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MASS FRAGMENTOGRAPHY AS AN APPLICATION OF GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY IN BIOLOGICAL RESEARCH

A. E. GORDON AND A. FRIGERIO
Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan (Italy)

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CONTENTS

1. INTRODUCTION

Mass spectrometry (MS) is a technique for separating charged masses or ions. In the mass spectrometers commonly used in organic chemistry, the material under investigation is partially ionized by bombarding the molecules with high energy electrons. These electrons, if they impart sufficient kinetic energy, can break molecular bonds and produce molecular fragments. Due to the nature of electron bombardment the great majority of molecular and ion fragments produced carry a positive charge, *i.e.* are cations.

Ions of different masses, but of similar charge, when accelerated acquire different momenta dependent on the relationship:

$$\text{momentum} = \text{mass} \times \text{velocity}$$

The flight path of such a moving, charged particle is affected by a magnetic field, the deflection being dependent on the accelerated ion's momentum. Therefore, ions of greater mass and consequently greater momentum are deflected less than smaller ions carrying a similar charge and subjected to the same accelerating voltage and magnetic field.

By placing slits in the flight path, only ions of a certain momentum can pass through and impinge on the electron multiplier. By varying either the ionic momentum (by changing the accelerating voltage) or the magnetic field, ions of different mass can be selectively focussed. By sweeping the field, the complete spectrum can be recorded. This, in essence, is mass spectrometry, an example being shown in Fig. 1.

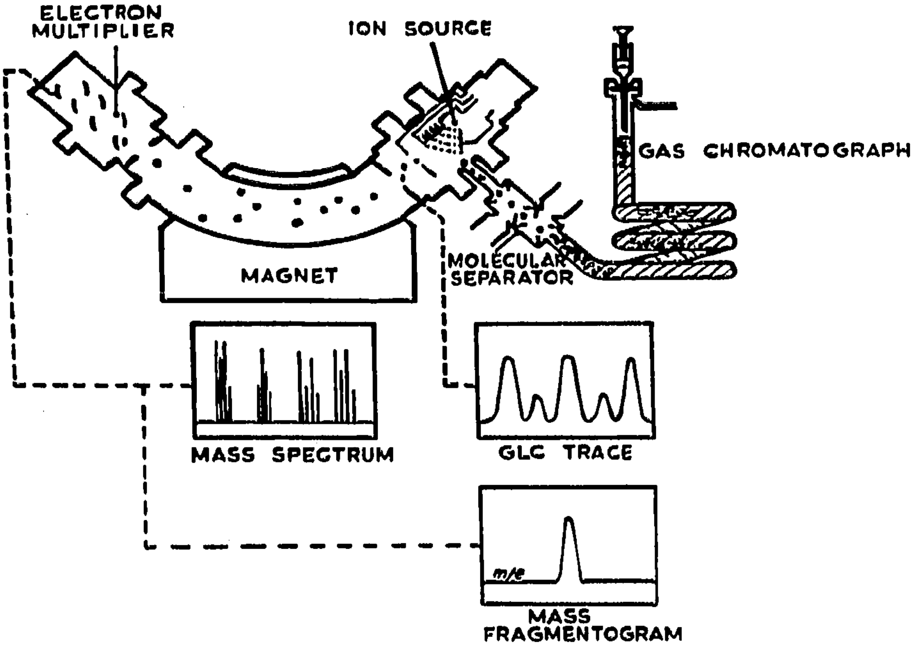

Fig. 1. GLC–mass spectrometer with mass fragmentography. GLC carrier gas (*e.g.* helium) is extracted by a molecular separator and the effluent enters the ion source. After electron bombardment, the positive ions are selectively focussed and recorded by the electron multiplier, thereby producing a mass spectrum. A measure of the total ion current is used for the GLC trace. A mass fragmentogram is produced by continuously focussing on one (SID) or a few (MID) masses only.

The fundamental equation in mass spectrometry using magnetic deflection is given[1] as

$$\frac{m}{e} = \frac{H^2 \cdot R^2}{2V} \tag{1}$$

which expresses the relationship between mass/electron charge (m/e), the intensity of the magnetic field (H), the radius of the ionic path (R) and the accelerating voltage applied to the ion (V).

When $e = 1$ and H = a constant value, we have:

$$m = K \cdot \frac{1}{V} \tag{2}$$

where $K = \dfrac{H^2 \cdot R^2}{2}$

The ion focussed by the spectrometer therefore varies inversely with the applied accelerating voltage, which suggests the possibility of focussing different ions by varying the applied voltage and keeping the magnetic field constant. By selecting the appropriate voltage, the corresponding ion is focussed[2]. By quickly switching the applied voltage to a number of pre-selected values, an equivalent number of corresponding ions will be focussed within a very short time interval. This, in essence, is mass fragmentography (MF), which is also known as single or multiple ion mass detection (SID, MID).

Mass fragmentography is therefore the simultaneous monitoring of one or more fragment ions rather than the scanning of the total ion spectrum as in conventional mass spectrometry.

Gas–liquid chromatography (GLC) is an important development that has been used to solve the increasing difficulties of achieving greater degrees of separation of biological compounds from biological tissues. There are other methods of chromatographic analysis (*e.g.* thin-layer, liquid and paper chromatography, electrophoresis) but GLC is the system most easily coupled directly to the mass spectrometer and therefore has been most widely used for this[3].

