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Review

Extending the molecular application range of gas chromatography

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Abstract

Gas chromatography is an important analytical technique for qualitative and quantitative analysis in a wide range of application areas. It is fast, provides a high peak capacity, is sensitive and allows combination with a wide range of selective detection methods including mass spectrometry. However, the application area of GC is limited because the molecules to be analysed have to be thermally stable and sufficiently volatile. Numerous molecules do not meet these requirements and hence are not amenable to direct GC analysis. Recent research has resulted in better chromatographic columns and methods for sample preparation that enable a significant expansion of the molecular application range of GC. The strategies exploited include conversion of (macro)molecules into smaller species and approaches to reduce the polarity of molecules. In this review we identify four generic routes for extending the applicability of GC. These include high-temperature GC, derivatisation, pyrolysis and thermochemolysis. The principles, recent developments and future perspectives of these routes are discussed and examples of applications using the different options will be shown. Life sciences, metabonomics and profiling strategies for sample characterization are identified as important future drivers for the continued development of GC.

Keywords: Gas chromatography; High-temperature GC; Derivatisation; Pyrolysis; Thermochemolysis; Applications; Application range; Future developments

Contents

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1. Introduction

Chromatographic separation methods are without any doubt the most frequently employed analytical techniques for compositional analysis. Both gas chromatography (GC) and liquid chromatography (LC) are widely used in a huge number of application areas in laboratories all over the world. LC and GC are complementary yet at the same time competing techniques. There is a significant number of applications that can equally well be solved by GC as by LC. For other applications clearly one of the techniques is to be preferred over the other. Which technique to select depends on numerous objective parameters such as the physico-chemical properties of the analytes, matrix properties, the presence of similar analytes, the required sensitivity and selectivity, etc., next to more subjective personal preferences. In practice the process of selecting between LC and GC is a process of multi-criteria decision making where incomparable properties have to be compared. Would I prefer the faster method running on the more expensive instrument, or the more reliable and selective method that unfortunately requires slightly more maintenance? As always when making a difficult choice a consequence of taking a decision is that the attractive features of the other option are lost. When resorting to LC one no longer benefits from the merits of GC. In this sense it is interesting to think about options for transferring applications from the LC to the GC domain.

The general consensus when comparing LC and GC is that GC is faster, provides higher separation efficiency, has better properties for combination with a wide range of sensitive and selective detectors and, finally, allows easier mass spectrometric identification. LC is generally stated to require less sample preparation, provide a better selectivity, have a much wider application range and be more rugged. Given these complementary advantages it is logical that in the past decades several attempts have been made to combine the advantages of LC and GC. One of the major justifications for research into supercritical-fluid chromatography (SFC) in the 1980s and 1990s was in fact the possibility to combine the advantages of LC and GC [1]. Right now SFC has secured itself a clear, albeit small position between LC and GC as is evidenced by the small and stable number of SFC papers appearing in scientific literature. Other examples of trying to combine the strengths of LC and GC include research directed towards LC with GC detectors [2], hyphenated LC–GC [3], comprehensive two-dimensional LC × GC [4], and unified chromatography [5,6]. Similarly to SFC, most of these attempts have been more or less successful, yet the methods have not (yet) gained widespread acceptance.

The main question that determines whether a compound can be eluted from a GC column is whether it can reach a sufficiently high concentration in the gas phase in the GC column at a realistic temperature. For very high molecular weight compounds this is evidently not possible. Polymers are thermally decomposed long before they reach a measurable vapour pressure. The same holds for highly polar molecules. Due to the strong intermolecular forces high temperatures are needed to vapourize polar molecules and the molecules might decompose on the GC column. It is for this reason that small but highly polar molecules, such as amino acids and sugars, cannot be analysed using GC. A possible solution is the use of chemical derivatisation techniques where the polar groups of the target molecules are converted into less polar moieties, which favourably affects the vapour pressure and the adsorption characteristics. Clearly the two factors that determine whether a compound can be analysed using GC are size and polarity. Only a limited range of relatively small and non-polar molecules is accessible for direct analysis by GC. By increasing the temperature at which the GC column is operated this range can be extended slightly, but at some point thermal stability of the compounds and/or the GC column becomes a limiting factor. Samples containing molecules outside the GC range have to be analysed using another technique, very often LC, or have to be pretreated first to make size and polarity compatible with GC. Derivatisation has already been mentioned as a method to convert polar species into less polar, GC-amenable analytes. Pyrolysis is a method to convert high molecular weight species into smaller fragments that fall within the application range of GC. Pyrolysis with simultaneous derivatisation, known as thermochemolysis, can be used for converting polar polymers into species suitable for subsequent GC analysis.

The application range of GC and the routes available to bring more molecules into the scope of GC are schematically depicted in Fig. 1. In the first route, high-temperature GC, the molecules in the sample are not changed. The chromatographic conditions are adapted to allow elution of the compounds that at lower maximum temperatures would be fully retained. The other routes, derivatisation, pyrolysis and thermochemolysis alter the properties of the molecules either slightly (derivatisation) or completely (pyrolysis and thermochemolysis). In Fig. 1 and in the rest of this review we will restrict ourselves to a discussion of these four generic routes for extending the applicability of GC. For certain specific applications alternative methods might be

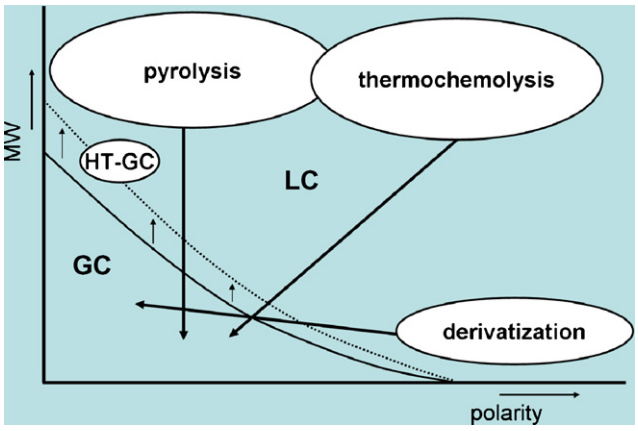

Fig. 1. Options for bringing more molecules into the application range of gas chromatography.

available such as e.g. acid hydrolysis in the study of polyesters or enzyme treatment to study glycosylation, etc. These specific options will not be discussed here.

In the present article we will review the various routes for extending the application area of GC. The rationale behind the work was the desire to open up the strengths of GC for even more solutes and analytical problems. The principles of the four generic options shown in Fig. 1 will be discussed and examples of how researchers have successfully applied the particular routes will be shown. In particular we will also try to describe how recent developments in the field of this review have impacted and will impact on the applicability of GC in existing areas and newly evolving research fields.

2. Bringing more molecules into the realm of gas chromatography

2.1. High-temperature gas chromatography

2.1.1. Gas chromatography at higher temperatures

The exact definition of high-temperature GC (HT-GC) is somewhat arbitrary and subject to some inflation over the years. In this contribution we consider separations with final temperatures below 340 °C as normal GC. In normal GC a wide range of stationary phases is available and various injection and detection devices can be used. In high-temperature GC the number of available stationary phases is much lower, normal fused-silica tubing with a standard polyimide coating can no longer be used, special injection techniques are needed, etc.

The main requirement that has to be met for a compound to be amenable to GC is that it should have sufficient vapour pressure at a temperature where both the compound and the GC column are still thermally stable. This statement, although in principle correct, needs to be refined slightly. Firstly, whether or not a compound is thermally stable will strongly depend on the conditions applied. Compounds that readily decompose or oxidize when in contact with air or water might show a remarkable stability in a highly inert GC column under the protective atmosphere of an inert carrier gas. Also with regard to the column a further refinement is needed. A GC column consists of at least three distinct chemical layers each of which is affected by the temperature of operation: the protective coating on the outside of the fused-silica tube, the deactivation layer and the stationary phase.

A clear example of the significant improvement of the thermal stability of compounds in an inert GC column under helium as compared to heating in air is seen when comparing results of thermal gravimetric analysis (TGA) with GC information. de Koning et al. reported a rapid weight loss of olive oil in TGA analysis at a temperature of 'only' 250 °C [7]. In GC the main constituents of olive oil, the triacylglycerides are routinely analysed using high-temperature GC at temperatures up to 380 °C. The weight loss in TGA clearly results from decomposition of the compounds in the air atmosphere.

2.1.2. Packed versus open-tubular columns

High-temperature GC is more or less the exclusive domain of the open-tubular column. The reasons are evident. Packing materials are more difficult to deactivate than fused-silica tubing. Moreover, the higher surface area of packed columns results in more retention, which obviously is undesirable for compounds that are already difficult to elute. Finally, the higher mass of stationary phase in a packed column results in much higher absolute levels of column bleed than in case of open-tubular columns. Additionally, of course, packed columns suffer from their standard disadvantages such as a lower speed and lower maximum plate numbers. An interesting new column format holding amidst between packed and open-tubular columns are the fiber-packed capillary columns recently proposed by Jinno and coworkers [8,9]. Three hundred-micrometer capillaries were packed with a bundle of polymer-coated filaments, in that way creating a miniaturised system that does not suffer from the traditional disadvantages of narrow-bore columns as e.g. limited sample capacity. Good results were obtained with Dexsil 300-coated Zylon fibers. The maximum allowable operating temperature for these columns was 450 °C and the successful application of 1-m long columns for the separation of an oligostyrene mixture and an oligo(methylmetacrylate) was shown. Oligomers of up to 14 and 17 units, respectively, could be eluted.

