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Gas Chromatography in the Functional Group Analysis of Organic Compounds

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The role and trends in the development of chromatographic methods for the functional group analysis of organic compounds are discussed. Attention is concentrated on the analytical possibilities of using chemical reactions of the test compounds together with gas-chromatographic separation (chemical-chromatographic method).
The bibliography includes 192 references.

CONTENTS

I.	Introduction	1007
II.	Retention parameters	1007
III.	Inverse gas chromatography	1008
IV.	Selective detectors	1008
V.	Chemical-chromatographic methods	1009

I. INTRODUCTION

With the introduction of gas-chromatographic methods appreciable changes have occurred in recent years in methods for functional group analysis, particularly for the functional group analysis of volatile organic compounds. The application of combined methods in conjunction with gas chromatography has revealed a number of new possibilities. These include the following: (1) an improvement of the resolving power of methods for functional group analysis which have been transformed from methods for group identification into methods for the identification of individual compounds: this has permitted the successful solution of problems which could hardly be solved by earlier methods[1,2]; (2) an improvement in the reliability of the method, in particular a decrease of the errors associated with the stage involving the intermediate isolation of the pure substance; (3) reduction of the time required for analysis; (4) decrease in the amount of substance necessary for functional group analysis.

The application of gas chromatography is useful and justified also in combination with any traditional methods for functional group analysis. For example, the employment of physical (spectroscopic and electroanalytical) methods, which are used at the outlet from the chromatographic column for the analysis of the eluate, makes it possible to proceed from group identification to the identification of individual compounds; the employment of chemical methods in combination with chromatographic procedures makes it possible to increase greatly the range of reactions studied, whose products may be not only coloured or gaseous substance but in general any volatile compounds; this of course greatly extends the scope of the method. In combination with chemical methods for functional group analysis, gas-chromatographic procedures can be used also as kinetic techniques, since the reactivities of isomers are frequently different.

II. RETENTION PARAMETERS

The retention parameter of a compound is its chromatographic constant, which is directly proportional to its distribution constant between the gas and stationary phases (the determination of this constant taking into account adsorption interactions has been discussed, for example, by Berezkin[3]). The retention parameters depend on the structure of the molecule of the compound, in particular on the size and number of functional groups present in the molecule. Various workers have successfully used additive schemes to calculate the retention parameters[4-8]. The possibility, in principle, of detecting certain functional groups on the basis of retention indices should be noted[9]; however, this is possible only provided that the structure of the carbon skeleton of the compound being analysed is first established and the procedure is not very reliable[10]. We may note that, in order to improve the accuracy, the relative rather than absolute retention characteristics (relative retention time, relative retention index, etc.) are usually employed in such cases.

The properties of retention parameters can be used for functional group analysis. For example, the linear variation of the logarithm of the retention parameter with the number of methylene groups in a molecule is generally known for homologous series[11,12]. This permits a relatively simple solution of one of the complex problems of functional group analysis—the determination of the number of carbon atoms in the hydrocarbon group. The solution of this problem by other methods is much more difficult. Sakharov and coworkers[13,14] have shown that fractional-linear functions can be usefully employed to characterise the variation of the retention parameters.

In assigning the components of the mixture under analysis to a particular class of organic compounds, the variation of the retention volumes on two[15,16] or more[17,18] stationary phases of different polarity can be of significant assistance: $\lg V_{1i} = A \lg V_{2i} + B$, where V_{1i} and V_{2i} are the retention volumes of the ith compound in a particular homologous series on the first and second phases and A and B are constants.

It has been suggested that organic compounds of different types can be characterised by graphical relations for differences between logarithmic functions of relative retention parameters, for example relations of the type $\Delta I_{12} = f(\Delta I_{34})$, where ΔI_{12} and ΔI_{34} are the differences between the retention indices of the given compound on phases 1 and 2 and phases 3 and 4 respectively. The points corresponding to compounds of a given class are located on the above graphs in specific characteristic regions, which can be

usefully employed to identify the functional groups in organic compounds[19].

The method of identification based on the variation of the retention time with the reciprocal of the absolute temperature in one chromatographic column, proposed by Arakelyan et al.[20], is fairly laborious but relatively reliable.

All the methods indicated above have been used successfully in analytical practice and have been described in detail in monographs on chromatography[21–24].

III. INVERSE GAS CHROMATOGRAPHY

Inverse gas chromatography (IGC) is based on the use of a direct interaction between standard compounds and the test substance and the establishment of definite relations between the chromatographic characteristics of the standard compounds and the structure of the compounds used as the stationary phase. IGC is particularly useful in the study of involatile compounds (high-molecular-weight compounds and compounds unstable at elevated temperatures).

