Introduction

In the majority of cases described in the foregoing reviews,1–6 chemical modification of the compounds under investigation was performed prior to mass spectrometric analysis, in a separate experiment, by using reactions in solution, the latter frequently being the same as those used for gas chromatography. Apart from being time consuming, derivatization reactions generally require rather large amounts of sample (micrograms). In this respect, a micromethod for the derivatization of organic compounds using reagent vapor appears to be a rather promising approach.7 Chemical or physical–chemical conversion, however, may be accomplished directly in the inlet system of a mass spectrometer (on-line). Such a procedure permits the analysis of submicrogram amounts of compound, which are accessible to mass spectrometric detection; it also saves time by eliminating the need for a separate derivatization step.

There are a number of places in a mass spectrometric device where the conversion of the compounds under study may be accomplished: (i) in the simplest case, the reaction may be carried out in a vapor phase microreactor connected directly to a mass spectrometer;
(ii) when gas chromatograph/mass spectrometer combinations are used, the conversion of the compounds can be made
(a) in a flash-heater
(b) in a vapor phase microreactor located before the gas chromatographic column (pre-column derivatization)
(c) on the chromatographic column
(d) in a vapor phase microreactor (or combustion reactor) situated between a chromatographic column and a mass spectrometer (post-column derivatization)

(iii) in liquid chromatograph/mass spectrometer combinations where post-column derivatization is of particular interest.

Thus, the use of on-line chemical transformations in mass spectrometry creates a basis for the development of fast, economical (in terms of the required amounts of sample and reagent) and effective analytical methods, the efficiency of which increases when chromatographic and mass spectrometric combinations are involved. In the case of gas chromatography/mass spectrometry (GC/MS), the name “reaction gas chromatography/mass spectrometry” (reaction GC/MS) was given to the method created on the basis of this procedure.8

The main reason for the chemical transformation directly in a gas chromatograph/mass spectrometer is to improve the analytical power of mass spectrometry for the analysis of mixtures. Further investigations showed, however, that this methodology offered new possibilities for the rapid study of the reactivity of gas-phase ions, of mechanisms of heterogeneous catalytic or other gas-phase reactions, etc.

All investigations which involve chemical modification of individual compounds or components of mixtures directly in the inlet system of a gas chromatograph/mass spectrometer (in a flash heater, in a special microreactor or on reaction or separation columns) belong to the field of reaction GC/MS. The latter is based on the use of vapor-phase reactions that proceed under the pressures available in the chromatographic part of the instrument. Hence, all the reactions that are utilized in reaction GC may also be used in reaction GC/MS. In principle, reaction GC/MS can be regarded as an extension of reaction GC.9,10 Distinct features and advantages, however, are peculiar to the former. First of all, one has to compare the volume of structural information that can be deduced by both methods. For instance, reaction GC involves chemical transformation into products with known chromatographic parameters or for simplification of mixtures by removal (subtraction) of components with particular functional groups. At the same time, reaction GC/MS involves such a modification of the compounds under study giving rise to products whose mass spectra reveal new or additional information about the structures of the original compounds. The most important advantage of reaction GC/MS is that it allows the synthesis of compounds labeled with stable isotopes (particularly deuterium) directly during the analysis which may be helpful in obtaining new structurally informative parameters and in studying the mechanisms of dissociative ionization and various vapor-phase reactions.

In reaction GC, the site of the chemical reaction is most frequently in a reactor situated ahead of the GC column. Only when the subtraction or conversion of components into derivatives, to which some specific detectors are sensitive, is used, the microreactor in reaction GC is located between the column and the detector. In reaction GC/MS, however, the location of a microreactor between the column and the mass spectrometer is most promising because it provides for an efficient structural analysis of each component within a mixture. The chromatogram thus registered corresponds to an initial mixture (which provides the quantitative analysis) whereas the mass spectra are due to the conversion products (which are used for structural identification). In this case, even those isomeric compounds that give rise to identical products can be analyzed. To illustrate the latter statement, Figure 1 shows three chromatograms of the alkene mixture

Figure 1. Chromatogram of an alkene mixture registered using (a) a by-pass, (b) a microhydrogenator between gas chromatographic column and a mass spectrometer and (c) a microhydrogenator located before column. 1: 2,4,4-trimethyl-1-pentene, 2: 1-octene, 3: 2-octene, 4: 1-nonene, 5: 1-decene; 1’: 2,4,4-trimethylpentane, 2’,3’: n-octane, 4’: n-nonane, 5’: n-decane.
registered by using by-pass (a) and the hydrogenation microreactor located between the column and the mass spectrometer (b). The chromatograms look almost identical. When the microreactor is situated ahead of the GC column, the chromatogram of alkanes is recorded [Figure 1(c)]. Instead of two peaks, 2 and 3, belonging to 1- and 2-octenes, it shows one peak due to n-octane.

So, reaction GC/MS differs strongly from reaction GC. It should be noted, however, that sometimes (for example, for the analysis of individual compounds), the separation column can be excluded and the method is converted into reaction mass spectrometry.

Reactions used in reaction GC/MS may be carried out in flash heaters, in microreactors or pyrolyzers, on reaction columns and within the conventional GC column.