2.1.3. Column preparation

Column technology for high-temperature open-tubular GC nowadays more or less is the exclusive domain of the column manufacturers, with most of the technology being proprietary. It is needless to say that column technology for HT-GC is not trivial. All chemical layers in and on the capillary need to be able to withstand the very high temperatures for prolonged periods without changing their chemical and mechanical properties. Two column materials are used for making open-tubular columns for high-temperature GC: metal and fused silica. Metal (Ultimetal or Silcosteel) has the advantage of excellent mechanical properties under all circumstances and at temperatures as high as 450 °C. On the other hand it has the drawback of very strong catalytic and adsorptive effects if not properly deactivated. Moreover, cutting the column is not as easy as for fused silica capillaries.

An alternative solution are the high-temperature columns made of fused silica having special protective coatings on the outside being based on either high-temperature polyimide or aluminium. The maximum temperatures for these protective coatings are around 430 °C for high-temperature polyimide and 480 °C for aluminium.

The stationary phases nowadays used in GC are predominantly based on polysiloxanes. The favourable properties of the polysiloxanes include a high-temperature stability, a good resistance to oxidation and rapid diffusion characteristics. Temperature stability of the polysiloxanes is limited by a mechanism known as 'back biting' [10]. The terminal silanol group of the flexible polymer bends back onto the own chain, cleaves a siloxane bond forming a volatile cyclic degradation product consisting of three up to eight siloxane units. From this mechanism it is clear that there are two routes to improve stability: minimize the formation of terminal silanol groups or make the chain stiffer so that it cannot bend backwards. Minimization of the terminal silanols requires end-capping of the end-groups of the siloxane chains and strictly water and air free carrier gases as these compounds easily form silanols at higher temperatures. Also the residual activity of the surface needs to be minimized. Residual silanols from the surface otherwise can easily move to the stationary phase initiating degradation reactions. Proper control of the surface chemistry hence is crucial.

Chain stiffening can be achieved in two ways: by the introduction of rigid and stable groups in the siloxane backbone or by inter-chain cross-linking. Important chain stiffening agents are the carborane groups introduced by the Olin Research Center in 1966 [11] and the phenylene or diphenylene ether groups, leading to silphenylene–siloxane copolymers. High-temperature phases that were developed in the past such as the fluoroalkyl/phenyl substituted phases [12] and hydroxyterminated diphenylvinylmethylpolysiloxanes [13] or other hydroxyterminated materials [14], have now all been replaced by these carborane and silphenylene phases.

Carborane–siloxane polymers are commercialised under the trade name Dexsil and were first used as GC stationary phases by Haken back in 1984 [15]. Especially copolymers of dimethylsiloxanes with $C_2B_{10}H_{10}$ carborane units turned out to exhibit an excellent temperature stability, yet with a limited inertness restricting their application to rather non-polar molecules. Columns with caborane stationary phases are commercially available from all major column manufacturers and have maximum allowable operating temperatures in programmed analysis of up to 480 °C [16]. The exact chemical compositions of the phases are not disclosed. Moreover, details of synthesis, deactivation and coating procedures are covered by patents and most of the real tips and tricks are kept highly confidential. Silphenylene-modified polysiloxanes have slightly lower maximum operating temperatures of around 380–430 °C. A drawback of introducing the phenylene group is that it changes the polarity of the stationary phase. Column manufacturers have recently managed to produce columns with maximum temperatures of 400 °C and even slightly higher with stationary phases that have the selectivity of 100% polydimethyl phases. Again details on the underlying chemistry are kept secret and only general information regarding the procedures applied are given. Key factors are indicated to be the catalyst used in the synthesis of the siloxanes, the purities of all reagents, the column surface and the quality of the deactivation layer [17]. Interesting details on the compositions of the carborane and silphenylene–siloxanes can be derived from detailed NMR studies on the materials by Kählig and Mayer-Helm [18] and Mayer et al. [19].

2.1.4. Instrumentation for high-temperature gas chromatography

Clearly the heart of a system for high-temperature GC is the chromatographic column. However, also the performance of the other parts of the chromatographic system is crucial. High quality pressure or flow regulators are necessary to minimize the ingress of air, cold spots should be avoided and heated autosampler trays are sometimes needed to prevent solubility problems as frequently encountered with high molecular weight analytes. Especially critical is the injection method. Cold on-column injection generally is the preferred technique as it eliminates discrimination against the most non-volatile compounds [20]. Good results for many high boiling compounds however, have also been obtained with programmed-temperature vapourisation injection (PTV) [21] and sometimes even with conventional split injection [22]. Retention gaps are frequently employed to protect the column against contamination by in-volatile material and to allow automated on-column injection onto columns with inner diameters of 320 μm or lower. The performance of the connector used to couple the retention gap to the analytical column is crucial: even the smallest leak results in irreproducible results and rapid column deterioration. For coupling fused-silica columns the best performance is generally obtained with glass press-fit connectors [23]. Unfortunately, even in the hands of experienced operators these often fail, especially in HT-GC applications [24]. The polyimide responsible for the actual sealing slowly oxidizes leading to leakage and oxygen ingress. It is for this reason that columns with integrated retention gaps and metal unions are promoted for HT-GC.

As regards detection, HT-GC has been coupled to numerous detection methods including the more exotic atomic emission detection (AED) [25] and inductively coupled plasma mass spectrometry (ICP-MS) [26]. The most frequently used detection methods are flame ionisation detection (FID), which basically can be used to temperatures as high as 500 °C without any modification, and the mass spectrometer. Whereas the use of FID in HT-GC is trivial, in HT-GC–MS more problems can occur. As an example, spectra recorded at the high source temperatures required to maintain chromatographic resolution and prevent condensation in the MS source can be different from those recorded at lower temperatures as a result of thermal rearrangements [27]. Sensitivity and chromatographic resolution can be lost if cold spots occur, which unfortunately often is the case [28]. An advantage of the use of MS detection is that the very low column outlet pressure allows operation of the system at a significantly enhanced flow rate [29]. This means the analytes can be eluted at lower final column temperatures which con-

tributes to a further expansion of the range of analytes amenable to (HT-)GC analysis [30].

2.1.5. Applications of high-temperature gas chromatography

2.1.5.1. Fossil fuels. One of the first application areas where (normal) gas chromatography was routinely applied was petrochemical and fossil fuel analysis. In the last decades, as a result of the increasing oil prices, also the higher-boiling oil fractions are now processed and converted to high-value end products. For the characterization of these heavy fractions normal GC no longer suffices and higher temperature systems are needed.

One of the most demanding applications of high-temperature GC in petro-chemistry is the so-called simulated distillation or SimDist method. In the strictest sense SimDist is not a chromatographic separation, but rather a physico-chemical measurement using GC. In GC SimDist the sample constituents are eluted in the order of their boiling point and detected using FID. Chromatographic retention times are converted into boiling points using *n*-alkanes with known boiling points as the calibrants. In this way boiling-point distributions can be obtained without having to use very laborious and slow laboratory-scale distillation methods. High-temperature GC SimDist methods cover the boiling-point range of 35–750 °C, corresponding to the boiling points of the *n*-alkanes from about C_5 to C_{120}. To allow complete elution of all compounds in boiling-point order with a minimal contribution of polarity, GC SimDist uses a thin film of a non-polar stationary phase. The film thickness varies between 0.05 and 0.15 μm in a wide-bore column e.g. 530 μm, resulting in a very high phase ratio and hence optimum elution characteristics for high boiling analytes. Moreover, the use of a 530 μm column greatly facilitates on-column injection. SimDist columns are typically only 5-m long, again with the aim to facilitate elution of non-volatile compounds. Linear temperature programmes are generally used starting at 45 °C or even lower and having final temperatures between 430 and 480 °C [31]. The separation of a Polywax 655 calibration standard containing *n*-alkanes in excess of *n*-C_{120} is shown in Fig. 2.

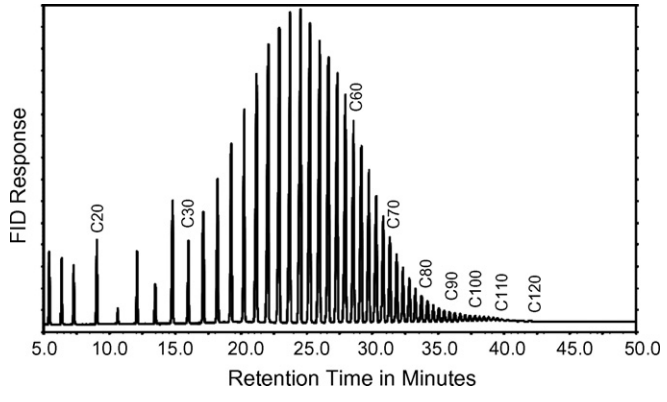

Fig. 2. Separation of a Polywax 655 standard for calibration in GC SimDist. Column: Varian CP-SimDist Ultimetal, $L = 5$ m, $d_c = 530$ μm, $d_f = 0.09$ μm. Temperature program: 40 °C (1 min) at 10 °C/min to 430 °C (10 min). Carrier gas: helium. Injection: PTV. Detection: FID. Courtesy Jaap Links and Jan Blomberg, Shell Global Solutions International B.V., Amsterdam, The Netherlands.