The IGC method permits the group identification of compounds used as the stationary phase. When the relative retention times of substances of different polarity (methanol, heptane, and toluene) are plotted in a triangular coordinate system using straight-chain paraffins, aliphatic alcohols, and acids as the stationary liquid phase, each of the above homologous series is characterised by a distinct range of values of the retention times for the standard compounds. We may note that certain isomeric compounds can also be distinguished with the aid of this method. Thus the spectra of the volatile standard compounds 1,3-butylene glycol and 1,4-butylene glycol and those of primary and secondary octyl alcohols are different. The above data indicate a fairly high sensitivity of the IGC method with respect to the nature and structure of the compounds. Similar results can be obtained also for polymeric systems.

The extensive factual data accumulated in gas chromatography on the application of polymers as stationary liquid phases for the separation of mixtures of volatile compounds lead to the conclusion that polymers can also be identified by the IGC method. Thus the retention volumes of phosphine are 1.75, 1.58, and 2.52 cm^3 on columns with silicone oils 702, VKZh-94 V, and PFMS-4 respectively[25]. In another example two polymeric compounds of the same type —poly(propylene glycol) with M = 1760 and poly(ethylene glycol) with M = 1540 — are characterised by markedly different retention parameters[26], which permits their identification by the IGC method.

The IGC method has been developed in greatest detail to characterise (identify) asphaltenes (M = 500–1000)[27].

Dorrence and Peterson[28] demonstrated the usefulness of employing in IGC chemical reactions with the stationary liquid phase investigated. In order to study the functional groups in asphaltenes, the asphaltene test specimen in the column was silylated, as a result of which the active hydrogen atoms in the hydroxy- and carboxy-groups were replaced by trimethylsilyl groups. Treatment of asphaltene with *NN*-bis(trimethylsilyl)acetamide at 160°C led to a sharp decrease in the specific coefficients for the interaction with the chosen volatile standards (phenol and propionic acid). This method can be used to characterise various polymeric compounds with functional groups.

We may note that, whereas the amount of test substance required for the IGC method on a packed column is about 0.4 g, it decreases to 3–4 mg when a capillary column is used[27]. The microversion of IGC is particularly important for polymers which are difficult to synthesize and in general for compounds which are difficult to obtain.

Since the problem of identifying polymers in may cases reduces to the determination of their molecular weights, we may mention the possibility of employing the relation between the retention parameters of volatile standard compounds and the molecular weight of the stationary liquid phase[29].

IV. SELECTIVE DETECTORS

One of the major requirements which must be met by detecting systems in gas chromatography, namely their universality, i.e. equal sensitivity to all the mixture components emerging from the chromatographic column, is a disadvantage in the identification of organic compounds. In functional analysis the main requirement which must be met by the detector is its selectivity and an ideal system would be one having specific sensitivity only to compounds of specified classes, whose signal would be proportional to the content of the corresponding functional group.

The automatic titrator[11] and the spectrofluorimetric detector[30] meet this requirement most closely. The first is designed for the selective determination of acids and bases in the eluate and is based, as its name suggests, on the automatic maintenance of a specified pH in the titration cell. The operation of the spectrofluorimetric detector, which is designed for the detection of aromatic groups, is based on the employment of the fluorescence[30] or ultraviolet light absorption[31] characteristic of aromatic compounds.

The detector with selective sensitivity to compounds having multiple bonds is of undoubted interest[32]. The detection principle involves passing the mixture of saturated and unsaturated compounds to be analysed through an ionisation chamber together with krypton, which is excited to a metastable state by radioactive emission. The metastable krypton atoms are capable of ionising the molecules of the unsaturated compound and in the presence of the latter an ionisation current is generated.

The electron capture detector, proposed for the first time by Lovelock and Lipsky[33], exhibits an enhanced sensitivity to alkyl halides, sulphides, carbonyl compounds with conjugated double bonds, organometallic compounds, nitriles, and nitro-compounds. The sensitivity of the detector to many types of compound reduces its value for the purpose of identification, but makes it irreplaceable in the determination of certain compounds present in nanogram amounts.