**On-line chemical reactions in a microreactor connected directly to a mass spectrometer**

**Reaction mass spectrometry**

This method, adopting direct connection of a microreactor to a mass spectrometer, may be useful for analysis of individual compounds. One of the frequent applications of such a system lies in the field of heterogeneous catalytic chemistry. In this case, the microreactor is packed with a catalyst under investigation and a compound is passed through the catalytic bed in the flow of inert or reagent gas. The conversion products are further admitted into the ion source of a mass spectrometer (a system for splitting the gas flow is usually used). The mass spectra registered are due to all the formed products. To simplify the identification, “soft”-ionization methods [chemical ionization (CI), field ionization (FI), photoionization] may be employed. The method is interesting in that it can sometimes allow the products of primary catalytic reactions to be identified. We will not give any references dealing with this method because many catalytic laboratories use it in everyday routine analysis.

In a further case, the system microreactor-mass spectrometer may be applied to the investigation of catalysts with the use of thermal desorption directly in the ion source. This method, named “mass spectral thermal desorption”, involves the following stages: adsorption of the organic compound on a catalytic surface, desorption of the initial compound and conversion products by increasing the temperature and their mass spectral detection. The method assists clarification of various mechanistic aspects of catalysis especially by observing the primary catalytic processes.

**Pyrolysis-mass spectrometry (Py-MS) or direct pyrolysis mass spectrometry (DP-MS)**

The method is particularly useful for the study of various macromolecules and polymers. A sample is usually introduced into the ion source through the direct inlet probe and the temperature is increased gradually until thermal degradation occurs and volatile pyrolysis products are admitted into the ion source. As thermal reactions proceed in close proximity to the ionization region and in the high vacuum, the probability of the occurrence of secondary reactions is reduced. This enables the detection of primary products better reflecting the structures of the macromolecules. Depending on the problem to be solved, a variety of ionization techniques [EI, CI, field desorption (FD), photoionization] may be selected to record the spectra. The application of the method will not be considered in detail because there are a number of excellent reviews and books on this subject (see, for example, a recent one, Reference 13). Note some of the problems that can be solved successfully by this method:

- study of mechanisms and kinetics of thermal degradation of macromolecules
- identification of synthetic polymers and biopolymers by using the “fingerprint” method
- determination of the monomer content of synthetic co-polymers
- unit length sequence and structure determination of co-polymers
- determination of additives in polymers
- determination of end groups and branching in macromolecules.

**On-line chemical reactions in the flash-heater of a GC/MS system**

In Reference 3, it was shown that alkylating agents such as tetramethylammonium hydroxide (TMAH), other tetraalkylammonium hydroxides, trimethylaminium hydroxide, and trimethylsulfonium hydroxide provide an efficient esterification of carboxylic acids, etherification of alcohols and N-alkylation of amino groups etc. under thermal conditions in the flash heater of a GC/MS system. The reaction can be accomplished at 250–300°C (the use of Curie-point pyrolyzer connected to a gas chromatograph/mass spectrometer is also efficient). Typically, the substrate is dissolved in a 0.2 M alcoholic solution of tetramethylammonium hydroxide and after 2 min, the solution is injected into the gas chromatograph for thermochemolytic reaction.

This reaction may include thermally assisted hydrolysis and alkylation of natural esters and synthetic polyesters, peptides and proteins. A further example is on-line derivatization with tetrabutylammonium hydrogen sulfate in the GC injection port (300°C) for mass spectrometric determination of napththalene and benzene monosulfonic acids (but not disulfonic) isolated from industrial effluents and river water samples by solid-phase extraction. Methylation of barbituric acids has been successively employed with trimethylaminium hydroxide, a reagent that forms N-methyl derivatives of molecules that have replaceable hydrogen atoms attached to nitrogen.

The same procedure following solid-phase microextraction in the presence of tetrabutylammonium
hydrogensulfate and alkylation inside the injection port of a GC/MS system was used for qualitative and quantitative determination of perfluorocarboxylic acids in different aqueous matrices.\textsuperscript{18} The ion-pairing reagent tetrabutylammonium hydrogenysulfate was added directly to the polydimethylsiloxane fibre used for solid-phase microextraction and served two purposes: it facilitated extraction by counterion association and provided alkylation of the adsorbed perfluorocarboxylic acids by thermal desorption in the injection port (300°C). In this particular case, GC/electron capture negative ionization (ECNI) mass spectrometry was used for the analysis.

Recently,\textsuperscript{17} dimethyl carbonate was suggested for on-line methylation of fatty acids in GC/MS. To profile the fatty acids, soybean oil and dimethyl carbonate were spread on to a solid support (quartz wool, K$_2$CO$_3$, or zeolite 13X) fitted into a quartz tube sample holder. The latter may be placed before the injection port or inside it. Thermal methylation was investigated at temperatures of 500, 700 and 900°C.