A detailed overview of advances in the use of HT-GC for the analysis of fossil fuels was published by Philp in 1994 [32]. Although not very recent, the paper still gives a good coverage of the applications of HT-GC in this area, in particular with regard to how the information obtained from HT-GC can be used in geochemical and environmental studies. As an example, Smith et al. [33] applied HT-GC for the characterization of methyl esters of the 4–8 ring C_{80} tetranaphtenic acids which contain information on the geochemical history of the samples. Hsieh, Philp and del Rio studied the high molecular weight fraction (>C_{40}) of crude oils in an attempt to elucidate the molecular structures of the molecules present in this fraction of the oil. The major high molecular weight compounds found were homologous series of *n*-alkanes, methylbranched alkanes, alkylcyclopentanes and hexanes, and alkylbenzenes [34]. Killops et al. [35] detected similar classes of compounds in a coal-source waxy oil and were able to correlate the levels of the C_{40}+ straight chain alkanes to the maturity of the oil. In their experimental work these authors encountered great difficulties with the solubility of the compounds in the extraction solvents. Solubility of the very high molecular weight species can be troublesome, even in the very strong solvents used, i.e. hot toluene and carbon disulfide. One final application of HT-GC in fossil fuel analysis is the analysis of geoporphyrins through coupled HT-GC with MS [36] or with ICP-MS [26]. In addition to this application still being highly relevant, it is also interesting to see that the two main problems these authors were faced which remain to be a nuisance to date: compounds are easily lost in transfer lines and band-broadening can occur in the interface.

2.1.5.2. Triacylglycerides and related lipidic material. Triacylglycerides (TAGs), also referred to as triglycerides, are the main constituents of edible oils and fats and are more recently also used in bio-fuels. TAGs are tri-esters where three fatty acids (FAs) are esterified to a glycerol backbone. Typical chain lengths of FAs found in vegetable oils range from 14 to 20. This results in TAGs with total carbon numbers typically between 46 and 56. Here the carbon number is defined as the sum of the alkyl chain lengths of the three FAs. In animal fats and fish oils TAGs with carbon numbers up to 70 can be present. As a result of the high molecular mass of the intact TAGs, GC analysis requires final column temperatures of 350 °C or higher. Elution is largely based on the carbon number, with weak selectivity towards the number of double bonds on some phases.

The first GC analysis of natural TAGs was published by Kuksis and McCarthy in 1962, at that time on packed columns [37]. Nowadays packed columns are rarely used anymore. TAG profiling using open-tubular columns is well established and routinely used for quality control purposes. It is also used in research studies where rare natural lipids are of interest (e.g. [38–40]). Finally, similar methods are also used to characterize related products such as modified oils [41], fat replacers based on sucrose fatty acid esters [42] or lubricants based on mixtures of edible and mineral oils [43]. The use of GC for TAG profiling has recently been discussed in detail in an excellent review by Buchgraber et al. [44].

There are three potential obstacles for obtaining reliable quantitative results in HT-GC analysis of TAGs. Firstly, as for all HT-GC applications, the selection of the solvent and the actual dissolution of the sample are not trivial. High carbon number, fully saturated TAGs are more difficult to dissolve and hence also more difficult to extract than their lower MW or more unsaturated counterparts. Secondly, sample introduction is critical and discrimination against boiling point can occur if the injection mode is not properly selected or optimised. This problem has been well documented in HT-GC, in particular also for TAG analysis (e.g. [45,46]). Finally, quantitation of the TAGs, either as pure compounds or as carbon number clusters, requires accurate response factors. van Oosten et al. studied FID response factors in the SFC mode to eliminate thermal degradation of the TAG molecules in the analysis and reported response factors of 0.8 for TAGs containing nine double bonds relative to saturated TAGs [47]. From these results it seems that for standard vegetable oils, where the individual TAG molecules hardly contain more than four or five double bonds, the actual FID response can be assumed to be unity. Unfortunately these results were not confirmed by a recent intercomparison study where response factors were measured for TAGs containing just two double bonds using different injection methods [48]. In the latter study the average response factors reported ranged from approximately 0.85 for the TAG palmitoyl-oleoyl-palmitoyl-glycerol (POP) to around 1.2 for stearoyl-oleoyl-oleoyl-glycerol (SOO). The positive finding of the study was that, at least if properly optimised, cold on-column, split and PTV injection show equivalent performance.

For the highly unsaturated TAGs lower responses are generally seen. Literature is not conclusive as to whether this is due to thermal instability or the result of polymerisation [49]. All authors agree, however, on the precautions that can be taken to minimize losses. Thermal stress should be kept to a minimum by eluting the compounds as rapidly as possible at the lowest elution temperature feasible. In practice this means column length should be minimized and the lowest possible film thickness should be used. The linear gas velocity should be as high as possible. To minimize the loss of column efficiency as a result of the use of a high linear velocity, hydrogen is the preferred carrier gas because of its much flatter plate-height curves as compared to helium [50]. Also the temperature-programming rate should be carefully selected. Experiments in our laboratory have indicated that elution temperatures increase if too high programming rates are used [51]. The lowest elution temperature was obtained at the highest linear velocity combined with a low programming rate. Unfortunately the programming rate cannot be made extremely low as this would result in very long analysis times and longer exposure of the analytes to a just slightly lower temperature. In practice the optimisation will be largely trial and error. The guideline derived by Blumberg and Klee [52] stating the optimum temperature-programming rate to be 10 °C/dead time could be a good starting point. However, it will predict impractically high programming rates at the dead times of 10 s or less obtained when very short columns are operated at high gas velocities.

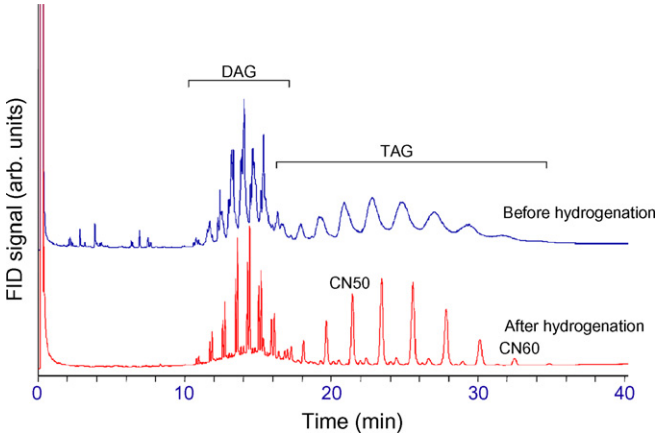

Fig. 3. Separation of a fish oil on a non-polar CPSil5-CB-low bleed HT-GC column before (A) and after (B) hydrogenation. Column: $L = 10$ m, $d_c = 320$ μm, $d_f = 0.12$ μm. Retention gap: $L = 1$ m, $d_c = 530$ μm, deactivated fused silica. Temperature program: 70 °C (2 min) at 10 °C/min to 370 °C (15 min). Carrier gas: hydrogen. Injection: cold on-column. Detection: FID. Abbreviations—DAG: diacylglycerides; TAG: triacylglycerides; CN: carbon number. Courtesy Ruud Poort, Unilever Food and Health Research Institute, Vlaardingen, The Netherlands.

As already mentioned earlier in this review, in HT-GC the possibility to use selective stationary phases is limited by the mere fact that there are not many polar polymers that are stable at high temperatures. TAG analysis is therefore almost exclusively done on non-polar stationary phases. Separations on such phases are based largely on vapour pressure, which in turn is strongly related to the molecular weight or the carbon number. Some separation based on the degree of unsaturation of the TAGs does occur, but on the short columns usually applied this only manifests itself as broadened peaks. An example of how different numbers of double bonds affect the separation is shown in Fig. 3. This figure shows the separation of a highly unsaturated fish oil before and after hydrogenation on a non-polar column. Before hydrogenation broad peaks are seen, each representing TAGs with identical carbon number. The large peak width is not an artefact but results from a slight dependence of the retention time of the TAGs on the number of double bonds. The higher the number of double bonds the shorter the elution time. After hydrogenation all double bonds are saturated and all TAGs of a certain carbon number elute at exactly the same retention time. A capillary column with a somewhat more polar stationary phases is available from Varian or Restek. This column is known as the CB-TAP (Triglyceride Analysis Phase, Varian) or Rtx-65-TG column (Restek) and allows to introduce additional selectivity for double bonds [53].

2.1.5.3. Miscellaneous applications. Many specialty chemicals are large molecules where the actual molecule has been carefully 'designed' to combine the various properties required. A good example of carefully designed molecules are surfactants: molecules with a polar, water-soluble part and a non-polar hydrophobic part. Several classes of surfactants can be analysed by HT-GC. HT-GC has for example been used to control the linear alkylbenzene sulfonate (LAS) sulfonation process. LAS is one of the most widely used surfactants with applica-

tions in numerous products ranging from laundry powders to surface cleaners and machine dish-wash products. Using high-temperature GC the levels of remaining raw material (LAB: linear alkylbenzene) and of the undesired dimeric by-product, the LAB sulfone, can be analysed in one HT-GC run [54]. HT-GC has also been successfully applied to other surfactants such as the ethoxylated alcohols, a group of surfactants consisting of a long-chain alcohol to which a chain of ethylene-oxide units has been grafted [25]. Important properties of the molecules that can be determined by HT-GC are the alkyl chain length distribution, the degree of ethoxylation and the weight distribution over the various homologues.