Using GLC we often have two or more peaks that are only partially resolved. Under these conditions it is not possible to study the corresponding mass spectrum because the eluted compounds are not pure. Taking repeated scans of the complete mass spectra during elution, and thereby reconstructing the elution pattern of the individual compounds, requires considerable time and patience[4] or the use of sophisticated on-line computer facilities[5].

Because of this, the idea was formulated of applying a simple device (an accelerating voltage alternator, AVA) that could continuously monitor just one, two or three characteristic ions (instead of the complete spectrum of the compound of interest) by automatically altering the applied accelerating voltage so swiftly that the different ions were all apparently recorded simultaneously[6].

In practice, the magnetic field is focussed manually on one of the mass fragments and then held constant, while the AVA is set so that the accelerating voltage is altered to focus upon one or two other mass fragments, as shown by the relationship:

$$m \propto \frac{1}{V}$$

For example, Fig. 2a shows a gas chromatographic recording of the two compounds, dehydroepiandrosterone and epiandrosterone. These very similar molecules have slightly different retention times in the GLC column and can only be partially resolved. After electron impact in the ion source of the mass spectrometer, the molecules are excited and fragmented. The pattern of fragmentation is characteristic of the original molecule. Dehydroepiandrosterone fragments produce an $m/e = 270$. Epiandrosterone produces a similar fragment, but due to the presence of two more hydrogen atoms this fragment is two atomic mass units heavier, *i.e.* $m/e = 272$. By focussing the AVA on $m/e = 270$ and 272, the separation of epiandrosterone and dehydroepiandrosterone can be attained (Fig. 2b)[6].

Chloro and iodo methyl mercuric compounds having similar retention times in a Carbowax 20M column have also been resolved by this method[7].

With the present instruments, up to four compounds with similar GLC retention times can be resolved in this way[8].

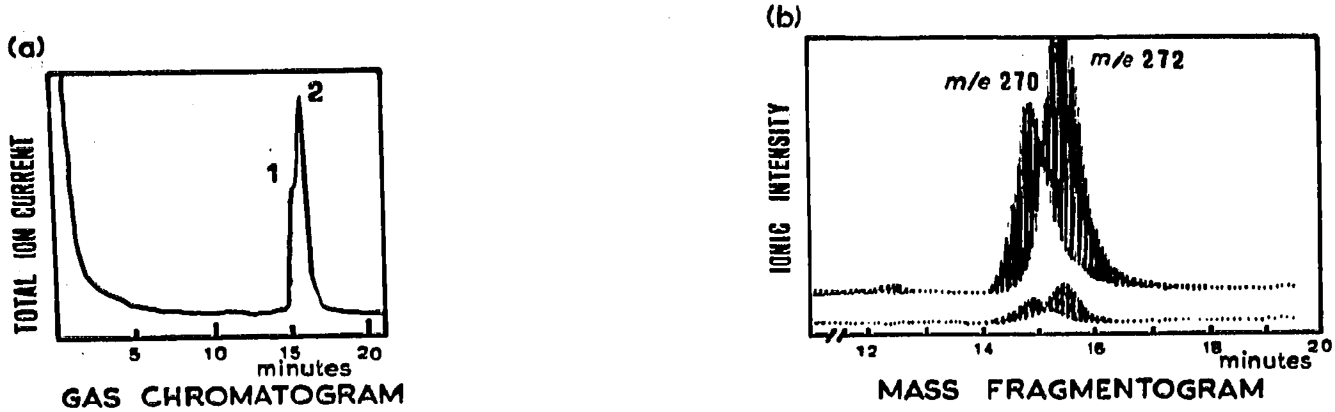

Fig. 2. (a) GLC of two partially resolved compounds, TMS-dehydroepiandrosterone (1) and TMS-epiandrosterone (2). (b) A continuous mass fragmentographic recording (MF) of $m/e = 270$ (TMS-dehydroepiandrosterone) and $m/e = 272$ (TMS-epiandrosterone) during elution of a mixture of the two compounds (see ref. 6, p. 1552).

2. APPLICATIONS OF MASS FRAGMENTOGRAPHY

With few exceptions, the use of MF as a single or multiple ion detector for GLC has mostly been applied in the field of qualitative and quantitative analysis of psychoactive agents and neurotransmitters together with their precursors and metabolites. Some of these compounds, the hallucinogens in particular, have behavioural effects when administered in only microgram quantities and therefore their adequate detection in biological tissue and urine requires the superior resources of MF.

MF has been applied in a number of ways, but it is most convenient to separate the applications into two groups: single ion detection (SID) and multiple ion detection (MID).

A. Single ion detection (SID)

In this capacity, the mass spectrometer can be used solely as a specific GLC detector. Only one mass fragment is focussed, usually the base peak (the AVA is not required), so that only when the compound leaving the GLC column has a mass spectrum showing an ion peak corresponding to that focussed upon in the spectrometer, is there a trace recorded on the UV monitor. This technique was first used by Henneberg[9,10] in 1959. The advantages are as follows.

(a) A sensitive GLC detector

This technique of single ion monitoring can increase the sensitivity of detection over that of conventional GLC methods, such as electron capture, flame detection and thermal conductivity, by a factor of 1,000 to 10,000. In this way substances not appearing on the conventional GLC trace can be monitored by the mass spectrometer.

An example is the detection of STP (2,5-dimethoxy-4-methylamphetamine, "serenity, tranquillity and peace"), a hallucinogen, as carried out in this laboratory[11]. Fig. 3 shows its mass spectrum, with a prominent peak at $m/e = 166$.