Quaternary nitrogen compounds such as acetylcholine and other choline analogs, can be degraded thermally to the corresponding tertiary amines in a flash heater of a gas chromatograph/mass spectrometer.\textsuperscript{18,19} In concluding this section, it should be noted that some other non-analytical thermal reactions can be performed in the flash heater of a GC/MS system in order to investigate the reactivity of compounds. For example, such an approach was used for the investigation of mechanisms of thermal decomposition of 2,1-benzoisothiazole 2,2-dioxides into azao-tho-xylene. The latter can rearrange or react with olefins to form tetrahydroquinoline derivatives. All the products are separated on the GC column and identified by using their EI mass spectra. By changing the temperature of the GC injector, it was possible to estimate the activation energy of the SO$_2$ elimination from the original sulfoxides.\textsuperscript{20}

**On-line chemical reactions in a microreactor located before the gas chromatographic column (pre-column derivatization)**

In such systems, the microreactor may be connected to a chromatographic column through an intermediate injection port. However, direct connection with a chromatographic column is more suitable because the microreactor can simultaneously serve as a flash heater. The principle of such pre-column chemical modifications is similar to that used in reaction GC. Its application, in conjunction with GC/MS, however, offers new possibilities because the presence of a mass spectrometric detector has the great advantage of operating with stable isotope-labeled compounds. In GC/MS, the subtraction reactor, which allows the removal of a particular compound type to simplify the mixture, may also be located between the injection port and analytical column. Pyrolysis GC/MS should be considered as another version of reaction GC/MS.

**Catalytic microreactions**

The most convenient reactions used are those which proceed over heterogeneous catalysts (for example, hydrogenation, hydrolysis, dehydrogenation, deoxygenation, desulfurization). In these cases, hydrogen or deuterium are the standard carrier and reagent gases. The reactions are carried out in microreactors that contain catalytic beds placed between the glass wool pads. The amount of catalyst required for complete conversion of the compound to be studied depends mainly on its dispersion and the length of the bed. The simple microreactor that can be used with fused silica capillary columns for both pre-column and post-column (see below) has been described.\textsuperscript{21}

The application of such reactions will be exemplified below for particular compound types.

As was noted in Reviews 1–5, olefins comprise the important class of compounds whose direct mass spectrometric structure elucidation is not a simple task. For example, EI mass spectrometry gives minimal information regarding the character of the carbon skeleton and the position and geometry of the double bonds. Therefore, a number of methods devised to obtain this information have relied on chemical derivatization prior to mass spectrometric analysis.\textsuperscript{1–5} Reaction GC/MS offers new possibilities for the on-line derivatization of olefins and allows creation of a rapid and efficient method suitable for submicrogram amounts of analyte.

The simplest approach is the use of a microhydrogenator with hydrogen as the carrier and reagent gas. This permits the conversion of olefins into the corresponding saturated compounds thus providing a more reliable determination of the carbon skeleton of olefins in a mixture because EI mass spectra of saturated compounds (for example, alkanes) are very sensitive to branching of the aliphatic chain and identification can be made with the use of mass spectral databases.\textsuperscript{22} In addition, this approach differentiates between unsaturated and isomeric cyclic compounds. The efficiency of such a methodology was shown for the first time by Teeter et al.\textsuperscript{23} and Issenberg et al.\textsuperscript{24} who inserted a microhydrogenator between the GC column and mass spectrometer in the study of \(\alpha\)-olefins and substituted cyclohexenols in mixtures. However, such a microreactor may be located ahead the gas chromatographic column. The main disadvantage of this position is that it does not permit isomeric olefins, which are converted to identical saturated compound, to be differentiated and quantified separately. As will be shown below, this problem can be overcome by using a microhydrogenator between the GC column and mass spectrometer.

For hydrogenation of olefins, supported Pt, Pd and Ni catalysts, which are normally applied for the vapor-phase hydrogenation, may be employed. For instance, 1–2\%Pt/chromaton and 1\%Pd/porous glass catalysts were used to achieve fast and quantitative hydrogenation of alkenes at 50–250°C without undesirable side reactions (for example, hydrogenolysis or isomerization)\textsuperscript{25}.
As was mentioned above, on-line hydrogenation in GC/MS allows olefinic and isomerichydrocarbon compounds to be distinguished because only the former undergo transformation. This approach, however, cannot be applied to cyclopropane compounds that undergo ring-opening reactions to form aliphatic products under the same hydrogenation conditions. At the same time, it was shown that hydrogenolysis of mono-substituted cyclopropanes containing the alkyl-type groups results in the formation of mixtures of branched-chain and straight-chain products in a ratio of nearly 94:6:

\[
\begin{align*}
\text{R} & \xrightleftharpoons{\text{H}_2/\text{Pd}} \text{(CH}_3\text{)}_3\text{CHR} + \text{CH}_2\text{CH}_2\text{CH}_2\text{R} \\
\text{R} &= \text{n-C}_8\text{H}_{17}, \text{CH}_2\text{CH}_2\text{COOH}_2\text{CH}_2\text{CH}_2\text{COOC}_2\text{H}_5
\end{align*}
\]

This fact makes it advantageous to locate a microreactor between the chromatographic column and the mass spectrometer. Although the mass spectra thus obtained arise by superposition of the spectra of both hydrogenolysis product types, interpretation is simplified because the branched-chain compound predominates over the straight-chain and, hence, the composite spectrum is practically identical to that of the former.