Other selective detectors, which can be divided into three main groups, exhibit a much higher specificity. Thermionic detectors, based on the increased conductivity of the flame in a flame-ionisation detector on combustion of organic compounds containing heteroatoms in the presence of alkali metal or alkaline earth metal salts, were used in early investigations mainly for the selective detection of halogen- and phosphorus-containing compounds[34,35]. When rubidium and potassium salts are used[36], the sensitivity for nitrogen-containing compounds increases, but the greatest sensitivity is achieved when caesium bromide is employed[37]. In the presence of caesium the thermionic detector may be used for the highly sensitive determination of silicon, tin, and lead compounds[38]. It has also been shown[39] that the signal of the thermionic detector with rubidium sulphate is proportional to the content of heteroatoms in the molecules of the substances being analysed and a formula has been proposed whereby it is possible to calculate the content of the heteroatoms (halogens, sulphur, phosphorus, and nitrogen).

The coulometric detector[40], which exhibits selective sensitivity to compounds containing halogens, sulphur, nitrogen and phosphorus, may prove to be of significant assistance in the identification of organic compounds. The detector design was developed further in studies by Stepanenko and Krichmar[41–44]. The ion-selective electrodes, which have recently come to be widely used in analytical chemistry, have also found applications in coulometric detectors for the determination of fluorine[45], sulphur, and chlorine[46]. Among other electrochemical detecting systems, mention should be made of a selective galvanic detector[47], which is sensitive to alcohols and aromatic and unsaturated hydrocarbons.

The group of spectroscopic detectors, a major advantage of which is (apart from a fairly high sensitivity in the determination of heteroatoms) a very high selectivity, has been developed vigorously in recent years. The simplest example of this group is the halogen detector based on the Beilstein effect, i.e. on the green light emission arising when halogen-containing compounds are introduced into the flame through a copper mesh[48]. The flame-photometric detector, which was initially proposed for the selective determination of phosphorus and sulphur[49], has been modified to determine many other elements. The operation of the detector is based on the measurement of the intensity of the characteristic emission lines of the flame in the presence of heteroatoms using a photo-multiplier. In the determination of sulphur- and phosphorus-containing compounds, 394 and 526 nm light filters are usually employed, but it has also been stated that the positions of these maxima have been determined inaccurately[50]. When the flame-photometric detector is sensitised by indium salts, its sensitivity to halogen atoms is increased selectively with a maximum in the region of 360 nm[51,52] or at 409.9 nm, which corresponds to the maximum in the emission spectrum of indium iodide[53]. The photometric recording of the flame emission from an alkali metal thermionic detector made it possible to distinguish chlorine, bromine, and iodine atoms[54] and the employment of metallic calcium ensures a selective determination of fluorine in the region of the spectrum near 529.9 nm.[55] Organomercury[56,57] and boron[58] compounds can also be analysed. It has been suggested that the disadvantage of the flame photometric detector, due to the mutual interference of the heteroatoms present (and also of their concentrations) on the observed emission, be eliminated[59] by measuring the ratios of the spectral line intensities for the elements being determined (at 256 nm for chlorine, 206.2 nm for iodine, 253.3 nm for phosphorus, 182.04 nm for sulphur, and 292 nm for bromine) to the intensity of the spectral line of the carbon atom at 247.9 nm, which is almost independent of the structure of the compound and the elements present in it[60].

Many other detectors are used for identification purposes (the mass-spectrometric detector has been particularly successful); selective detectors were surveyed in detail in an earlier review[61].

One should note that almost all specific detectors (with the exception of those mentioned at the beginning of this section) have only an indirect bearing on functional analysis, since they cannot show unambiguously within which functional group the given heteroatom is located. Therefore their application is more effective in elemental analysis of organic compounds, particularly when it is necessary to determine substances present in microamounts. However, there is no doubt that the detecting systems developed so far have assisted in the identification of unknown compounds. The preliminary introduction into the test compounds of a specific heteroelement using reactions characteristic of the given functional group (for example the introduction of bromine at the site of a double bond) with subsequent employment of a detector selective for the element introduced can probably be of some interest. When the given heteroatom is detected in the compound being identified, the presence of the expected functional group may be postulated with considerable certainty.

V. CHEMICAL-CHROMATOGRAPHIC METHODS

The combination of chemical and gas-chromatographic methods appears to be the most effective and flexible procedure in functional group analysis. There has been a steady increase in the proportion of studies involving chemical-chromatographic methods among all the investigations on gas chromatography. Fig. 1 shows that the contribution of these methods increased in 1972 almost by a factor of 1.5 compared with 1966 (data quoted in the bibliographic section of the Journal of Chromatography were used to plot this graph).