An important application of HT-GC in polymer industry is the determination of extractables. These include residual solvents and monomers, but also small oligomers and additives. Simoneau used HT-GC in the determination of the extractability of polymer additives from food-contact materials [55]. Similar work was also done for pharmaceuticals where heat-cured siliconized glass containers are used to store parenteral formulations [56]. In extracts from such vials polymethylsiloxanes with up to 45 units or a molecular mass as high as approximately 3500 Da could be analysed using HT-GC.

A final important application area of HT-GC is the rapidly growing area of life sciences. Although especially researchers in this area have a strong preference for LC and LC–MS-based methods, a large number of interesting applications of HT-GC in this field has been published. Very often the compounds involved are lipid-derived and/or glycosylated compounds that require at least some sample pre-treatment to improve their volatility and/or reduce their tendency to adsorb. Choi et al. for example determined high molecular weight urinary anhydrogen glucuronides using HT-GC–MS after methylation and silylation [57]. Olsson et al. used the same technique to study the ceramide composition of mono- to tetra-glycosylceramide glycolipids using again methylation [58]. The latter authors also studied oligosaccharides in foods, diets and intestinal contents using HT-GC. Oligosaccharides with up to seven sugar units could be analysed after reduction and methylation [59]. Numerous other applications from several other areas have also demonstrated the good performance of modern HT-GC.

2.1.6. Future perspectives

The foundations for high-temperature GC were laid in the 1980s. Meanwhile reliable columns have become commercially available that are widely used in the application areas reviewed above. HT-GC is mature and it is difficult to imagine further large developments in the area. Some practical problems still need to be resolved, such as e.g. the lack of reliable column connectors and the problems in hyphenating HT-GC to special detection methods including MS. Other problems are of a more fundamental nature and will be difficult, if not impossible, to resolve. Few molecules are stable at temperatures in excess of 480 °C, even in optimally deactivated and inert systems. A further extension in terms of the molecules that can be separated using HT-GC is hence not very likely. The same holds for higher polarity phases for HT-GC. Stationary phase bleed might be reduced a little further, but phases with selectivities really different from the current ones are unlikely to be developed. HT-GC is an important technique, it has established a secure place in the toolkit of the modern analytical chemist, but it is not an area where major break-throughs are to be expected. HT-GC is close to reaching its fundamental limits.

2.2. Derivatisation

2.2.1. Principles of derivatisation

Many compounds that contain polar and reactive groups are not sufficiently volatile and stable for direct analysis by gas chromatography. Moreover, if these polar groups have strong hydrogen-donating or -accepting properties they are likely to interact with active sites present in every GC system, in the worst case resulting in irreversible adsorption. Numerous categories of compounds exist that contain such polar groups. To make these compounds amenable to GC the technique of chemical derivatisation was developed already decades ago. For one of the first reviews see e.g. Poole and Zlatkis [60]. Derivatisation refers to the process of blocking the polar groups of molecules to improve their characteristics in terms of volatility, adsorptivity and stability. Derivatisation can also be applied to impart more favourable properties for detection, to reduce the volatility of highly volatile species, to introduce chemical selectivity next to the GC selectivity or to form diastereoisomers for chiral separations. Here we will focus only on the use of derivatisation strategies for reducing the adsorptivity and increasing the volatility and stability of polar analytes. By clever selection of the reagent at the same time also the detection properties sometimes can be altered. In this section of the review we will not include methods for derivatisation that use pyrolytic methylation, although sometimes difficult to distinguish from normal derivatisation. Pyrolytic derivatisation procedures are discussed in more detail in a later section of this review.

In the last decade the importance of derivatisation in chromatography has dwindled considerably. This is especially due to the development of novel detection devices for liquid chromatography, with MS detection of particular importance. With recent progress in LC–MS(-MS) many of the compounds that required derivatisation to make them suitable for GC can now be conveniently done with little or no sample preparation by LC–MS. Knowing that derivatisation is usually one of the most time-consuming and labour intensive steps in the analytical procedure, it is tempting to replace GC applications by LC methods that do not require derivatisation, even if the equipment needed is much more expensive. There is one area of research that can result in a revival of derivatisation in GC: the area of metabonomics. Because of its significantly higher peak capacity GC is ideally suited for profiling applications [61]. The drawback of the labour intensive and tedious derivatisation reactions here is more than outweighed by the increase of the peak capacity and the easier MS identification of peaks correlating with pharmaceutical or food interventions. Given the large sample numbers that generally have to be analysed in metabonomics studies automation of the derivatisation procedure is a prerequisite in this area.

2.2.2. Manual and automated derivatisation reactions

A vast body of literature is available to aid the analyst in selecting a derivatisation procedure in GC. Good reviews are presented by several authors. Particularly valuable are reviews by Wells on recent advances in non-silylation derivatisation techniques for gas chromatography [62], a review by Little on artefacts in trimethylsilylation derivatisation reactions and ways to avoid them [63], reviews on alkylchloroformates by Husek et al. [64,65] and last but not least the book by Knapp [66]. Although published in 1979, this book even to date still is the standard work on derivatisation reactions for chromatography. It is actually amazing how little has changed in the almost three decades after the publication of the book.

Without any doubt the most popular derivatisation method is silylation. Its main assets are the universality in terms of compounds, the highly volatile nature of the by-products of the reagent and the ease of the procedure. Its main shortcomings are the requirement for the sample to be dry and the limited stability of the derivatives when exposed to moisture in the air or in the solvent used to dilute the sample after derivatisation. The reaction time needed, typically between 30 and 60 min, is not really a problem as a large number of samples can be prepared in parallel. As indicated the derivatives obtained are somewhat sensitive to moisture. Also, they might decompose in poorly deactivated columns or injectors, a property exploited by Donike in his well-known test mixture for column and injector inertness [67]. The range of polar groups that can be derivatised using silylation reactions includes alcohol functionalities, carboxylic acids, thiols, amines, phosphorus, sulfur groups, etc. The popularity of silylation has grown even further after diazomethane was abandoned because of its carcinogenic and explosive properties.

Common silylation reagents are BSA [*N*,*O*-bis(trimethylsilyl)acetamide] and BSTFA [*N*,*O*-bis(trimethylsilyl) trifluoroacetamide] which are often used in combination with a catalyst such as trimethylchlorosilane (TMCS). Also slightly modified reagents are used, such as for example *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [68]. Less common silylation reagents described include for example *N*,*N*-dimethyl-trimethylsilyl-carbamate which was used by Eze and Torkos for the reaction with alcoholic mycotoxines such as deoxynivalenol (DON) [69] or trimethylsilylbromide that was used by Coene et al. for the derivatisation of alkyl lysophospholipids [70].

A second widely used group of derivatisation reagents are the alkylchloroformates. In particular these have recently gained widespread use as derivatisation reagents in GC amino acid analysis. The true application area of the alkylchloroformates, however, is much wider as has convincingly been demonstrated by Husek in various reviews [64]. One of the major advantages of alkylchloroformates is that the derivatisation reaction proceeds smoothly in aqueous media including physiological fluids as e.g. urine. Numerous other derivatisation reagents have been described for a wide variety of other species. A more complete overview, however, is beyond the scope of the present review.

From the viewpoint of laboratory efficiency, virtually all derivatisation reactions present a serious concern. Automating derivatisation reactions, or more in general automating wet-chemical sample preparation methods, is very difficult. It is for this reason that solid-phase microextraction (SPME) has attracted considerable attention in recent years. SPME not only provides a route to automate extraction, it also allows automation of the derivatisation step. In SPME several options exist to automate derivatisation. Derivatisation can be done in the liquid prior to the SPME equilibration in the vial. Salgado-Petinal et al. used this derivatisation route for the determination of alkylthiols in water. SPME was performed after thioethers had been formed though the reaction of the target analytes in a dinitrobenzylation reaction [71]. Similar strategies yet involving other reagents were also applied for the analysis of e.g. aldehydes in drinking water [72] or for the determination of methylmalonic acid and glutaric acid in biological fluids after derivatisation with diethylsulfate [73]. Other workers used derivatisation in the fiber. Examples of this include work by Okeyo and Snow on the analysis of estrogens and anabolic steroids in urine. Here the fiber was first equilibrated in the urine sample followed by headspace derivatisation using BSTFA [74]. Rodriguez et al. applied the same approach using MTBSTFA as the reagent for derivatisation of acidic herbicides in water [75]. Finally, a method for in-injector derivatisation was described by Jaber et al. [76] who applied it for the determination of phenols and polyphenols in rain water samples. Analytes were extracted from the water in the immersion mode. Just before inserting the SPME fiber into the injector 2 μl of the silylating reagent MDBSTA was injected into the hot injector and a good silylation was obtained.

Following the success of automated SPME with derivatisation semi-automated stirbar sorptive enrichment methods were also adapted to allow inclusion of a derivatisation step. Stopforth et al. for example used in situ stir-bar extraction with derivatisation with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine for the determination of 4-hydroxynonenal, a marker for oxidative stress, in urine [77]. Baltussen et al. previously had applied a similar method using derivatisation with acetic anhydride for the determination of phenols in water [78]. Novel applications of stirbar sorptive extraction with in situ derivatisation in biomedical and environmental analysis were recently reviewed by Kawaguchi et al. [79].