Fig. 4 shows the GLC recording using the total ion monitor of the mass spectrom-

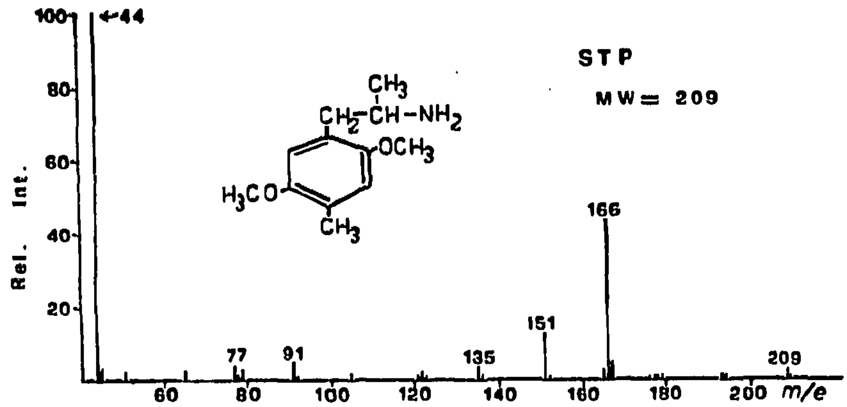

Fig. 3. Mass spectrum of STP (2,5-dimethoxy-4-methylamphetamine).

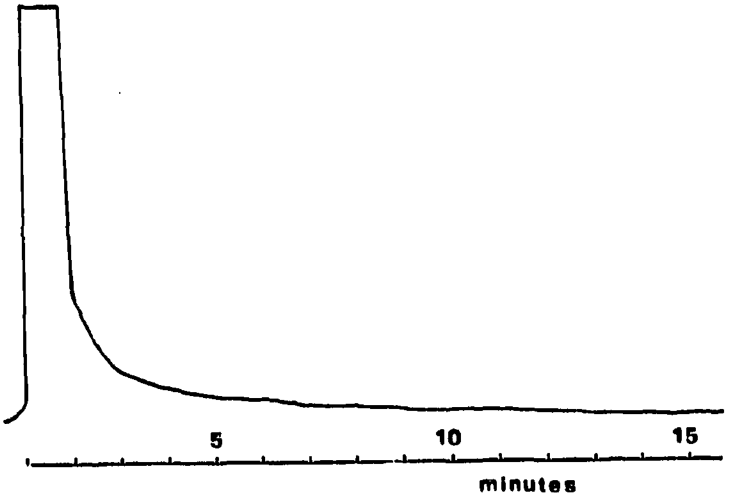

Fig. 4. GLC of 1 ng of STP. Column: OV-17 (3%) on Gas-Chrom Q; isothermal conditions at 140°; detector, total ion monitor of mass spectrometer.

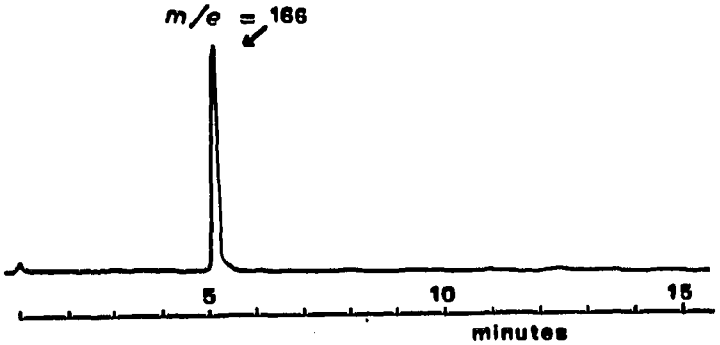

Fig. 5. Mass fragmentogram of 1 ng of STP: single ion detection with mass spectrometer.

eter as a detector. The retention time of STP under isothermal conditions at 140° is 5 min, but no peak could be observed after 1 ng had been applied to the column.

Fig. 5 shows the recording of the mass fragmentogram of STP under the same conditions as above. The mass spectrometer was used as a single ion detector and focussed on $m/e = 166$ ($m/e = 44$, although more abundant, is not characteristic of STP alone).

The sensitivity of this method allows less than 100 pg of STP to be detected when applied to the GLC column[11].

In a similar way, DDT can be detected in trace amounts as low as 10 pg (ref. 12) and other examples are shown in Table 3.

(b) SID with a high resolution instrument

In this system a high resolution instrument, capable of a resolution in the order of 100,000 (AEI MS9), has been used for the estimation of *p*-tyramine, a precursor of catecholamines. The sample was placed directly in the mass spectrometer (direct inlet system, DIS) and not applied to a GLC column; the principle is similar however.

When an extract from brain was evaporated from the probe, a double peak at $m/e = 108$ was observed. By using high resolution, the mass spectrometer was focussed on $m/e = 108.0575$, an ion fragment corresponding to *p*-tyramine, thereby distinguishing it from the interfering lipid hydrocarbon with an ion fragment $m/e = 108.0939$[13]. High resolution is therefore useful when more than one fragment ion with a similar nominal m/e ratio is present.

(c) Multi-compound monitoring

SID may also be used when a number of compounds have a different retention time in the GLC column, but on ionization produce a similar ion fragment with the same m/e ratio. This proved useful in the analysis of GLC peaks containing phenylalkyl compounds. By simultaneously focussing on $m/e = 91$ (the tropylium ion formed by cleavage of the β bond[14]) only those compounds yielding a fragment $m/e = 91$ indicative of $C_6H_5\text{–}CH_2^+$ will be recorded. The coincidence of a GLC and SID peak indicates the presence of phenylalkyl compounds. An example is shown in Fig. 6.