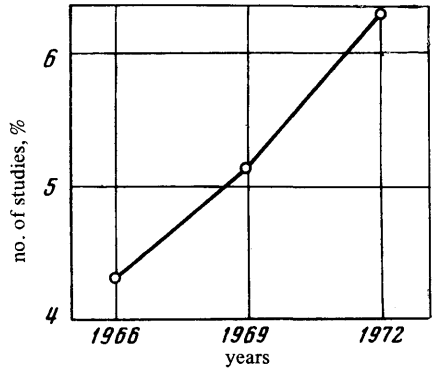


Figure 1. The number of studies on chemical-chromatographic analytical methods in relation to the total number of publications on gas chromatography.

Various methods are used to carry out the chemical reactions: reactions carried out outside the chromatographic system, reactions before the column, reactions in the column, and reactions after the chromatographic column. The choice of the reactions and of the conditions governing their occurrence is governed by the problem to be solved.

The effect of the reaction of the analytical mixture before the chromatographic process, involving a selective removal of components, makes it possible to assign the latter to specific classes by comparing the chromatograms obtained with and without the treatment of the sample by the selected reagents. This method has been called the substraction method.

The substraction process is carried out in a small column (reaction loop) filled with the selected chemical reagent, usually deposited on an inert carrier, like the stationary liquid phase. Under these conditions, it is even possible in many cases to use volatile reagents when the latter are deposited on a strongly adsorbing carrier[62,63].

If the sample analysed is introduced simultaneously into two columns connected in parallel having detector cells operated in a differential mode (the reaction loop is used in series with only one of the columns), then the chromatograph will show only the components eliminated[64]. The system suffers from the disadvantage that it is necessary to achieve an absolutely exact reproducibility in all the working parameters of both chromatographic columns; this can be obviated by including the reaction loop between the two detector cells[65] and by employing one column. The main requirement which the system must meet in this case is a small volume, which should be comparable to the peak width.

It is quite unnecessary that the compounds removed should be fully retained in the reactor in the form of involatile compounds. Even if reactions result in volatile products, the latter will have different elution times from those of the initial compounds, which can be clearly shown by subsequent chromatography. Moreover, the incomplete adsorption in the reaction column may be used to secure additional information about the structure of the compounds analysed. Thus the incomplete removal of *cis*-dienes in a reactor with maleic acid chloride makes it possible to distinguish them from the *trans*-isomers, which react faster and are completely eliminated in the reactor[66]; the employment of a reactor with boric acid ensures the separation of primary and secondary alcohols from their tertiary isomers[67,68].

The fillers in the reaction loops, used for the selective removal of a wide variety of different classes of compounds, have been described in detail in the literature[69,70].

Tricyclohexyl borate has been suggested for the selective absorption of alcohols[71]; metal hydrides can be used for the same purpose[72–74] and it has been noted[73] that aluminium hydride and lithium tetrahydroborate remove all compounds with oxygen-containing functional groups, except ethers. The complete elimination of aldehydes from the mixture analysed is achieved when *o*-dianisidine[74], sodium tetrahydroborate[73], or hydroxylamine hydrochloride[72] are used. The difference in the reactivity of boric acid towards various alcohols has been used to separate tertiary alcohols from primary and secondary alcohols[73,74] and the employment of sodium tetrahydroborate, which reacts slowly with ketones, makes it possible to distinguish the latter from aldehydes[73]. Prokopenko et al.[75] showed that the selectivity in the elimination of different oxygen-containing substances in reactors containing boric acid and lithium tetrahydroaluminate depends on the amount of the reagent. Thus tertiary alcohols can be completely removed in a reactor with a high boric acid content.

The method for the preliminary absorption of alcohols and carbonyl compounds in a reactor filled with 40% of 3-nitrophthalic anhydride and semicarbazide on Celite-545, placed directly before the separating chromatographic column[76], is also of interest.

The formation of adducts with urea is a characteristic reaction for the removal of n-alkanes[77,78] and methyl esters of aliphatic acids[79]. Ohtaki et al.[80] showed that all hydrocarbons with a straight-chain structure can be removed by the reaction with urea. In the subsequent treatment (reactions with hydroborates and oxidation) the olefins can be isolated in the form of the corresponding alcohols and can be determined gas-chromatographically. The overall olefin content in the initial mixture can probably be found also by determining the amount of hydroborate which has reacted.

Columns with *NN*-di-(2-cyanoethyl)formamide[81], mercury perchlorate[82], and alumina[83] have been used for the selective retention of aromatic hydrocarbons. Mercury perchlorate is also an effective absorber for unsaturated compounds[82,83]. It has been suggested that the group isolation of organomercury compounds be carried out either by including in the chromatographic system a short reactor with a powdered metal or by removing them by reaction with various sulphur-containing compounds[84].