An area where automated derivatisation is already routinely performed is in fatty acid analysis of edible oils and fats. The fatty acids or triacylglycerides are dissolved in an organic solvent to which a solution of sodium methylate in methanol is added [80]. Rapid hydrolysis and methylation occur already at room temperature. Finally, also phase-transfer catalytic (PTC) methods for extraction and derivatisation can provide interesting strategies towards automated sample preparation [81]. In PTC ion pairs are formed between the (polar) analytes of interest and an ion-pairing agent such as a quaternary ammonium salt. The ion pair is more soluble in the organic phase and hence is 'dragged' through the interface into the organic phase where the reaction occurs.

2.2.3. Future perspectives

A very significant fraction of all analyses done by GC requires derivatisation. If no derivatisation methods would be available the applicability of GC would be very limited. Still, sample

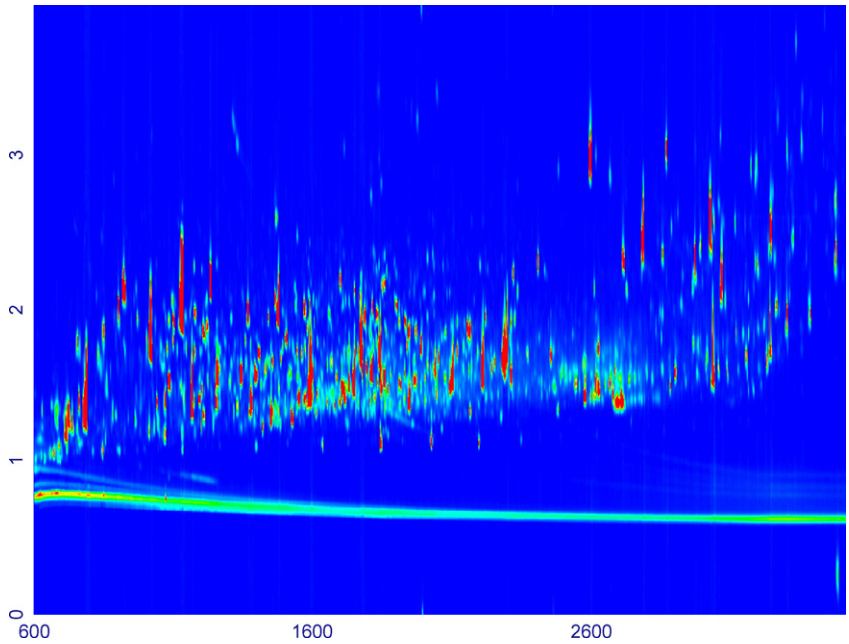

Fig. 4. GC × GC–time-of-flight MS analysis of a plant extract (traditional Chinese medicine) after oximation and silylation. For more details see Koek et al. [82].

preparation, of which derivatisation is just one step, is known to be one of the most expensive and error-prone parts of a chromatographic analysis. Even simple methylation or silylation reactions occasionally fail. It is for this reason and because of the difficulties in automation that researchers try to avoid derivatisation. For many years there has been little or no alternative for derivatisation. In the last decade however, LC–MS(-MS) has developed into a mature technique that is now accessible for regular laboratories. From the efficiency point of view it is often more attractive to invest once in the expensive technique of LC–MS(-MS) and run all samples in their native form than having to derivatise every single sample. This is particularly true for target compound analysis. A drawback of LC–MS in quantitative analysis still is ionisation suppression. However, if the resolving power is not sacrificed too much and labelled analogues are available, LC–MS can be fast, reliable and accurate. For profiling applications of chromatography, such as e.g. in the rapidly developing area of metabonomics, the higher peak capacity and increased information content of a GC chromatogram makes GC the method of choice. Indeed a number of new derivatisation protocols have been developed in recent years to support metabonomics research. Since even the metabonome of simple species can be highly complex, multiple chemical derivatisation routes are often needed [61]. More work is clearly needed in this area to avoid the problems of incomplete derivatisation of sterically hindered groups. Additional research is also needed to improve the hydrolytic stabilities of the derivatisation products. Finally, the strong increase of the molecular mass of the compounds as a result of the multiple groups attached during derivatisation will have to be dealt with. Anyhow, the peak capacity and hence information content of the GC profiles recorded is huge, especially in case of GC–MS or even more so with the ultimate profiling tool GC × GC–MS. In the metabonomics area derivatisation will continue to expand. In all other areas it is more likely to decrease further, mainly as a result of the growth in LC–MS and the aversion to manual sample preparation. An example of an impressive GC × GC profile of a traditional Chinese medicine obtained after oximation and silylation is shown in Fig. 4 [82].

2.3. Pyrolysis

2.3.1. Principles of pyrolysis

For GC to be applicable, the molecules of interest need at least some degree of volatility, a requirement polymers clearly do not meet. Still, GC can be used to study polymeric material if suitable sample preparation is applied. Pyrolysis is a thermal pre-treatment method widely used in the study of (bio)macromolecules with GC. In a pyrolysis treatment the non-volatile material is subjected to a rapid, destructive thermal treatment to yield smaller, much more volatile fragments which fall within the application boundaries of GC. Once formed, the compounds are rapidly flushed out of the heated zone to avoid secondary reactions. The degradation products are hence characteristic for the polymer and can be used to determine specific compositional or structural parameters of the original material [83,84]. Nowadays pyrolysis in combination with capillary GC and GC–MS is commonly used for the characterization of a wide range of (polymeric) materials, ranging from synthetic polymers to complex environmental mixtures and bio-macromolecules. Simultaneously, pyrolysis GC can also be used for the determination of impurities and additives in complex polymeric mixtures. These molecules are in principle amenable to direct GC characterization, but are not accessible for GC analysis as they are trapped in the solid polymer. Pyrolysis here basically replaces extraction.

2.3.2. Instrumentation for pyrolysis-gas chromatography

In most conventional pyrolysis-GC configurations the pyrolysis device or pyrolyzer is physically mounted on top of the GC-instrument [85]. The carrier-gas flows from the pyrolyzer through a heated transfer line to the GC part of the instrument sweeping with it the fragments formed during pyrolysis. On the detection side of the GC, MS is nowadays commonly used. Nevertheless, also FID, AED, flame photometric detection (FPD) and nitrogen–phosphorus detection (NPD) have been used, in particular for specific applications [86–89].

Based on the way the sample is heated three major types of pyrolyzers can be distinguished. The two fastest types are the Curie-point and the filament pyrolyzers. The Curie-point pyrolyzer uses a ferromagnetic metal coil or ribbon and a high frequency generator for extremely rapid heating of the samples to a well-defined, fixed final temperature. The sample is deposited directly onto the Curie-point metal. Heating here requires less than 0.4 s [90]. The filament-type pyrolyzer uses a pulse of a high electrical current sent through a resistive piece of metal (frequently platinum). In both the Curie-point and the filament-type pyrolyzer the heating rate is very high and too fast to be controlled. The final temperatures are well defined, yet the sample mass applied can affect the actual heating profile and a temperature overshoot can occur in the filament pyrolyzer [91]. In the third type of pyrolyzer, the microfurnace pyrolyzer, the sample is placed in a small cup which is inserted into a small furnace. The furnace temperature is increased rapidly until the user-selected pyrolysis temperature is reached. It then is kept constant for a time again to be set by the user. In the microfurnace pyrolyzer it takes several seconds to reach the set pyrolysis temperature. For certain polymers this 'slow' heating can result in increased secondary reactions as compared to the other pyrolyzers [85]. However, for the furnace pyrolyzer the positioning of the sample and the sample amount is less critical. This significantly improves the quantitative characteristics of the method [92].

Irrespective of which pyrolysis devices are used, some form of interfacing between the pyrolyzer and the GC is needed. Such interfaces often have large internal volumes and, if poorly designed, cold spots can occur [93]. This can induce losses of pyrolyzates, in particular of the heavier fragments with relatively high boiling points. To overcome this problem, Górecki and Poerschmann [94] developed a novel pyrolysis method called in-column or non-discriminative pyrolysis. In this technique, pyrolysis is carried out inside a metal precolumn which is directly connected to the analytical GC column. Rapid heating is obtained by passing a pulse of an electrical current from a capacitive-discharge power supply through the tubing. The major advantage of this instrument design is that no interface is needed between the pyrolyzer and the GC. This provides an elegant way to avoid losses of larger pyrolysis products between the pyrolysis zone and the GC column. Therefore, the range of pyrolysis products amenable to analysis with the non-discriminating system is limited by the GC separation rather than by the pyrolyzer hardware applied [91]. Another approach for performing pyrolysis without an interface between the column and the pyrolyzer is the use of a rapid heating PTV injector as the pyrolysis device [89]. Also this configuration minimizes the risk of losses of higher molecular mass components. Other advantages of the PTV pyrolysis approach are its simplicity and versatility. The major advantage, however, is that the PTV injector allows combined solvent elimination and pyrolysis in one device thereby facilitating pyrolysis of dilute polymer solutions [95]. PTV pyrolysis also makes it possible to perform combined (multistep) thermal desorption and pyrolysis allowing detailed characterization of polymeric materials in one run [96]. PTV pyrolysis with GC × GC analysis of an olive oil after thermal desorption is shown in Fig. 5. Finally, in case of PTV pyrolysis the pyrolysis device does not interfere with the usual sample introduction device. The GC system can be rapidly converted to a Py-GC without having to mount or dismount parts of the equipment. Disadvantages of the PTV method are the temperature limitation of 600 °C and the relatively slow heating rate (30 °C/s) which potentially could result in enhanced secondary reactions.