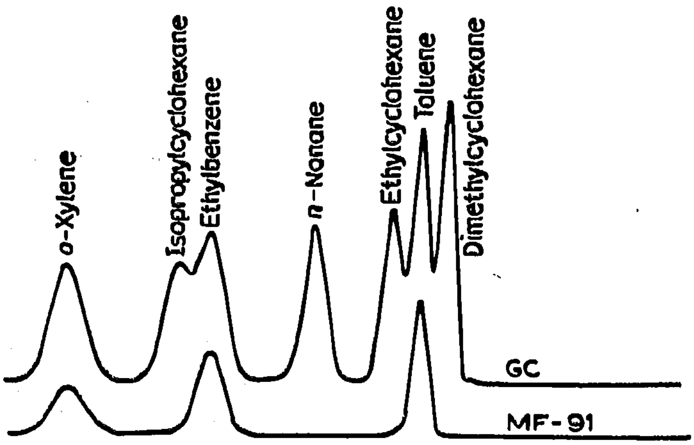

Fig. 6. GLC and MF recordings of a mixture of compounds. The mass spectrometer was continuously focussed on $m/e = 91$, indicative of phenylalkyl groups. (see ref. 10, p. 20).

This method has also been used for the identification of paraffins, alcohols, esters and acids by HENNEBERG[10]. A similar application using a quadrupole mass spectrometer has also been reported[15]. The oestrogens have a similar structure to one another and on ionization can afford the same ion of *m/e* = 216. By focussing the mass spectrometer on this ion, when 17β-estrenol and some of its 17α-analogues are applied to the GLC column, a trace on the UV recorder is observed with peaks corresponding to the different retention times of the compounds. In this way, more than one compound can be analysed from a single injection of the sample solution into the GLC[16].

B. Multiple ion detection (MID)

For MID, the mass spectrometer is focussed on two, three or four mass fragments within 10–30% of the mass range[8,17,18], or up to eight mass fragments over the whole mass range using a quadrupole mass spectrometer[10]. To illustrate this, Fig. 7 shows the mass spectrum of a compound with prominent peaks at *m/e* = 264, 266 and 276, within a 10% range.

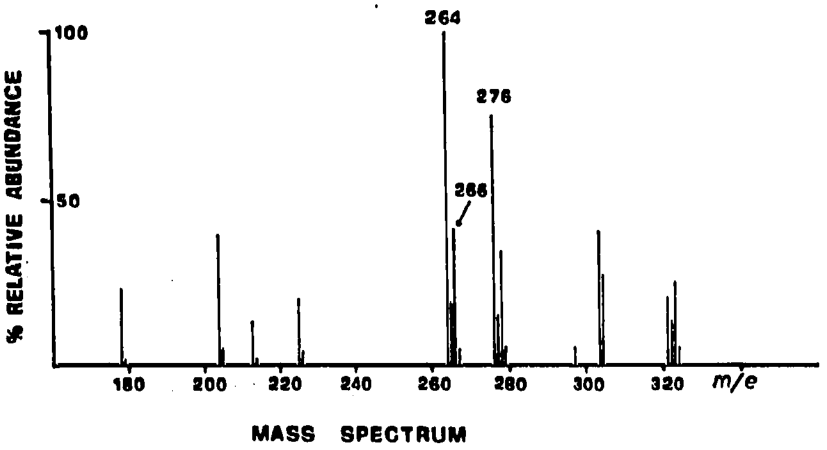

Fig. 7. Mass spectrum of compound with prominent peaks at *m/e* = 264, 266 and 276.

In Fig. 8a is shown the trace from the UV recorder of an instrument fitted with an AVA focussed on the three prominent peaks *m/e* = 264, 266 and 276. This can be seen more clearly in the diagrammatic representation in Fig. 8b (recent developments in instrumentation have greatly facilitated MF recordings[8]).

The applications of such a system are as follows:

(a) Mass spectra building by successive mass fragmentation scans

When compounds are only available in very small quantities, quantities too small to give an acceptable mass spectrum when scanning in the conventional way, partial mass fragmentograms obtained by focussing on usually only three spectral lines can be repeated by refocussing on different groups of mass fragments until a complete mass spectra is built up.

This technique has been successfully employed to obtain the complete mass spectra of the demethylated and oxidatively deaminated metabolites of chlorpromazine[17,20].