A novel method has been proposed for the determination of the overall admixture of involatile organic substances[85].

In many instances it is useful to employ a reaction column with phosphoric acid, which permits the selective removal of nitrogen-containing organic bases from complex mixtures[86].

Examples of the determination of alcohols, ketones, aldehydes, esters, and aromatic hydrocarbons by reactions in a syringe have been described[87].

Qualitative chemical tests after chromatographic separation constitute a less common way of identifying the components of complex mixtures. One of the reasons for this is the low sensitivity of colour reactions, so that considerable amounts of the substance analysed, not less than 20–100 μg, have to be used[88]. It is possible to specify the two most interesting procedures for rapid qualitative tests. The first consists of the subdivision of the eluate from the chromatographic column into several approximately equal streams which are directed to test-tubes with group reagents for a specific class of compounds[88]. A colour change in one of the test tubes at the instant of the emergence of the peak is evidence that the substance belongs to the given class. The second method provides for the employment of a sorbent layer impregnated with the reagent for the given functional group and moving continuously relative to the stream of eluate from the column[89]. If the eluate contains a compound of the given class, then a coloured spot appears on the sorbent layer. By changing the reagent impregnating the sorbent and by comparing the results of the chemical study with the separation chromatograph, it is easy to determine the class of the compound corresponding to each peak. The advantages of the first method are the possibility of testing each peak for several functional groups and also the possibility of determining the nature of the components present at microconcentrations insufficient for the appearance of an appreciable colour by the successive introduction of several samples (provided that the colour of the solution remains stable for a sufficiently long time). The sensitivity of the method based on the subdivision of the eluate stream can be improved significantly by concentrating the substances in short porous glass traps filled with alumina[90]. When the flow rate through the trap is of the order of 5 ml min^{-1}, the majority of compounds investigated (aldehydes, ketones, alcohols, ethers, and aromatic and sulphur-containing substances) are trapped by the layer of packing 2–3 mm thick in the form of a concentrated band, which makes it possible to reduce the detection limit to the level of 0.1–1 μg. The specific reagents used for qualitative tests after chromatographic separation have been discussed[69,70].

All the methods for group analysis described above are applicable only to mixtures of volatile compounds which can be subjected to gas-chromatographic separation. In order to separate the components of mixtures of involatile or high-boiling compounds and assign them to specific groups, special procedures are used, many of which are also carried out in non-chromatographic reactors. Thus a number of procedures (esterification, decarboxylation, silylation, etc.) have been developed or applied in the gas chromatography of involatile compounds and also of highly polar substances whose separation is difficult. In particular, acetylation[91], silylation[91,92], and methylation[93] are used in the gas-chromatographic analysis of hydroxy-compounds; carboxylic acids are converted into the more volatile methyl esters or decarboxylated, etc. Without going into the details of these procedures (reactions of this kind for many classes

of compounds have been described in the literature[94]), we may say that the chemical reactions themselves can be regarded as one of the methods for group identification. In fact, the reagents employed are usually highly selective, for instance, when aldehydes or ketones are identified among the reaction products on treatment of the solid mixture with, for example, iodic acid and subsequent chromatography[95,96], it is possible to conclude with a high degree of certainty that the initial mixture contains α-hydroxycarboxylic acids.

Yet another useful feature of the above procedures should be indicated, namely the possibility of securing additional information about the qualitative composition of the mixture analysed on the basis of gaseous side products which are at present disregarded. Thus, in the conversion of phenols into methyl ethers by the reaction with diazomethane[97] nitrogen is evolved, ammonia is a side product in the silylation of involatile hydroxy-compounds[98], and in the decomposition (described above) of α-hydroxycarboxylic acids by iodic acid to yield the corresponding ketones or aldehydes[95,96] (which are not always convenient for chromatography) an equivalent amount of carbon dioxide is formed, etc.

Pyrolytic methods give rise to extensive possibilities for the identification of unknown compounds. Compounds which permit qualitative and quantitative inferences concerning the presence of specific functional groups in the specimen are as a rule formed in the pyrolytic decomposition of the substance analysed.

The thermal decomposition of organic compounds in the presence of sulphur vapour at 850–880°C gives products which yield information for the identification of many functional groups[99]. Thus the products formed in the pyrolysis of aldehydes, ketones, ethers, esters, alcohols, sulphonic acids, amino-compounds, and acids have been investigated in this way. The nature and ratios of the volatile pyrolysis products (nitrogen, hydrogen sulphide, carbon monoxide, carbon dioxide, carbon disulphide, and sulphur dioxide) are largely determined both by the structure of the initial compounds and by the presence of functional groups in the molecules.