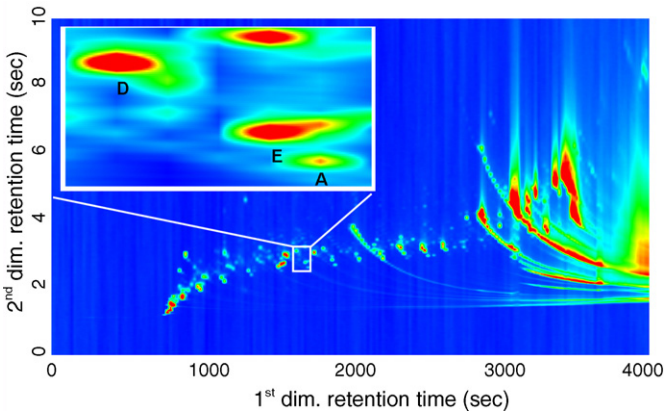

Fig. 5. PTV pyrolysis of an olive oil sample after thermal desorption. GC × GC allows the highly detailed characterization of the pyrolyzate. For more details see de Koning et al. [7].

Another recently introduced method for pyrolysis is laser pyrolysis. In laser pyrolysis, thermal degradation of macromolecules is effected by the interaction of laser energy with the sample. The laser shot generates a high-temperature plume. This causes a thermal shock which in turn produces a range of pyrolysis products [97]. An important advantage of laser pyrolysis is that it allows selective targeting of specific areas and layers of the sample [98,92]. Nevertheless, studies which have utilized this technique are limited because of the difficulties in interfacing the instrument to GC, the cost of the equipment and the fact that not all samples produce degradation products with laser irradiation.

2.3.3. Mechanism of pyrolysis

Pyrolysis is the process of converting large molecules into smaller fragments by thermal energy in the absence of oxygen. Which products are actually formed during pyrolysis depends on the molecular structure and the bond-dissociation energies of the polymeric materials [85]. Hence, the degradation products contain information about the composition and the molecular architecture of the original macromolecules. Three mechanisms

responsible for the breakdown of the polymeric material can be distinguished: side-group scission, monomer reversion (or depolymerisation) and random scission [99]. Side-group scission refers to the release of side-groups from the backbone of the polymer. Monomer reversion is in fact a reversed polymerisation process: the pyrolysis fragments are the original monomers of the polymer. Random scission finally refers to the breaking of the backbone of the polymer at random and varying positions. Also other mechanisms can take place, such as for example cross-linking. In general these mechanisms are undesired. The original pyrolysis products formed should not be allowed to react with each other. This would result in the formation of the so-called secondary reaction products which complicate the interpretation of the pyrograms.

2.3.4. Applications

The major advantage of pyrolysis (Py)-GC in comparison to other analytical techniques is that only a minimum sample preparation is required. As a result of this, pyrolysis treatment is fast. An additional advantage is that the equipment is relatively cheap, both in terms of purchase as well as with regard to operational cost. The array of different materials that has been analysed by Py-GC is enormous and includes for example plastics, paints, micro-organisms, rubbers, wood, etc. Detailed reviews on applications of pyrolysis GC have recently been prepared by Wang [100], Wampler [85], Moldoveau [101] and Baron et al. [102]. Below a small selection of representative applications is discussed to demonstrate that the application of pyrolysis sample pre-treatment indeed allows bringing important classes of molecules and analytical problems into the domain of GC.

One of the oldest and most important application areas of Py-GC is the study of synthetic polymers. The key goal of using pyrolysis here is the identification and differentiation of polymers with respect to their chemical composition and molecular structure. Wampler et al. [103] and Wang and Smith [84] for example nicely demonstrated that monomer peaks and monomer peak ratios can be used for composition determination of copolymers of ethylene–propylene and styrene–acrylate. Tsuge and Ohtani [104] showed that Py-GC can be used for sequence determination and to obtain stereoregularity (tacticity) information of polystyrene and polymethylmethacrylate (PMMA). An extensive bibliography on Py-GC of synthetic polymers is published by Haken [105].

While Py-GC is extensively used for the identification of polymers, the study of compounds present in the polymers, like antioxidants and hindered amine light-stabilizers, is becoming equally important [106]. The determination of these polymer additives can be carried out using Py-GC eliminating all sample preparation steps otherwise needed [107]. Herrera et al. [108] developed a combined thermal desorption and pyrolysis method for the qualitative determination of additives in different acrylonitrile–butadiene–styrene materials. Using such combined strategies, information on volatile materials present in the polymer, on the additives and on the actual polymeric material itself can be obtained in one single run.

In forensic sciences Py-GC is already used for a long time as a standard technique for the identification of a wide range of solid materials [109]. Py-GC is very useful here because it allows an easy comparison of different solid materials through their pyrograms. Analytical pyrolysis greatly simplifies the study of solid materials by volatilizing the polymeric content and delivering it to the GC column, along with additives such as plasticizers, while leaving the fillers behind. Moreover, because the pyrograms are generally very complex and high-resolution GC–MS can be applied for their recording, highly detailed fingerprints are obtained allowing the detection of even only subtle differences between materials. Since the main question in forensic sciences often is whether two samples are identical or not, pyrolysis fingerprinting is a uniquely suited tool for forensic researchers. To quote a few examples of forensic applications: Wampler [110] used Py-GC for the comparison of automobile paints by their Py-GC fingerprints. Pyrolysis-GC–MS is also used as a suitable technique for the differentiation of colorless polyacrylonitrile-based fibers with similar morphology [111]. Furthermore, Py-GC is extensively used in the analysis related to documents. It has for example been used to characterize inks [112] and toner materials used in printers and photocopiers [113–116].

Py-GC is also applied in the investigation of art falsification. In this research field the technique is used to obtain chemical, physical and structural information of the materials used. A clear advantage of Py-GC in this application area is that it only requires minute sample amounts making it a very suitable technique for the analysis of precious art work [117]. Especially the ingredients of paints, varnish, coatings and natural binders used in artwork provide valuable information to confirm authenticity or to aid in restoration work [118,119]. One example where Py-GC–MS was used in artwork authentication was the characterization of binding media and protectives from Coronelli's terrestrial globe. The analytical results revealed the presence of a range of original materials, such as natural gums and animal glues together with industrial products, such as synthetic germicides [120]. Based on the information provided by Py-GC it could be unambiguously concluded that at least parts of the artwork were a falsification.

Py-GC has also been applied to study biological and environmental samples like bacteria and micro-organisms [121,122]. In this area Py-GC is mostly used to provide information about the origin of the sample as well as for sample differentiation. The use of Py-GC–MS here can avoid time-consuming laboratory sample work-up. Sugden et al. [123] for example used flash pyrolysis for a rapid screening of bacterial species for the presence of bacteriohopanepolyols (BHPs) in this way replacing a laboratory method using solvent extraction with subsequent manual derivatisation.

2.3.5. Future perspectives

The huge number of publications and the wide range of application areas already indicate that pyrolysis is a powerful analytical tool. The application of Py-GC results in a significant expansion of the application area of GC. It enables the use of GC for the study of synthetic as well as natural macromolecules. Still, the applicability of GC in combination with pyrolysis can be increased even further when the instruments would be capa-

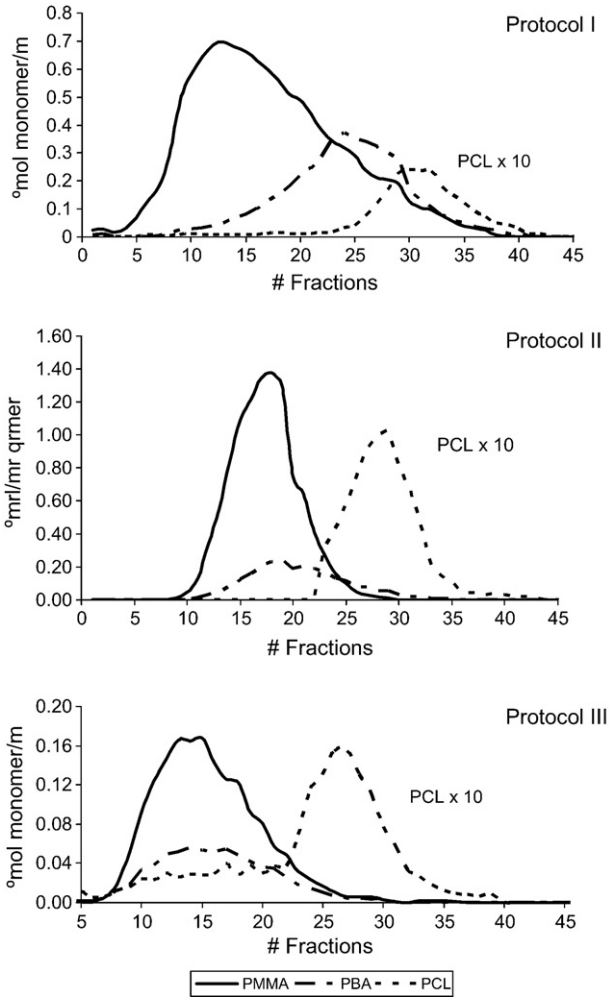

Fig. 6. SEC–Py-GC–MS plots of polymethylmethacrylate–polybutylacrylate–polycaprolactone (PMMA–PBA–PCL) *ter*polymers prepared according to three different synthesis routes. For more details see Kaal et al. [95]. Reprinted from J. Chromatogr. A with permission.

ble of analysing large series of samples fully unattended. In addition to this also the quantitative performance of pyrolysis treatment should be improved and data-treatment should be made easier.