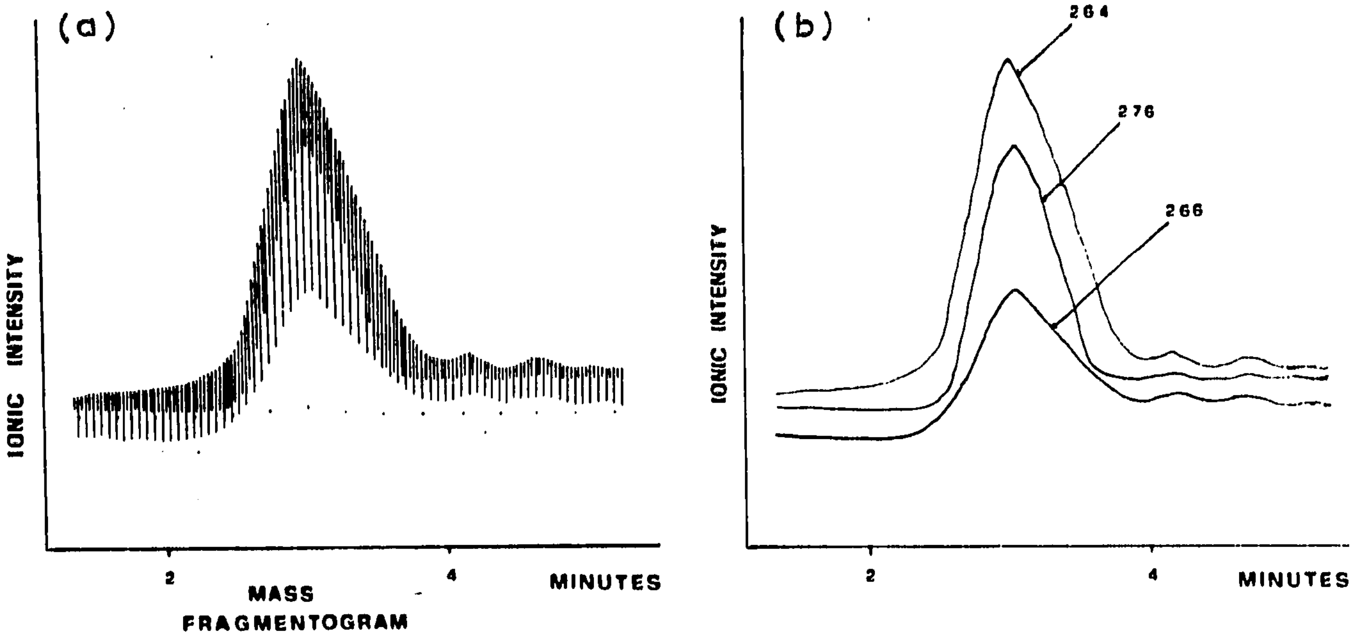

Fig. 8. (a) Mass fragmentogram of compound produced by focussing the mass spectrometer on ions of *m/e* = 264, 266 and 276. (b) A diagrammatic representation of Fig. 8a.

(b) Predicted precursor or metabolite search

Certain precursors and metabolites of naturally occurring and synthetic compounds can be hypothesized from a knowledge of the biochemistry and the already partially known metabolism of the substances in question. By predicting for these compounds a hypothetical chemical structure, either preparation of the synthetic homologue with subsequent spectral analysis or an already acquired knowledge of the compound's spectra can be used in MF to predict the existence and the ratio of certain ion fragments. In this way, the existence of hypothesized precursors or metabolites in biological material can be established and substantiated. This method has been successfully applied to the identification of 10-hydroxynortriptyline, desmethylnortriptyline and demethyl-10-hydroxynortriptyline, metabolites of nortriptyline, a widely used antidepressant[21,22] and to precursors of the hallucinogen mescaline, and tetrahydroisoquinolines[23].

(c) Quantitative estimation of nanogram and sub-nanogram levels of compounds with a high degree of specificity

This procedure is similar to that outlined using SID, but with the added advantage of greater specificity. The greater specificity is achieved by focussing on more than one mass fragment characteristic of the molecule, thus making more sure of the compound's identity. One of the channels of the AVA may also be used to monitor the intensity of a fragment ion derived from an internal standard or marker.

It is in this field that MF has been most widely used. Examples of its applications are reported in Tables 1–4.

Fig. 9 shows how this method has been used for the quantitative estimation of the anti-depressant imipramine[24].

(d) Multiple ion detection and stable isotopes

For carrying out quantitative estimations for metabolic studies the ideal reference compound should be chemically similar but in some way distinguishable from the compound under investigation. Deuterium and ^{15}N-labelled compounds with little or no discernable isotope effect are admirably suited and are probably the most important tools for the future in the hands of the biochemist working in mass spectrometry.

(1) Labelled analogues. The ^{15}N- or deuterium-labelled analogues of the compound under investigation may be metabolised, extracted, have similar retention and absorption properties in the GLC column and show similar fragmentation patterns to the unlabelled compound. After fragmentation, however, due to its extra mass, the analogue fragment ion will have a higher and, therefore, different *m/e* ratio, distinguishable from the unlabelled fragment. Suitably labelled analogues are, therefore,