The use of a hydrogenolysis microreactor located ahead of the chromatographic column may be helpful in structure elucidation of cyclopropane-containing compounds of biological origin and was shown to be efficient in the study of hydrogenolysis processes of arylfurlylcyclopropanes.

Aliphatic and aliphatic alcohols are the other type of compounds whose EI mass spectra are not very informative. In particular, the spectra of such alcohols are often devoid of molecular ion peaks because of ease of dehydration under EI conditions. Primary alcohols may sometimes be mis-identified as olefins whereas secondary and tertiary alcohols cannot be distinguished from ethers. The use of derivatization reactions (mainly, silylation and acylation) facilitates mass spectrometric identification of alcohols. However, they do not always allow determination of the carbon skeleton. To solve this problem, the following catalytic reactions, accomplished in a vapor-phase microreactor, may be recommended:

(a) catalytic dehydrogenation that can proceed over copper dust (320°C) and under a stream of helium

(b) hydrodeoxygenation of alcohols over fused iron catalyst

The reaction gives rise to the formation of hydrocarbons with retention of the carbon skeleton. Mass spectrometric determination of the structure of hydrocarbons thus obtained facilitates analysis of the carbon skeleton of the alcohols under study.

The use of an on-line pulse microreactor located ahead of the chromatographic column and with deuteron as both carrier and reagent gas offers the possibility for the rapid study of mechanisms of heterogeneous catalytic reactions. This method permits the deuteration as well as chromatographic separation and mass spectrometric analysis of all reaction products in the same experiment. It was used, for example, for the investigation of the gas-solid-phase hydrogenation mechanism for aliphatic ketones over a fused iron catalyst. Hydrogenation of carbonyl compounds to alcohols is supposed to involve “ketonic” and/or “enolic” surface forms. When the reaction proceeds in deuterium gas, deuterium atoms should be added to carbon/oxygen and carbon/carbon bonds. Because the resulting alcohols show negligible M+ peaks in EI mass spectra, on-column silylation with BSTFA was used which permitted complete elimination of active hydrogen (deuterium) from the hydroxyl groups. EI mass spectra of the trimethylsilyl ethers thus obtained showed abundant [M–CH3]+ ion peaks that allowed the number of deuterium atoms in the molecules to be determined and the above-mentioned mechanisms to be quantitatively discriminated.

There are reports describing the use of on-line derivatization in combined LC/GC/MS systems. In this case, derivatization reactions are accomplished inside the appropriate interface between the LC and GC systems. Such a methodology, involving derivatization in a loop-type interface, was developed for structure elucidation of drug degradation products and carboxylic acids. In a further paper, two approaches for on-line derivatization of stilbene hormones, quinoxaline-2-carboxylic acid, sulfamethazine and 2-naphthoic acid with the use of silylation (BSTFA, MSTFA and MTBSTFA), alkylation (TMAH or dimethylformamide dimethacetal) and acylation (Ac,O/El,N) were employed. One of them involved pre-mixing of the derivatization reagent and the analytes in the HPLC effluent ahead of the GC pre-column; another approach involved independent reagent delivery after deposition of the desalted analytes in the GC pre-column. The former is limited by reagent-mobile phase compatibility, whereas the latter may be prone to absorption problems.

Pyrolysis gas chromatography/mass spectrometry (Py-GC/MS)

The method is usually used for structural investigation of high molecular weight compounds or those with extremely low volatility. However, Py-GC/MS has been applied to the study of the thermal reactivity of low-molecular weight compounds. This method allows the on-line thermal decomposition of a sample, chromatographic separation of the products and mass spectrometric determination of their structures to be accomplished in a single experiment.

The experimental device used to perform such investigations comprises a pyrolysis unit connected to
the flash heater of a GC/MS instrument. Three types of pyrolyzers may be employed: furnace isothermal, resistively heated filament and inductively heated filament (Curie-point) pyrolyzers.\textsuperscript{35} The former device has the disadvantage that it should be held isothermally at the desired pyrolysis temperature and the sample should be introduced into the hot volume. To avoid the simultaneous introduction of atmospheric oxygen giving rise to oxidation of the sample, special devices have been developed. Filament type pyrolyzers provide very rapid (less than 5–10 s) heating of a filament coated with a sample up to desired temperature and pulse introduction of pyrolysis products into the gas chromatographic column. This is necessary to retain the best resolution with capillary columns. In Py-GC/MS, all the decomposition products are passed through a GC column, separated and admitted into a mass spectrometer that is used for their identification.

For the study of thermal behavior of low molecular weight compounds, the furnace-type pyrolyzer is used. It is preheated up to the desired temperature and a liquid sample is introduced in a hot zone with a syringe. It is not possible to cite here all the literature concerning this methodology. As an example, the thermal isomerization and decomposition of 3,3-diethyl-2,4-dimethyl-3-silathietane were studied at temperatures ranging from 300 to 530°C.\textsuperscript{36} Structures of the products were determined by EI mass spectrometry.

As mentioned above and in Reference 3, thermal hydrolytic alklylation of natural and synthetic high molecular weight esters, polyamides as well as proteins and other compounds with tetraalkylammonium hydrohides can be achieved in both flash heater and Curie-point pyrolyzer.