Laser pyrolysis has great potential. Especially the possibility of controlled pyrolysis at a specific position is very useful. Also the ability to obtain information on material from specific layers contributes to the attractiveness of laser-pyrolysis GC. Another approach with great potential is the hyphenation of liquid-phase separation methods and Py-GC. A recently developed system for automated on-line size-exclusion chromatography (SEC)–Py-GC–MS allowed obtaining combined molecular weight/chemical-composition distributions of organic- and water-soluble copolymers. Some results obtained with this system for a *ter*polymer consisting of polymethylmethacrylate (PMMA), polybutylacrylate (PBA) and polycaprolactone (PCL) are shown in Fig. 6 [95]. Also other liquid-phase separation methods such as e.g. gradient RPLC were hyphenated to Py-GC using similar principles [124].

An improved level of detail can also be obtained through the use of comprehensive two-dimensional GC (GC × GC) instead of the commonly used 1D Py-GC. This will improve the separation of the complex pyrolyzates dramatically [125], which is especially relevant for the characterization of samples from environmental or biological origin where a large number of different pyrolysis products can be expected. GC × GC will improve the identification and quantification of the analytes of interest and will also enable group-type identification/classification and quantification.

Finally, chemometrical methods can greatly improve our ways of extracting information from complex pyrograms [126]. Advanced chemometrics can aid in the interpretation of the complex fingerprints as well as in detecting small differences or similarities in pyrograms. With the proper chemometrical tools it will be easier to convert detailed data sets obtained for complex samples into useful information. GC and GC × GC here provide the high resolution and excellent sensitivity after pyrolysis has converted the molecules into the smaller species required for these high-resolution techniques.

2.4. Thermochemolysis

2.4.1. Principles of thermochemolysis

Pyrolysis of macromolecules usually produces a wide range of chemical species widely differing in polarity and molar mass. For these compounds to be suited for GC they must be of sufficiently low molecular weight and polarity. For this reason, polar polymers creating polar pyrolyzates constitute a potential problem in Py-GC. To overcome the limitations of conventional pyrolysis in the analysis of polar polymers, Challinor [127] introduced a technique called thermochemolysis in 1989. This technique is in fact a pyrolytic degradation technique with in situ derivatisation. The reagent used in the original publications was tetramethylammonium hydroxide (TMAH). Co-pyrolysis of this compound with the polymer sample avoids carboxylation and results in the in situ generation of methyl esters of carboxylic acids and hydroxyl groups. Owing to these reactions significantly more materials now become accessible to pyrolysis GC [128].

Virtually all applications of thermochemolysis-GC used TMAH as the reagent. More recently also other reagents have been used for the modification of polar groups. These included for example dimethyl carbonate (DMC), hexamethyldisilazane (HMDS) or other quaternary ammonium salts. Fabbri et al. for example [129] used DMC in combination with a catalyst (zeolite 13X) for the production of fatty acid methyl esters from complex samples under pyrolytic methylation conditions. Ranz and Lankmayr [130] used the quaternary salt ammonium anilinium hydroxide as a reagent for the determination of acidic herbicides. Besides pyrolysis-methylation also pyrolysis-silylation can be an alternative. Oseta-Cortina and Doménech-Carbó [131] for example successfully applied on-line trimethylsilylation using HMDS for the characterization of the main diterpenoid components of natural resins present in artwork by means of thermochemolysis-GC–MS. Thermochemolysis is excellently suited for fingerprinting purposes and for the elucidation of the

structure of polar polymers. It is less applicable for truly quantitative analysis. The repeatability of the quantitative results is poorer than in regular applications of chromatography. A notable advantage of thermochemolysis-GC–MS compared to other derivatisation methods is that it is fast and sample pre-treatment is virtually absent. Standard sample preparation methods often are time consuming and frequently require toxic or corrosive substances which may pose environmental and health concerns [129].

2.4.2. Mechanism of tetramethylammonium hydroxide thermochemolysis

TMAH thermochemolysis is a combination of pyrolysis with in situ methylation of polar groups using the methyl donor TMAH. The exact reaction mechanism is not fully understood. It is now believed that the process consists of thermally assisted chemolysis, rather than true pyrolysis followed by methylation of the pyrolysis products [132]. A widely used abbreviation to describe the mechanism is THM: thermally assisted hydrolysis and methylation. To some extent the word "thermally" here is confusing since it has been demonstrated that TMAH already promotes hydrolytic ester and ether bond cleavage at room temperatures or slightly above [133]. What is clear, however, is that pyrolysis in the presence of TMAH can take place at milder conditions than required for classical pyrolysis [134]. Fewer secondary reactions occur under the milder conditions, which has advantages for tracing back the original structure of macromolecules. The following scheme outlines the most likely reaction mechanism for thermochemolysis. Here A–B represents the hydrolysable polymer molecule [135]. The reaction starts from the OH^- ion generated from the TMAH. The chain of subsequent reactions is given below:

- Hydrolysis:

$$OH^- + A\text{–}B \rightarrow A^- + B\text{–}OH$$

- Formation of TAA salts:

$$A^- + R_4N^+OH^- \rightarrow R_4N^+A^- + OH^-$$

$$B\text{–}OH + R_4N^+OH^- \rightarrow R_4N^+OB^- + H_2O$$

- Thermal dissociation to alkyl derivatives:

$$R_4N^+A^- \rightarrow AR + R_3N$$

$$R_4N^+OB^- \rightarrow BOR + R_3N$$

As can be seen from these reaction equations only hydrolysable bonds react with the reagent. It is for this reason that only these bonds yield products in the presence of a quaternary ammonium hydroxide that are different from those obtained in conventional pyrolysis. Once more, it should be emphasized that the mechanism of thermochemolysis is not yet fully understood. Ishida et al. [136] for example showed that methanol used to dissolve the sample and the TMAH can affect the reaction. The contribution of methanol to the THM reaction resulted in the formation of a considerably greater amount of methyl esters relative to the methyl ethers. The methyl derivatives here apparently are formed not only by hydrolysis but also through methanolysis. The authors also showed that inorganic salts can affect the THM process: KCl and KOH for example hindered the formation of dimethyl derivatives. Most likely this multitude of factors affecting the thermochemolysis process explains the poorer repeatability that is frequently seen in THM experiments.

2.4.3. Practical aspects of thermally assisted methylation

To perform thermochemolysis experiments standard pyrolysis devices can be used. For a successful reaction the sample and the reagent, commonly TMAH, must be thoroughly mixed prior to analysis and rapidly heated to a temperature sufficiently high for the reaction to occur. The absence of a thorough contact between reagent and sample results in incomplete hydrolysis and alkylation reactions, a low reaction yield and poor repeatability [137]. Obtaining an adequately mixed sample and reagent mixture is less trivial than one might expected and a variety of approaches for sample reagent/mixing have been applied. In our hands, the best results were obtained by first dissolving the polymer in water, adding this to a TMAH water solution and apply vortex homogenization prior to analysis.

THM reactions proceed successfully at ambient temperature or slightly above. However, to obtain a sufficiently intense pyrolysis the use of higher temperatures is necessary. Unfortunately, at higher temperatures the TMAH reagent can evaporate resulting in a separation of the reagent and the macromolecules leading to a failure of the reaction. To avoid this, the sample and TMAH can be mixed first and incubated for 10 min at 100 °C in an ambient atmosphere prior to the pyrolytic treatment [135]. To eliminate the manual sample handling steps we developed an automated version of this using a multistep PTV temperature treatment.

Another approach is to perform the reaction off-line in sealed ampoules in an oven followed by an extraction step [138,139]. There are some advantages of this off-line approach in comparison to the other methods. The huge amounts of TMAH and its decomposition product trimethylamine for example do not enter the GC column and the isolated products can also be used for further characterization e.g. by NMR. Irrespective of which approach is used for the THM reaction it is important to use fresh TMAH solutions and store the reagent properly.

2.4.4. Applications

Thermochemolysis is relatively simple and inexpensive and therefore easy to implement in any laboratory process. Another advantage of the technique is that it can yield information on a wide range of different compounds otherwise not accessible to GC. The applicability of THM-GC is enormous; the reaction can be used for the characterization of all materials that undergo at least some chemical degradation when heated in the presence of a quaternary ammonium salt. Therefore, this technique is implemented in a number of different application areas

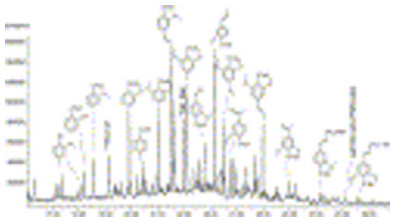

Fig. 7. Total ion chromatogram of a wood sample coming from historic Pisa (Italy) obtained using thermochemolysis with HDMS as the reagent. For more details see Colombini et al. [142]. Reprinted from Microchem. J. with permission.

including environmental, biological and food analysis as well as in the growing area of proteomics. It has been applied to an enormously wide range of materials including wood products, lipids, polysaccharides, cellulose, soil, proteins, waxes, etc. A detailed review on applications of THM-GC has been published by Challinor [135] in 2001. Below a short selection of applications is given to demonstrate the benefits of thermochemolysis and to show that it indeed has enabled a further expansion of the application domain of GC.