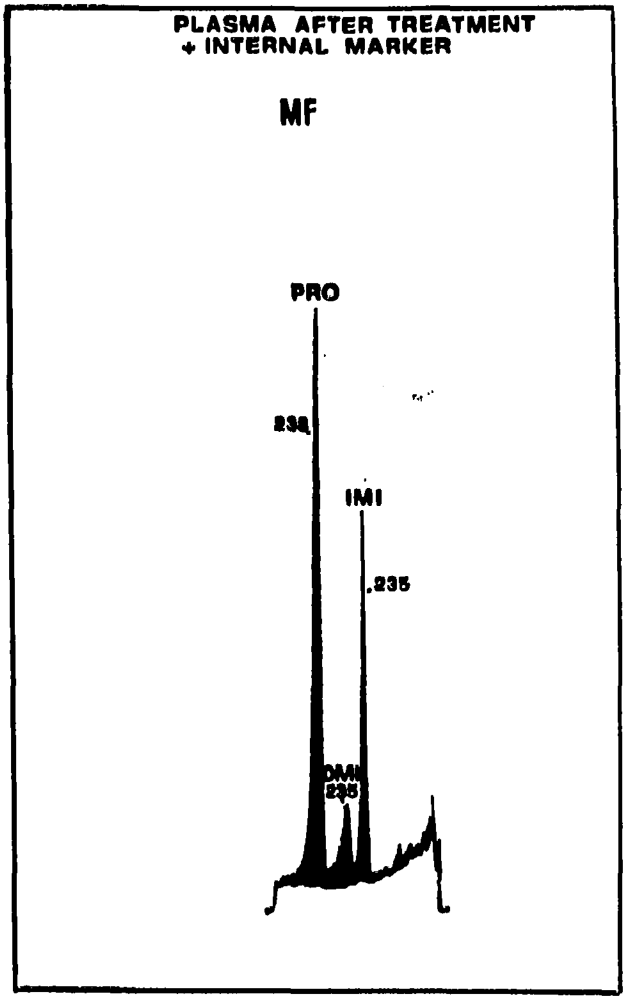

Fig. 9. Mass fragmentogram showing the quantitative determination of imipramine (IMI) in human plasma using a method of multiple ion detection (MID). The mass spectrometer was focussed on mass fragments of $m/e = 235$ and $m/e = 238$. Promazine (PRO) was used as an internal marker. The second peak of the fragmentogram is that of desmethylimipramine (DMI), a metabolite of imipramine. The method is capable of detecting as little as 10 ng imipramine/ml of plasma after extraction in *n*-hexane. Greater sensitivity was achieved by amplifying the signal from the AVA via the peak matcher unit and recording the output on the pen recorder normally used to monitor the total ion current.

useful for the quantification and estimations of recovery of the endogenously occurring compound. The first application of MF with MID was one using this technique[6]. The technique has also been used to estimate endogenous plasma concentrations of prostaglandins[25], with the added refinement that a large excess of labelled compound may also act as a carrier as well as an internal standard marker, thereby reducing the loss of endogenous compound through column absorption, etc.[26].

The investigation of metabolic pathways and the discovery of previously unknown metabolites using GLC–MF has great possibilities and, as yet, has only been little used (metabolites of nortriptyline using deuterium- and ^{15}N-labelled nortriptyline)[27]. This system may also be used for biogenic amine turnover studies. The acquisition of suitably labelled compounds is, however, still difficult and usually requires a special synthesis.

(2) Derivatized analogues. Many compounds have to be derivatized before they are suitable for GLC analysis. Suitable internal standards can be made by forming stable isotope analogue derivatives from suitably labelled reagents. Because of their very similar physical and chemical nature they are not as prone to different behaviour in molecular separators and GLC columns, such as different rates of diffusion and absorption, the biggest drawback of other internal markers.

By using reagents labelled with deuterium such as [$^2H_3$]methoxylamine, the equivalent [$^2H_3$]methoxy-derivative can be formed from a pure reference compound and used as an internal standard. The equivalent proton reagent is used on the biological sample. By focussing on mass fragment m for the derivatized sample and $m + 3$ for the methoxy derivatized internal standard marker, accurate quantifications of endogenous levels of biological compounds can be made.

This method has already been successfully employed in the estimation of prostagladin E_1 (ref. 25). It may also be possible to use reagents such as bis(trimethylsilyl) acetamide (BSA) and trimethylsilylimidazole (TMSI) labelled with deuterium[28].

(3) Naturally occurring stable isotopes. Twenty five percent of naturally occurring chlorine has an atomic weight two units greater [^{37}Cl] than the most abundant isotope [^{35}Cl]. Therefore, all fragments and molecules containing chlorine should give rise to two ions at a ratio of 3:1. By simultaneously focussing on fragments m [^{35}Cl] and $m + 2$ [^{37}Cl], a greater specificity can be obtained, as has been illustrated with chlorpromazine[17,29] and chloroform[30]. The increase in specificity can also be used in fragments containing sulphur and carbon atoms which also have naturally occurring stable isotopes, but unfortunately the proportion of other isotopes in relation to the most abundant is much smaller. Nevertheless, this has helped in substantiating the identity of thiocyanate derivatives of amphetamine and methylamphetamine[30].

Fig. 10 shows a GLC recording and fragmentogram of chlorpromazine, illustrating the greater specificity of detection by focussing on the molecular ions m/e = 318 and m/e = 320. The difference in mass of the two similar molecules is due to the two isotopes of chlorine. The molecular ions are therefore in the ratio of 3:1, *i.e.* [^{35}Cl]:[^{37}Cl]. The method followed was essentially similar to that first proposed by HAMMAR *et al.*[17]. The sensitivity of detection using this method is less than 1 pg of chlorpromazine[29].