The literature contains many applications of Py-GC/MS for the study of high molecular weight compounds, particularly synthetic polymers.\textsuperscript{3,37} We will not cite all these papers because there are a number of excellent reviews and books devoted to this specific problem. Note only structural information that may be deduced in the course of investigation of synthetic polymers by Py-GC/MS:

- unit sequence distribution in co-polymers
- character of bonding of repeat units (“head-to-tail”, “head-to-head” etc., isomerized polymerization)
- stereoregularity of branched polymers
- chemical nature of end-groups
- branching and cross-linking structures of macrochains
- character of curing
- identification of additives and contaminations etc.

\textbf{Elemental analyzer-isotope ratio mass spectrometer (EA-IRMS)}

For bulk stable isotope analysis of various substances, a combination of elemental analyzers with a mass spectrometer is used. A sample is usually combusted by a flash-heater and the reaction products pass through a chemical trap in a flow of helium. This so-called “continuous flow” system, however, may be realized by insertion of a gas chromatographic column between the elemental analyzer and a mass spectrometer. Such combinations enable the isotope ratios of carbon and nitrogen as well as their atomic percentages to be determined. The isotope ratios of sulfur, oxygen and hydrogen may also be determined.\textsuperscript{38}

\textbf{On-column derivatization in GC/MS}

\textbf{On-column derivatization}

For the investigation of polar compounds within mixtures, derivatization reactions described in the foregoing reviews are carried out in separate experiments by using solution chemistry. However, some of them may be conducted directly within a GC column itself by using a subsequent injection of the derivatizing agent. Such a procedure is not time-consuming and the analysis is accomplished during the time needed for the analysis with the aid of conventional GC/MS. Some authors believe that such reactions proceed in the flash heater of a GC/MS system. However, in our opinion, gas-liquid phase reactions take place when the liquid stationary phase participates as a solvent.

On-column synthesis of trimethylsilyl ethers of alcohols with the aid of BSTFA in GC was reported earlier.\textsuperscript{39} A similar procedure was used for gas chromatographic/mass spectrometric analysis of alkanols,\textsuperscript{40} aliphatic diols HO(CH\textsubscript{n}\textsubscript{2})\textsubscript{2}OH (\textit{n}=4–10)\textsuperscript{41} and alkoxyalkanols RO(CH\textsubscript{n})\textsubscript{2}OH (R=CH\textsubscript{3}, tert-C\textsubscript{4}H\textsubscript{9}).\textsuperscript{42} In all these cases, the sample and agent BSTFA were successively introduced at intervals of 5 s; a threefold excess of the agent provided quantitative silylation. On-line esterification of carboxylic acids by BSTFA dissolved in ethyl acetate has also been described as a time-saving measure during repetitive quantitative analysis.\textsuperscript{43}

It was shown many years ago that similar methodology could be used to prepare N-acyl derivatives of amines directly in the GC column.\textsuperscript{44} On-column formation of trifluoroacetyl and heptafluorobutyl derivatives by using (CF\textsubscript{3})\textsubscript{2}CO and C\textsubscript{4}F\textsubscript{5}COCI, respectively, was suggested for the rapid characterization or identification of aliphatic and heterocyclic amines.\textsuperscript{45}

On-line esterification of carboxylic acids may be also used in GC/MS analysis. For example, on-column synthesis of alkyl esters (and most likely alkyl ethers of phenols) may be achieved by using N,N-dimethylformamide dialkylacetals.\textsuperscript{46} For on-line vapor-phase esterification in GC/MS, reagents prepared from alcohols and BF\textsubscript{3}-ether appear to be promising because they permit the introduction of different alcoholic residues (in particular, deuterium labeled).\textsuperscript{47}

It is well-known that silylation agents can provoke enolization of carbonyl compounds and the formation of enol silyl ethers. The reaction can proceed within the GC column and enable mass spectra of enol-ethers obtained from aldehydes and ketones to be recorded. On-column formation of imines was observed in the study of carbonyl compounds by NH\textsubscript{3} chemical ionization GC/MS. Respective imines were also formed by the injection of an amine immediately after the introduction of a carbonyl compound.\textsuperscript{49}
A double injection derivatization technique has been applied to the GC/MS analysis of phenolalkylamines. They were firstly O-trimethylsilylated by coinjection of the sample with MSTFA, then on-column acylated with N-methyl(trifluoroacetic acid) introduced in a second injection.

On-column thermal reaction in the gas phase was suggested for the rapid derivatization of polyfunctional compounds belonging to α-butyl- and phenylboronic acids. In this case, solutions of the acids in ethyl acetate were added to the mixture of analyzed compounds, mixed for 30 s and injected into the GC/MS system. An improved yield of cyclic boronates was achieved when triethylamine or pyridine was added to the mixture.

**On-column deuteration**

Introduction of a deuterium label sometimes allows additional structural information to be deduced from the mass spectra. Corresponding addition or exchange reactions may proceed directly on the gas chromatographic column connected to a mass spectrometer. In addition, the presence of a mass spectrometric detector permits the study and optimization of deuteration processes at submicrogram level. Usually, packed chromatographic columns are used to perform such experiments. In the simplest case, a chromatographic column pre-saturated with CH₃OD can readily replace active hydrogens in carboxylic acids, alcohols and amines for deuteration.