The pyrolysis products (hydrogen, carbon monoxide and dioxide, methane, water, etc.) of substances in an inert gas atmosphere and their ratios also characterise the functional groups in the initial specimen[100–102]. Thus ketones and aldehydes give rise to CO, alcohols to CO_2 and H_2O, esters to CO_2, H_2O, and CO, acids to CO_2, mercaptans to hydrogen sulphide, halogen-containing compounds to the corresponding hydrogen halides, and the decomposition of amines results in the evolution of ammonia[101]. However, since the quantitative aspects of the formation of characteristic products were not determined in the published investigations, the methods based on pyrolytic decomposition are at present applicable mainly to qualitative analysis only.

On thermal decomposition in the presence of a catalyst [copper(II) oxide], hydrazine derivatives evolve methane; this made it possible to devise a method for their quantitative chromatographic determination[103].

The possibility of employing the effects of electron impact and of electric discharges in functional analysis has been studied[104,105]. It has been shown that, by decomposing the specimen analysed in an electric discharge, it is possible to assign the substance to a particular homologous series[104]. The study of the products formed in the decomposition of alcohols, aliphatic and aromatic hydrocarbons, ethers, esters, ketones, and aldehydes in an electric discharge established that methane is formed from all the test compounds and acetylene is generated by compounds with an ethyl group. The decomposition products of all aromatic compounds contain benzene and carbon monoxide and dioxide are present in those of oxygen-containing compounds.

Examples of the employment of thermal decomposition methods for the analysis of certain other groups will be discussed below.

It is necessary to consider especially methods for the quantitative determination of functional groups. In principle any classical gas-volumetric analytical method can be modified for chromatography but it would be very wrong to assume that the only problem is to replace one method by another, even if the latter is more up to date and more automated (although these advantages are important, particularly in routine determinations). Chemical-chromatographic methods give rise to distinctly new possibilities. These include simultaneous qualitative (from the retention time) and quantitative (from the signal intensity) determination of the desired reaction product in the complex reaction mixture, the possibility of the simultaneous determination of several reaction products with similar chemical properties, which in chemical methods can be determined only as a single group, the separation of the desired reaction product from side products, which is frequently a potential source of errors in classical methods, the possibility, in principle, of employing reversible reactions, since one of the products is continually removed from the reaction system by the carrier gas, a high sensitivity of the determination, which makes it possible to carry out a complete analysis of microspecimens, rapidity of the determinations, and finally the possibility of complete automation of the entire analytical process.

The use of chemical reactions (or specific thermal decomposition), leading to reaction systems of two types, has now been described for the quantitative analysis of functional groups in organic compounds. If the reaction products are compounds with physicochemical properties similar to those of the other ingredients of the reaction mixture, then an aliquot part of the latter is chromatographed. This procedure involves a decrease of the sensitivity of the determination (only part of the mixture is analysed), the possibility of loss of the required product and hence of a decrease in the accuracy of the analysis, and also additional difficulties in the automation of the analytical process. It is more convenient although not always possible to employ reactions which lead to the formation of either a gaseous or a volatile product, which characterises specifically the functional group being determined. The final product (or products) are in this case directed to chromatographic analysis either immediately from the reactor or after intermediate concentration.

The employment of chemical-chromatographic methods for the quantitative determination of specific functional groups is discussed below.

1. Determination of Active Hydrogen

The quantitative determination of active hydrogen is based on the known reactions of the compounds analysed with a Grignard reagent or with lithium tetrahydroaluminate. In the first case the reaction results in the evolution of methane, the gas-chromatographic determination of which is fairly simple. It is noteworthy that all the errors of the classical method[106], associated with additional gas evolution (for example, the evolution of ethane appearing as a result of the decomposition of the Grignard reagent) can be readily eliminated in the final chromatographic step[107–110].

The employment of lithium tetrahydroaluminate[111–114], which reacts with active hydrogen atoms in several seconds even at room temperature, is of considerable interest. Methyl, ethyl, n-propyl, and n-butyl ethers, tetrahydrofuran

(THF), dioxane, and ethylmorpholine[115] can be used as solvents, which greatly extends the range of applications of the method compared with the classical chemical determination. THF is a particularly satisfactory solvent for many organic compounds[111,116].