Tables 1–4 show a list of some of the compounds that have been studied using mass fragmentography. Indication is given as to whether, (i) the compounds were in-

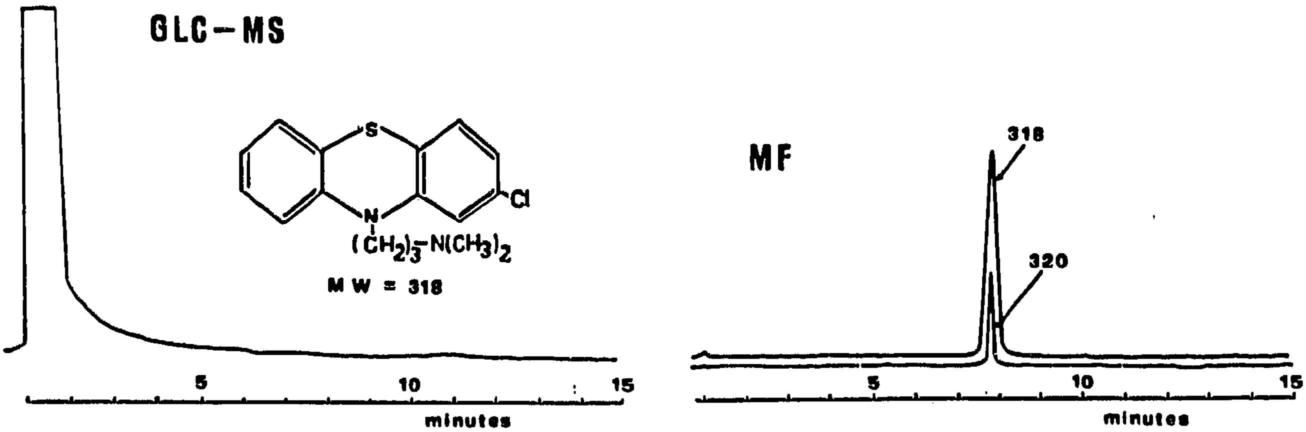

Fig. 10. A GLC–MS recording and a mass fragmentogram (MF) of 1 pg of the drug chlorpromazine. The instrument was focussed on molecular ions of $m/e = 318$ and 320. The ratio of the peak areas is in accordance with the naturally occurring isotopic abundance of $[^{35}\text{Cl}]:[^{37}\text{Cl}]$.

TABLE 1

BIOGENIC AMINES IN BRAIN INVESTIGATED BY MASS FRAGMENTOGRAPHY

See text for details. Stable isotopes not used.

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*	*sensitivity*	*Remarks*
Acetylcholine	31	MID	+	1 ng/g	Estimation of acetylcholine in rat brain. Propionyl and butyrylcholine not detectable.
Catecholamines	32	MID	+	1–60 pg	Estimation of dopamine and noradrenaline. PFPA[a] derivatives prepared and α-methyl homologues used as internal standards. Levels in rat brain regions given.
Catecholamines	19	MID	+	50 pg	Detection of dopamine and noradrenaline. The α-methyl homologues used as internal standards. Quadrupole spectrometer used.
Catecholamines	23	MID	—	—	Identification of hydroxy, methoxy and dimethoxy phenethylamine compounds derived from a mescaline-producing cactus.
Catecholamines	33	MID/SID	—	10 pg	Isothiocyanate and TMS derivatives. Detection of dopamine and noradrenaline.
p-Tyramine	13	SID	+	1 pg	Estimation of *p*-tyramine in different regions of rat brain. A high resolution instrument used for specific detection. Compound evaporated from probe (DIS).
Indole alkylamines	34	MID	+	200 pg	Estimation of serotonin, N-acetylserotonin, 5-methoxytryptamine and melatonin. Areas investigated were pineal gland, specific brain areas and blood.

[a] PFPA = pentafluoropropionic anhydride.

vestigated by single (SID) or multiple ion detection (MID), (ii) if quantitative estimations were carried out (+ or —) and where possible, the degree of sensitivity achieved, (iii) if a stable isotope such as deuterium or [^{15}N] was used and (iv) general remarks relating to the procedures or findings.

TABLE 2

PSYCHO-ACTIVE COMPOUNDS INVESTIGATED BY MASS FRAGMENTOGRAPHY

See text for details.

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*		*Stable isotope used*	*Remarks*
				sensitivity		
Marihuana constituents and metabolites	35	MID	+	200 pg	—	Estimation of Δ^1THC^a in urine and plasma.
Marihuana constituents and metabolites	36	MID	+	8 ng	—	Detection of Δ^9THC in biological material. Search for monohydrated and O-methylated metabolite of Δ^9THC.
Marihuana constituents and metabolites	37	MID	—	—	—	Detection of THC without derivative formation.
Mescaline and Tetrahydroisoquinolines	23	MID	—	—	—	Identification of mescaline and other compounds from a phenolic alkaloid extract of peyote.
Mescaline and Tetrahydroisoquinolines	33	MID	—	50 pg	—	Formation of the isothiocyanate derivative.
LSD	37	SID/MID	—	15 pg	—	Absorption on GLC column variable.
Psilocin	37	SID	—	—	—	Formation of the isothiocyanate derivative.
STP	11	SID	+	100 pg	—	Estimation as free base.
STP	37	SID/MID	—	10 pg	—	Formation of the isothiocyanate derivative.
Chlorpromazine and metabolites	17	MID	—	1 pg	—	Identification of chlorpromazine and metabolites desmethyl and didesmethylchlorpromazine in plasma and red blood cells.
Imipramine	24	MID	+	50 pg	—	Estimation of imipramine in human plasma (10 ng/ml). Promazine used as an internal standard.