Further types of deuteration columns providing basic H/D exchange includes suspension of KOD or Ba(OD)₂ in the stationary liquid phase (for example, polymethylsiloxane liquid) and operates at 20 to 300°C. To completely convert hydroxides to the DO-form, D₂O vapor is periodically injected into the column. The column permits the exchange of enolizable hydrogens in aldehydes and ketones by deuteration. Shifts in mass numbers of the M⁺ peaks and the peaks of the ions due to α-cleavage and McLafferty rearrangement allow the extent of substitution at α-position to carbonyl group to be determined.

**Carbonyl compounds obtained from primary or secondary alcohols in a dehydrogenation microreactor (see foregoing section) may be further passed through such a column allowing enolizable hydrogens to be exchanged providing a characteristic parameter regarding the substitution pattern at the carbon atoms. The methodology was used, for example, for the investigation of some steroid alcohols (hydroxyprogrenes, hydroxyandrostanes).**

Figure 2 shows examples of EI mass spectrum of a ketone recorded after pre-column dehydrogenation of 6-ethyl-3-octanone and of the deuterium-labeled analog obtained after passing the ketone through deuteration exchanged column.

The same deuterium exchange column included in a GC/MS system was tested for deuteration of fluorenes, furans and thiophenes. It was shown that at 200°C, fluorenes undergo complete exchange of the 9-H atoms by deuteration whereas hydrogen atoms at position 5 of furans and thiophenes are replaced by deutério under such gas-liquid phase conditions.

Reaction columns providing acid-catalyzed H/D exchange have also been described. Such a packed column containing D₃PO₄ in Carbowax 20 M was used to study acidic H/D exchange. It was shown that the extent of exchange depends on temperature and, at 150°C, 2-substituted thiophenes underwent complete replacement of the H atom at C-5 for deutério (furans and fluorenes remained unaffected). It should be noted that even fused-silica capillary columns are capable of performing H/D exchange.

Searching for an approach to determine the double bond position in alkenes by EI mass spectrometry, a packed reaction column permitting the addition of a deuterium molecule to a double bond was tested. It was supposed that the EI mass spectra of the vicinal di-deuteroalkanes thus obtained from alkenes should permit the determination of the position of the deuterium label and, hence, the location of the double bond in the alkenes under study. As supported heterogeneous catalysts do not provide the selective addition of deuterium to the double bond owing to extensive isotope scrambling, homogeneous Wilkinson's catalyst, (Ph₃P)RhCl, was taken to prepare the reaction column. This catalyst is widely used in solution chemistry for selective addition of a deuterium molecule to the double bond without scrambling. To employ the catalyst in reaction GC/MS, it was pre-heterogenized by coating the solid support (Chromaton) with a dispersion of (Ph₃P)RhCl in the stationary liquid phase (Carbowax 20M). A column was
packed with the catalyst system and was connected to a mass spectrometer. Alkenes were passed through the column in a stream of deuterium gas. The particular catalytic system provided the selective addition of deuterium to mono- or disubstituted double bonds at 50–80°C and the recorded EI mass spectra showed that dideuteroalkanes possess extremely high isotopic purity. A disadvantage of such a column is the rather low operating temperature which does not allow higher olefins to be investigated.

It should be noted that this catalytic system only permits the deuteration of olefins with a mono- and disubstituted double bond (the latter fact, however, is useful for distinguishing between such compounds and tri- and tetrasubstituted olefins or carbocycles).

The mass spectra of vicinal dideuteroalkanes provide reliable information on the position of deuterium atoms and, hence, on the location of a double bond in the original olefin. It should be noted that for a successful mass spectrometric location of the label in deuterated alkanes, the fragments arising from the loss of small alkyl radicals should be used because they are normally due to simple carbon–carbon bond cleavage. The fragments formed formally from the loss of large radicals (even C₃H₅) are usually of little use because they can also arise from rearrangement processes.

The efficiency of the suggested approach is demonstrated by a comparative analysis of the mass spectra of isomeric dideuteroheptanes registered by the deuteration of isomeric heptenes (Figure 3). The qualitative differences between them are seen to be very large and they are converted in quantitative yield. The origin of characteristic fragments in the spectra of dideuteroalkanes produced from 1-, 2- and 3-alkenes is demonstrated by the respective cleavages:

\[
\begin{align*}
\text{CH}_2\text{DCHDCH}_2\text{Cl} & \rightarrow \text{CH}_2\text{DCHDCHDCHCl} \\
\text{CH}_2\text{DCHDCHCH}_2\text{Cl} & \rightarrow \text{CH}_2\text{DCHDCHDCHCHCl} \\
\text{CH}_2\text{DCHDCHDCHDCHCl} & \rightarrow \text{CH}_2\text{DCHDCHDCHDCHDCHCl}
\end{align*}
\]

Figure 3. EI mass spectra of selectively deuterated (a) 1-heptene, (b) 2-heptene and (c) 3-heptene.