The method for determining active hydrogen was largely the same in all the studies mentioned. A solution of lithium tetrahydroaluminate in a suitable solvent is poured into the reaction cell, a sample of the substance analysed is positioned without coming into contact with the solution (for example the specimen can be put in a dish resting on the reactor stopper[114]), the reaction cell is flushed with the carrier gas and then disconnected from the chromatographic system, and the sample is plunged into the solution. After the completion of the reaction, the cell is reconnected to the chromatograph and the hydrogen evolved is determined in a column with activated charcoal or molecular sieves. A cell designed as a standard attachment of a chromotograph[117], which we believe to be the most convenient for the analysis of liquid substances, has been described for reactions with lithium tetrahydroaluminate.

However, we may note that the high reactivity of lithium tetrahydroaluminate can in many cases constitute a disadvantage, since certain compounds which do not contain active hydrogen are converted into substances containing such hydrogen as a result of the reduction by the reagent.

In this connection we may mention the methods for the determination of active hydrogen linked to a silicon atom, based on the hydrolysis of the organosilicon compound in the presence of potassium hydroxide for 15s and accompanied by the evolution of an equivalent amount of hydrogen, which is determined gas-chromatographically[118].

2. Determination of Amino-groups

Various modifications of the gas-chromatographic method for the determination of primary amino-groups in organic compounds, based on the well-known deamination reaction first used for the quantitative determination of amines by Van-Slyke[124], have now been proposed[119–123]. The nitrogen evolved as a result of the reaction can be readily separated from the accompanying gases in a column with 5 Å molecular sieve for routine analyses, reaction systems[122,123] are preferable in which provision is made for the stirring of the solution during the deamination reaction, a bubbler-type reactor is used (which guarantees freedom from errors associated with the solubility of nitrogen in the reaction mixture), and provision is also made for the absorption of nitrogen oxides which corrode the metallic components of the chromatograph. The duration of analysis does not usually exceed 15 min, the error is not more than ±0.3–0.4%, and the sensitivity of the determination reaches 0.07 mg of amino-nitrogen.

A modification of the method[125], in which solid sodium nitrite deposited on Celite C-22 is suggested instead of the nitrite solution normally employed, is of interest. In addition an internal standard (THF) is introduced into the reaction solution, which obviates the necessity of daily calibration of the apparatus.

The reaction with acrylonitrile has been suggested for the determination of amino-groups[126]; the excess of acrylonitrile which has failed to react is determined gas-chromatographically on a column with poly(ethylene glycol)-300 at 100°C. A similar method, also based on determination of an excess of the reagent which has failed to react, has been proposed for the analysis of relatively involatile primary amines by reaction with benzaldehyde[127]. All the characteristics of the method for the determination of amino-groups with a final gas-chromatographic step are superior to those of the classical Van-Slyke method. An important advantage of the former is also that it fully eliminates errors associated with the presence in the gas mixture of nitrogen oxides and other gaseous substances[128–130] which are not absorbed by an alkaline potassium permanganate solution and which constitute a potential cause of unduly high results.

3. Determination of Alkoxy-groups

The determination of alkoxy-groups in the form of the corresponding alkyl iodides, which are formed by the Zeisel reaction[131] when the compound analysed is refluxed in hydriodic acid, constitutes the basis of almost all gas-chromatographic methods. The classical chemical analysis of alkoxy-compounds is one of the most time-consuming and laborious methods in functional group analysis and yields only the overall content of alkoxy-groups. The ease of gas-chromatographic separation and quantitative determination of alkyl iodides (for example, the separation of 24 C_1–C_{10} alkyl iodides has been described[132]) from the very beginning indicated the likely usefulness of chromatography in analysis, which has been reflected also in the number of publications on this problem[133–147]. In early investigations the alkyl iodides were usually subjected to intermediate concentration in cooled traps with the liquid solvent (n-heptane) and as early as 1961 the contents of C_1–C_4 alkoxy-groups were determined by this procedure[137]. Silica gel[139,141], kieselguhr[140], and Apiezon L on silica gel[143] were employed to concentrate the mixture using high-temperature desorption at 150–170°C. Ehrenberger[140] was able to determine as many as five C_1–C_4 alkoxy-groups present simultaneously. To improve the accuracy of the analysis, the gas-chromatographic separation was employed together with gravimetric[139] and iodometric[140] determination of the amount of alkyl iodide, the accuracy of determination improving to ±0.05 abs.%. The overall duration of analysis is determined by the duration of the reaction with hydriodic acid and ranges from 1 to 3 h.