TABLE 2 *(continued)*

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*	*sensitivity*	*Stable isotope used*	*Remarks*
Nortriptyline and metabolites	18	MID	—	50 pg	—	Identification of nortriptyline as trifluoroacetyl derivative.
Nortriptyline and metabolites	8	MID	—	—	—	Identification of dehydronortriptyline as a probable product of 10-hydroxynortriptyline, a metabolite of nortriptyline.
Nortriptyline and metabolites	21	MID	—	—	—	Identification of desmethyl-, 10-hydroxy- and 10-hydroxydesmethylnortriptyline, metabolites of nortriptyline, in plasma, CSF[b] and urine.
Nortriptyline and metabolites	27	MID	—	50 pg	+	Illustration of the advantage of internal standards labelled with the stable isotopes of deuterium and [^{15}N].
Nortriptyline and metabolites	47	MID	+	—	—	Quantitative determination of nortriptyline and demethylnortriptyline in the same human plasma sample after HFBA[c] derivative formation. Sensitivity <10 ng/ml plasma.
Chloroform	30	MID	+	1 pg	—	Identification in "kidnapped murder victim".
Barbiturates	38	MID	—	1 ng	—	Identification of brallo-, sero-, vin-, apro- and cyclobarbitals in blood and liver.
Amphetamine and methylamphetamine	30	MID	—	—	—	Identification and detection of amphetamine and methylamphetamine in urine samples 40 h after the ingestion of 2–4 mg.
Methamphetamine and fenfluramine	48	SID/MID	—	—	—	Methamphetamine, fenfluramine and their N-dealkylated metabolites: effect on monoamine concentrations in rat tissues.

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[a] THC = tetrahydrocannabinol.
[b] CSF = cerebrospinal fluid.
[c] HFBA = heptafluorobutyryl.

TABLE 3

PESTICIDES AND POLLUTANTS INVESTIGATED BY MASS FRAGMENTOGRAPHY

See text for details. No stable isotopes used.

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*	*sensitivity*	*Remarks*
Aldrin	39	SID	+	500 pg	Detection using a quadrupole spectrometer.
DDT	12	SID	—	10 pg	
Methyl mercuric halides	7	MID/SID	+	<5 ng	Estimation of methyl mercuric chloride and iodide in fish after benzene extraction. Limit of 1 μg Hg/kg fish. SID used on small quantities.
Tetraethyllead	40	SID	+	10 ng	Estimation of lead alkyls in petrols. Capillary column used.

TABLE 4

OTHER COMPOUNDS INVESTIGATED BY MASS FRAGMENTOGRAPHY

For details see text.

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*	*sensitivity*	*Stable isotope used*	*Remarks*
Steroids	41	SID	+	1 ng	—	Estimation of oestetrol in pregnancy urine, using low electron bombardment energy for greater specificity.
Steroids	6	MID	+	20 ng	—	Estimation of TMS derivatives of dehydroepiandrosterone and epiandrosterone.
Steroids	16	SID	+	10–40 pg	—	Simultaneous investigation of CMDMS[a] ethers of a mixture of estrenols.
Steroids	42	SID	—	0.4–40 ng	—	Detection of derivatives of Reichstein's substances 'S'.
Purines	43	MID	+	10 ng	—	Estimation of uric acid, xanthine, hypoxanthine, allopurinol and oxypurinol in skeletal muscle of treated and untreated gout patients, using DIS and a high resolution instrument for greater specificity with multiple peak-matching circuits.

TABLE 4 *(continued)*

Compound	*Ref.*	*SID/MID*	*Quantitative estimation*	*sensitivity*	*Stable isotope used*	*Remarks*
Hydroxy acid mixtures	12	MID	—	—	—	Detection of a mixture of hydroxy-, dihydroxy-, hexa- and pentadeconate methyl and TMS ethers after plant cutin hydrolysis.
Unsaturated fatty acids	44	MID	—	—	—	Detection of petroselenic, oleic and vaccenic acid polymethoxy methyl esters.
Fatty acids	45	MID	—	—	+	Resolution of perdeuterated ($C_{21}[^2H_{42}]O_2$) and monoprotium ($C_{21}H[[^2H_{41}]O_2$) methyl 3,7,11,15 tetramethyl hexadecanate.
Prostaglandin E_1	25	MID	+	3 ng	+	Deuterated methoxy derivative used as internal standard.
Prostaglandin E_2 & Prostaglandin $F_{2\alpha}$	26	MID	+	250 pg	+	Stable isotope used as an internal standard and carrier in the ratio of 1000:1, thereby facilitating quantitative estimation and reducing column absorption of the endogenous substance. The method may also be used for the determination of plasma concentrations.
Prostaglandins F	46	MID	+	200 pg	+	Computer function of endogenous and labelled compound used for greater specificity.
Glucose	3	MID	+	100 ng	+	Resolution of $[^2H_7]$glucose and glucose TMS derivatives. The first use of mass fragmentography with MID.

[a] CMDMS = chloromethyl(dimethyl)silyl

3. CONCLUSIONS

The important contribution of gas-liquid chromatography-mass fragmentography is that this new technique enables us to determine both the identity and the quantity of chemical compounds in biological material to a far greater accuracy and sensitivity than by any other method.

As illustrated, the technique is also capable of identifying new, and as yet unknown, or perhaps unpredicted, metabolites as well as substantiating the existence of hypothesized structures. This valuable tool, therefore, is capable of greatly increasing our knowledge of drug metabolism, toxicology, drug interactions and general body metabolism.

With the further development of appropriate computer programs and the greater sophistication of present instruments, mass fragmentography will make the elucidation of these problems even simpler.

4. SUMMARY

The application of mass fragmentography, as a specific detector in gas–liquid chromatography, is reviewed. The different techniques and their suitability in aiding research in toxicology, metabolism studies and the identification of drug intermediates are illustrated with reference to psycho-active compounds.

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