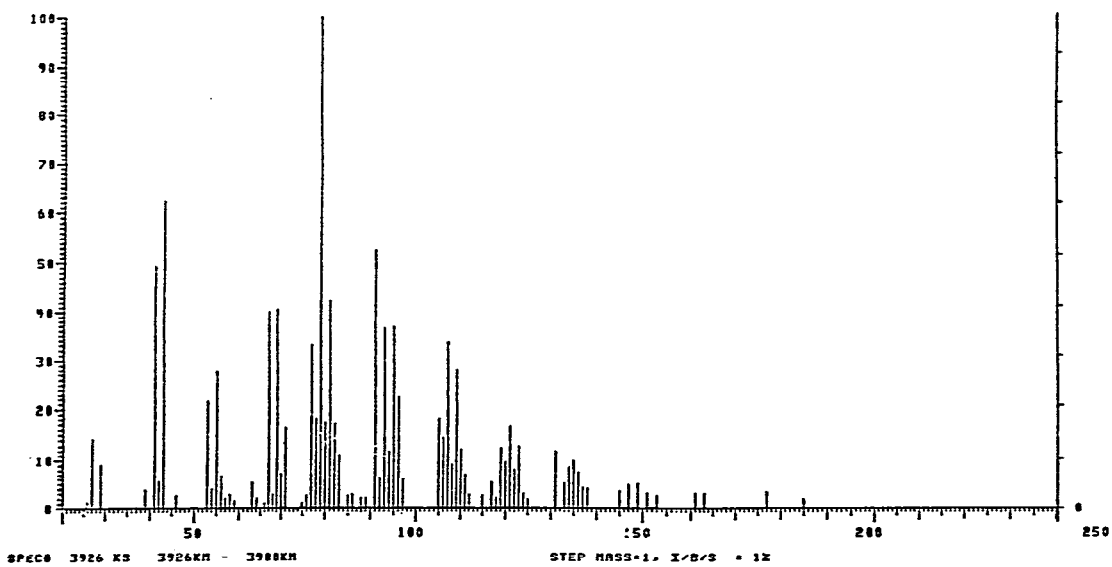


Fig. 21. Electron-impact mass spectrum of caryophyllene oxide

which shows no molecular ion at *m/e* 220 but only a large number of uncharacteristic fragment ions. To detect such a compound by mass fragmentography is almost impossible.

Fig. 22 shows the spectrum of the same compound obtained with chemical ionization. The fragmentation is strongly reduced and new intense masses appear at *m/e* 203 and 221, which is the M + H ion. These masses yield good responses in mass fragmentography.

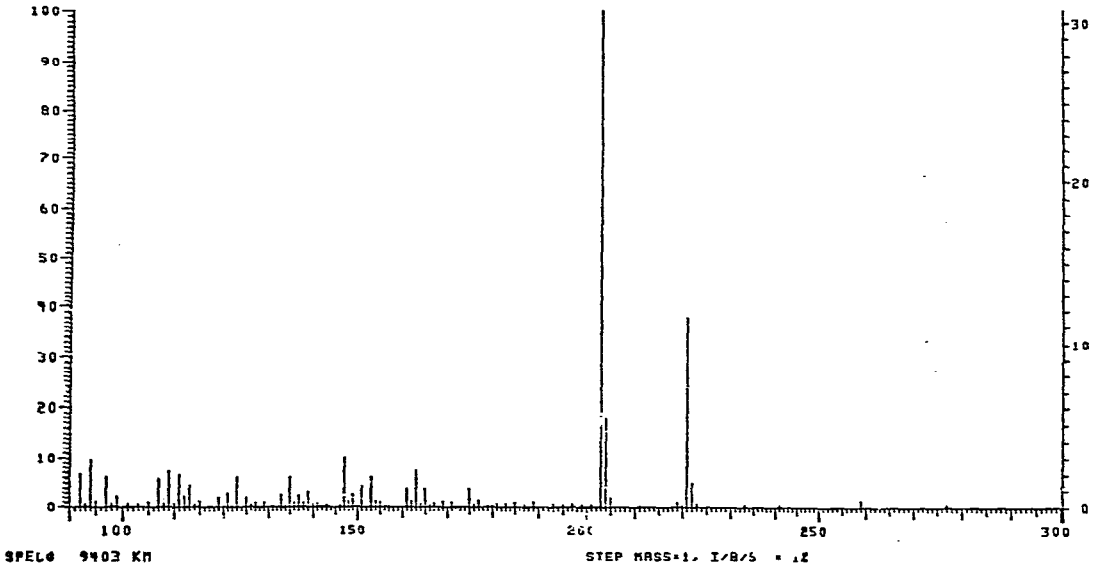


Fig. 22. Chemical-ionization mass spectrum of caryophyllene oxide, obtained with isobutane.

4. CONCLUSION

The application of GC–MS in analytical chemistry is still increasing in spite of the high investment costs. This is due to the fact that the time required for confirming and identifying compounds in complex mixtures can be reduced to a few hours instead of several weeks.

The rapid analytical response of such a system not only yields economic benefits, but is also very important in, *e.g.*, clinical chemistry, where a rapid response from the laboratory may save a patient's life[56].

A subject that has not been discussed in this paper is the combination of high-performance liquid chromatography with mass spectrometry (HPLC–MS). Although many different interfacing techniques are being developed, there is still a long way to go before useful HPLC–MS systems will become available[57–60]. The technology of interfacing and the techniques of ionization required are much more complicated than in GC–MS. The introduction of capillary columns in HPLC might be helpful in realizing a simple interfacing technique so that the application of HPLC–MS could be achieved.

5. SUMMARY

A brief historical survey is given, together with a review of the main requirements for obtaining an optimal GC–MS system. Special attention is paid to the many different coupling techniques that have been developed during the last 15 years. The necessity for computerization and the various operational techniques applied in GC–MS are discussed and illustrated. Examples derived from the daily practice of an analytical laboratory are used to illustrate the different methods applied in GC–MS analyses of complex mixtures, such as low- and high-resolution multi-component GC–MS analyses, computer-aided mass fragmentography, spectrum subtraction procedures, interference problems occurring in multiple and single ion detection (low and medium resolution) and chemical ionization.

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