Wood is one of the materials which is extensively studied using thermochemolysis-GC. Wood is a complex mixture of a wide range of chemical compounds such as e.g. cellulose and hemicellulose, lignin as well as several other minor organic compounds such as lipids, phenolic compounds and terpenoids. The detailed chemical characterization of wood is important for several reasons such as sample differentiation, determination of the origin of species as well as for the investigation of the biodegradation of wood [140,141]. In wood research the characterization of the lignin, a polyhydroxyphenolic macromolecular substance, is of primary importance. Unfortunately, TMAH-based thermochemolysis-GC does not appear to be the ideal approach for the characterization of lignins because the methylation of the phenolic groups makes them indistinguishable from the methoxy-groups already present in the wood (Fig. 7) [142]. To overcome this problem, Filly et al. [143] used isotopically labelled [^{13}C]TMAH which permitted the differentiation of the original and the added methyl groups. Other solutions reported by Colombini et al. [142] and Ishida et al. [144] were the use of tetrabutylammonium hydroxide (TBAH) and HMDS for in situ derivatisation instead of TMAH to avoid indistinguishable groups.

An interesting application area of thermochemolysis is the determination of the chemical composition of humic and fulvic acid. As discussed previously, thermochemolysis to some extent overlaps with derivatisation. Lehtonen et al. investigated tetramethylammonium hydroxide treatment in the study of lake aquatic fulvic acids [145]. TMAH treatment was performed off-line at 100 °C without significant thermochemolytic degradation (ensured with a solvent extraction). In addition to this also the product distributions at three pyrolysis temperatures (300, 450 and 600 °C) were studied. The degradation of TMAH-treated fulvic acid was found to be strongly thermally controlled. The number of degradation products and their abundance increased significantly when the pyrolysis temperature was raised from 300 to 600 °C. In the definition used in this review the treatment at 100 °C is a derivatisation whereas treatment at a temperature of 300 °C or higher clearly is thermochemolysis.

THM-GC–MS has also been used for the structural characterization of peptides and proteinaceous materials [146,129]. These applications clearly indicate the potential of THM-GC–MS in the life-sciences area. TMAH effectively methylates amino, carboxylic and hydroxyl groups, yielding primary amino acid methyl esters. However, the results from these studies also show that partially methylated amino acids still exist after the reaction procedure. This indicates a limitation of the technique. The fact that multiple reaction products are obtained complicates the interpretation of the chromatogram considerably. For fingerprinting purposes however, as for example would be applied in the initial stages of metabonomics research, the additional detail might be an advantage. An overview of the most important application areas of thermochemolysis and a selection of applications for each of these areas is given in Table 1.

2.4.5. Future perspectives

After the introduction of THM-GC in the late 1980s the technique is now frequently employed in several application areas. Especially for screening purposes of complex mixtures of biological and environmental origin it is very useful. A lot of information can be obtained in just one single GC run at a minimum of sample preparation. Without thermochemolysis the use of Py-GC for the characterization of polar macromolecules would be virtually impossible. Nevertheless, the limitation of thermochemolysis is in the quantitative evaluation of the pyrograms. This drawback is caused by the incompleteness of the reactions and the occurrence of a multitude of varying secondary reactions caused by differences in the matrix and non-optimised experimental conditions. Quantitative use of the data is even further complicated by the serious lack of understanding of the reaction mechanisms. Future work on the understanding and optimisation of thermochemolysis will allow a further development of the technique from a rapid and clean screening method to a reliable tool for the quantitative characterization of complex mixtures.

Thermochemolysis-GC holds great promise for use in life-sciences applications. Because of the huge complexity of samples from this area multidimensional methods will be needed. One here could envisage LC separations comprehensively interfaced to thermochemolysis-GC. Alternatively, as mentioned before in the future aspects of pyrolysis, also the incorporation of GC × GC and the use of chemometric methods will further improve the performance of thermochemolysis techniques. Powerful statistical methods are needed to convert the pyrograms into even more useful information.

3. Epilogue

GC is a mature technique. This by no means should be misinterpreted as it is dull and tiring! GC is more efficient, faster, more robust and more reliable than many of the newest additions to the family of chromatographic methods. Of course it is not our intention to criticise the other chromatographic techniques

Table 1
A selection of recent applications in the most important application areas of thermochemolysis

Material	Reagent	Study background	Reference
Humic and fulvic acids			
Humic acid fraction isolated from polluted sediments	TMAH	Characterization of the molecular structure of humic acids	[147]
Humic substances from soil and peats	TMAH/TEAAc	Comparison between humic substances from various sources	[148]
Soil humic acids	TMAH	Differentiation of genetically different soil humic acids	[149]
Humic acids extracted from sewage sludge	TMAH	Structural characterization of humic acids	[150]
Lipid constituents of peat humic acids and humin	TMAH/TEAAc	Distinction between chemically bound and trapped acids	[151]
Soil residue	TMAH	Semiquantitative GC–MS analysis of soil humic acids	[152]
Wood and wood module compounds			
Phenolic extractives in wood	TBAH	Direct determination of phenolic compounds	[144]
Lignin (oak)	^{13}C-TMAH	Determination of phenolic composition	[153]
Lignin	TMAH	Characterization of fresh vs. degraded barks	[154]
Spruce sapwood	TMAH	Characterization of lignin and polysaccharide fraction	[155]
Lignin	BSTFA	Determination of cinnamyl alcohol	[156]
Sapwood	TMAH	Determination of chemical changes in polysaccharides and lignins	[157]
Proteinaceous			
Proteinaceous material in kerogens	TMAH	Differentiation of kerogen origin	[158]
Insoluble residues of geochemical samples	TMAH	Identification of protein remnants	[159]
Proteinaceous moieties kerogen	TMAH	Detailed characterization	[160]
Bacteria, algae and marine sediments			
Freshwater microalgae	TMAH	Determination of chemical structure	[161]
Marine sedimentary organic matter	TMAH	Qualitative characterization	[162]
Sediment cores	TMAH	Determination of molecular composition of lignin phenols and selected acids	[163]
Coastal marine sediments	TMAH	Determination of the chemical composition	[164]
Bacterial biomasses	TMAH	Profiling fatty acids	[165]
Coastal sediments	TMAH	Comparison of polycyclic aromatic hydrocarbon distributions and sedimentary organic matter characteristics	[166]

and those preferring these. Each chromatographic method has its own merits as well as its own problems and future needs. But GC should be the method of choice for the separation of thermally stable and volatile compounds. In this article we have identified ways to allow other applications to escape from the drawbacks of their current methods and start to benefit from the strengths of GC. These routes are not giant strides, but use small incremental improvements that can be adopted and implemented readily in all laboratories.

Running the column to higher temperatures is an obvious route for extending the application range of GC. HT-GC is well established. From the practical perspective there are a few items that could be further improved to make HT-GC simpler and more reliable, but the technique is approaching its fundamental limits. Derivatisation is a route that allows bringing more polar molecules within the scope of GC. For obvious reasons derivatisation is under pressure. The chemistry of derivatisation reactions is occasionally difficult to control with moisture and air sometimes leading to undesired effects. LC–MS is a logical option to consider if automated and reliable derivatisation proofs to be difficult. Ionisation suppression remains to be an issue there. For profiling applications of chromatography, such as e.g. in the rapidly developing area of metabonomics, the higher peak capacity and increased information content of a GC chromatogram makes GC the method of choice. The peak capacity and hence information content of GC or GC × GC profiles is huge. It is this huge peak capacity that allows the use of entirely new strategies for extracting information from samples: profiling instead of target compound analysis. Derivatisation will remain necessary to allow the use of GC or of hyphenated and comprehensive chromatographic system including at least one GC dimension in metabonomics. Automatisation of sample preparation procedures incorporating a derivatisation step is an important research subject. Advanced autosamplers offer great possibilities here.

Gas chromatography is not really widely used in the analysis of (bio)macromolecules, yet pyrolysis and thermochemolysis are attractive methods. Recent improvements have allowed the use of both methods for obtaining quantitative data and combined molecular weight/chemical-composition distributions of

organic- and water-soluble copolymers through hyphenated and comprehensive SEC–Py-GC and LC–Py-GC. Although the quantitative performance of the methods has been improved more work is needed, especially in thermochemolysis-GC. Also here automation remains to be an issue. PTV pyrolysis can provide solutions to a number of these problems. Two-dimensional GC (GC × GC) instead of the commonly used one-dimensional Py-GC can further improve the separation of complex pyrolyzates. This is especially relevant for the characterization of samples of environmental or biological origin where a large number of different pyrolysis products can be expected. GC × GC will also allow improved identification and quantification of the analytes of interest and finally will enable group-type identification/classification and quantification. Combination with chemometric methods can greatly improve our ways of extracting information from the complex pyrograms. With the proper chemometric tools it will be easier to convert detailed data sets into useful information. GC and GC × GC here provide the high resolution and excellent sensitivity after thermal treatment methods have converted the molecules into smaller fragments. Life sciences is an important application area that could further benefit from developments in pyrolysis and thermochemolysis. In that sense it is a key driver for the development of the routes identified here and of GC more in general.

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