The same deuteration column was used for on-line preparation and for measuring the mass spectra of dideutero derivatives from olefins in order to elucidate mechanisms of EI-induced fragmentation of the corresponding saturated analogs, as was carried out for alkylcyclobutanes and some silacycloalkanes.

### Derivatization/degradation in a microreactor situated between a chromatographic column and a mass spectrometer (post-column derivatization)

For the analysis of chromatographically separable mixtures, the most interesting and promising position of a microreactor is between the analytical column and the mass spectrometer. In this case, the chromatogram registered is that of the original mixture, whereas the mass spectra correspond to the products (if the reaction has taken place). It is very important that this microreactor location permits independent investigation of all components that are transformed to identical products.

In order to retain the separation power of the GC column, chemical reactions, used in conjunction with GC/MS, should be fast, selective, and quantitative. There are cases, however, when high selectivity is not necessary and side reaction can facilitate structure elucidation.

Many heterogeneous hydrogenation catalysts satisfy these requirements. For post-column conversion of olefins the same microhydrogenators may be used that have been described for pre-column modification. As mentioned above, Teeter and Issenberg were the first to introduce a microhydrogenator between the GC column and mass spectrometer for the analysis of various unsaturated
Compounds. It is worth mentioning that post-column hydrogenation in GC/MS may also be accomplished in a tube prepared from hydrogen permeable alloy (for example, Pt/Ir). In this case, hydrogenation proceeds on the walls of a tube, carrier and reagent hydrogen being pumped through the walls. In addition, such tubes may be used as the interface between a gas chromatograph and a mass spectrometer.

The post-column position of the microhydrogenator does not give rise to significant broadening of registered chromatographic peaks. This is demonstrated by a comparison of chromatograms presented in Figure 1(a) (conventional GC of alkenes) and Figure 1(b) (the same mixture but with post-column hydrogenation). It should be noted that the chromatogram shown in Figure 1(c) has been recorded with a microhydrogenator located ahead of the GC column. Peaks 2 and 3 for isomeric octenes are converted to one peak for octane.

By combining vapor-phase hydrogenation and GC/MS with selected ion monitoring, the position of the hydroxyl group in molecules of unsaturated hydroxy acids was determined. These acids were first converted into methyl ester trimethylsilyl ether derivatives. The mass spectra of the latter revealed very intense (frequently, base) peaks due to the scission of C–C bonds adjacent to the trimethylsilyloxy group. This enabled not only unambiguous determination of the position of the hydroxyl group in the original acids but also highly sensitive detection of the acids in amounts below 20 pg.

To differentiate between compounds containing five- and six-membered carbocycles, dehydrogenation was suggested (catalyst 20% Pt/C, temperature 320°C, reaction and chromatography in a stream of carrier gas—95% helium and 5% hydrogen). This was accomplished in a microreactor located between the GC column and the mass spectrometer. Because only six-membered carbocycles without quaternary carbon atoms usually undergo aromatization, the distinction between cyclohexane and cyclopentane hydrocarbons can be made. In the paper, the following conversions demonstrated the efficiency of the method: n-hexylcyclopentane → no conversion; n-hexylcyclohexane → n-hexylbenzene; 1,4-di(tert-butyl)cyclohexane → 1,4-di(tert-butyl)benzene; cis-hydrindane → indane; trans-decaline → naphthalene; cyclopentylcyclohexane → cyclopentylbenzene; dicyclohexyl → diphenyl; 1,2-dicyclohexylethane → 1,2-diphenylethane; 1,1-dicyclohexylethane → 1,1-diphenylethane. It is also important that the aromatic hydrocarbons reveal the characteristic fragmentation pattern which assists structure determination. For example, the EI mass spectra of alkylicaromatic compounds reveal intense M+ peaks and provide information about branching at the α-C atom in a substituent. This cannot be deduced from the spectra of alicyclic compounds.

Cyclic sulfides comprise another class of compounds whose EI mass spectra are insufficiently informative and, in particular, do not always allow the determination of the ring size of the heterocycle and the position of substituents.

For on-line conversion of cyclic sulfides in a microreactor (catalyst Raney nickel; carrier and reagent gas hydrogen) situated between the GC column and the mass spectrometer, catalytic hydrodesulfurization was suggested. The registered mass spectra were of the corresponding hydrocarbons providing some information regarding the skeleton of the original sulfides. The use of deuterium as a carrier and reagent gas at low temperatures (up to 80°C) provides labeling of the carbon atoms originally attached to the sulfur atom accompanied by only a small degree of deuterium scrambling. If the EI mass spectra of the labeled hydrocarbons thus recorded allow the position of deuterium in their molecules to be established, the sites of sulfur attachment may be determined.

The use of deuterium for identification of polycyclic sulfides seems to be useless because mass spectrometric determination of the position of deuterium in alicylic hydrocarbons is not always possible. Nevertheless, hydrodesulfurization also facilitates the structure determination in this case. For instance, post-column hydrodesulfurization (commercial AP-56 catalyst, 300–325°C, carrier and reagent gas hydrogen) was used for structure elucidation of substituted thiabicyclononanes within a synthetic mixture. As a result, for each eluted stereoisomeric 8-methyl-7-thiabicyclo[4.2.1]nonane (A), identical mass spectra of ethylcycloheptane were recorded. At the same time, the mass spectra registered for separated 8-methyl-7-thiabicyclo[4.3.0]nonanes (B) and 7-methyl-6-thiabicyclo[3.3.1]nonanes (C) corresponded to a mixture of n-propylcyclohexane and n-propylbenzene (the product of additional dehydrogenation):

It should be noted that, because only six-membered carbocycles undergo aromatization, cyclohexane and cyclopentane or cycloheptane rings can be distinguished.

Post-column hydrodesulfurization may be used with success for the differentiation of isomeric alkylthiofenes whose EI mass spectra are known to be similar. A further version of post-column modification has been suggested by Ligon and Grade. They described the principle involving the addition of reagents to the region between the capillary gas chromatograph and the mass spectrometer. The combination delivers very narrow spikes.
of reagent which are scavenged efficiently. The authors noted that the reactions used (bromination, deuterium exchange, silylation, acylation) should proceed at GC oven temperatures, atmospheric pressure and must be complete in less than 1 s and include, for example, the D$_2$O provided deuterium exchange of active hydrogen atoms in phenols, various alcohols, amines, amides, acylation of amines by acetyl chloride, benzoyl chloride, (CF$_2$CO)$_2$O, silylation of alcohols with BSTFA, bromination of olefins and aromatic compounds.

Gas chromatography/combustion/isotope ratio mass spectrometry (GC-c-IRMS)

Since the late seventies,$^{72,73}$ post-capillary column degradation followed by mass spectrometric measurements has become a very powerful tool for the determination of the isotope ratios of carbon, nitrogen, oxygen, sulfur and hydrogen. These systems include some kind of combustion device allowing complete oxidative decomposition of the gas chromatographically separated compounds into low molecular mass gases—CO$_2$, N$_2$, CO, SO$_2$. $^{74}$ The gases are further admitted to the mass spectrometer for the determination of $\delta$C, $\delta$N, $\delta$O and $\delta$S values, respectively. More recently, the same methodology has been applied to determine the isotope ratio of hydrogen. $^{75}$ To accomplish complete combustion, eluents from the gas chromatographic column are introduced into a high-temperature reactor (about 1450°C) and hydrogen and oxygen are quantitatively converted into H$_2$ and CO, respectively. Additional oxidation is used for the isotope analysis of carbon, nitrogen and sulfur.

The main requirement for the determination of isotope ratios by this method is good gas chromatographic resolution of the compounds under analysis. Sometimes, this may be achieved by using preliminary derivatization. For example, trimethylsilylation of steroids provided well-resolved GC signals and reproducible $^{13}$C/$^{12}$C measurement in testosterone metabolites. $^{76}$ For the determination of $^{15}$N and $^{13}$C content in labeled urea by GC-c-IRMS, preliminary conversion of urea into 2-hydroxy-5-cyano-pyrrole (reaction with malonaldehyde) followed by methylation with CH$_3$N$_2$ was employed. $^{77}$

Post-column derivatization in HPLC/MS

Post-column derivatization is a well-known procedure in HPLC. $^{78}$ Because liquid carriers are used in HPLC, this enables various solution reactions to be utilized for derivatization. Among them, the reactions proceeding by addition of a liquid reagent to the effluent, as well as photochemical, solid-phase and electrochemical reactions are particularly employed. It should be noted, however, that this methodology is not always acceptable owing to high demands in speed and efficiency of the reactions employed and the levels of detection required where an amount of sample is limited. To be used for post-column derivatization in HPLC/MS, the reactions should be fast, compatible with the mobile phase and flow rates, quantitative and reproducible. The experimental system should be applicable to a wide variety of reactions, should provide only a minimal loss in chromatographic resolution and the reaction should take place in a small total volume. $^{79}$ The latter paper describes on-chip derivatization that meets these requirements and represents one of the techniques used to perform pre-ionization derivatization in HPLC hyphenated to MS through an ESI interface.

In the majority of cases, post-column derivatization in HPLC has been used to convert the analyte into a compound that can be detected by a particular detector (the introduction of fluorescent or UV absorption groups is frequently used in practice). Commonly, such methodology is used for the detection of separated components of a mixture, for providing the specificity, for discriminate detection of selected compounds of interest, for increasing the sensitivity of detection and for quantitation.

In principle, there is no necessity to perform such derivatization in the analysis by a combination of HPLC/MS, because mass spectrometry is a universal detector for various types of compounds. At the same time, post-column derivatization in HPLC/MS may be used to convert the compounds under study in an ionizable form, to improve the detection and to enhance the information contents of mass spectra recorded with the use of “soft”-ionization. Many examples of this kind of methodology will be given in the forthcoming reviews.

Note that the chemical reaction interface in LC/MS was developed for analyzing isotopically and intrinsically labeled compounds. $^{80}$ The suggested device used a variable frequency microwave generator, a multi-modal microwave cavity, that allows the plasma to remain lit when liquid sample from the LC column is introduced, i.e. the instrument is capable of atomizing liquid samples introduced directly from an LC column without solvent removal before the plasma support gas is removed prior to mass spectrometry.

References