In 1968 Chumachenko and Kolesnik[148] showed that, in the reaction of organic substances with hydriodic acid in a hermetically sealed tube at 130°C, alkyl iodides are quantitatively split off in the course of 5–10 min, which made it possible to develop methods for the rapid determination of methoxy-[145] and ethoxy-groups[146] in organic compounds. The apparatus is illustrated schematically in Fig. 2. The sample of the substance (1–2 mg) was placed in a 10 × 0.8 cm test tube, 0.3 ml of hydriodic acid (or a mixture of 50 mg of potassium iodide and 0.05 ml of orthophosphoric acid) was poured in, the tube was closed, and kept at 130°C for 10–15 min in the determination of ethoxy-groups or 5 to 12 min in the case of methoxy-groups. The stream of nitrogen was then switched to flush the reactor and the alkyl iodides split off were transferred by the carrier gas to a column with Polysorb-1, where they were separated. Absorption tubes with potassium carbonate and calcium chloride were used to absorb moisture and the acid vapour. The entire apparatus was made of glass and Teflon. The overall duration of the analysis was 15–20 min or 25–30 min in the determination of methoxy- and ethoxy-groups respectively and the accuracy was at least to within 0.2 abs.%. The determination of the relatively volatile methyl and ethyl iodides was possible when these were transferred directly by the carrier gas from the reactor to the chromatographic

column, but in the analysis of C_3-C_5 alkoxy-groups intermediate concentration will probably be a necessary step.

The method for the determination of alkoxy-groups in carboxylic acid esters which are difficult to hydrolysed, based on their alkaline fusion at 240–360°C in the presence of sodium alkoxide, merits consideration[149]. The alcohols evolved in this process are trapped in a cooled trap and then analysed gas-chromatographically. Fig. 3 presents a chromatogram for the separation of the alcohols formed in the analysis of a mixture of polychloroacrylates. Calculation showed that the reaction proceeds with quantitative liberation of the alcohols from the corresponding esters. The alkaline fusion method has been used also to determine the alkoxy-groups in polysiloxane[150].

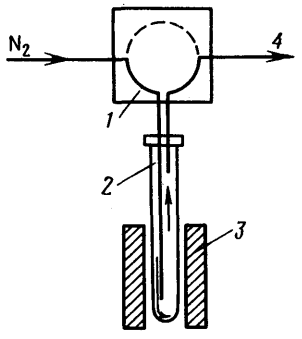


Figure 2. Apparatus for the determination of alkoxy-groups[148]: *1*) diaphragm valve; *2*) reactor; *3*) heater; *4*) outlet to chromatographic column.

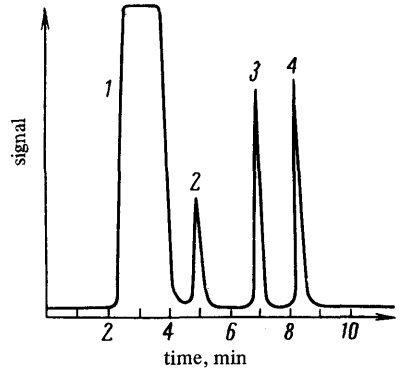


Figure 3. Chromatogram of the products of the alkaline fusion of poly(methyl α-chloroacrylate), poly(ethyl α-chloroacrylate), and poly(isopropyl α-chloracrylate)[149]: *1*) water; *2*) methanol; *3*) ethanol; *4*) isopropyl alcohol.

A method has been described for the determination of ethoxy-groups by oxidising the specimen with CrO_3 and subsequent analysis of an aliquot part of the reaction solution for acetic acid[151].

Among other methods, mention should be made of the determination of hydroxy-ethyl groups in the form of acetanilide, formed quantitatively from the initial specimen on pyrolysis carried out at 400°C for 10 min[152], and also the determination of alkoxy-groups by a hydrogenation method[141]. The latter procedure is particularly attractive, since the alkoxy-groups are eliminated in a flow reactor, which significantly reduces the duration of the determination and the saturated hydrocarbons formed on hydrogenation can be extremely conveniently separated and detected. The substance analysed enter a hydrogenation tube containing 2.0–2.5 ml of the catalyst (5% of platinum on kieselguhr or 1.5% of platinum on porous glass) and heated to 325°C. On hydrogenation, the alkoxy-groups are split off in the form of hydrocarbons, which either enter a 5 cm column with activated charcoal at 20°C (determination of C_1-C_2 alkoxy-groups) or a 50 cm column with silica gel (determination of C_3-C_5 alkoxy-groups).

It is noteworthy that, despite their inadequate reproducibility, the gas-chromatographic methods for the determination of alkoxy-groups are fairly widely used nowadays and in the immediate future may completely replace classical chemical analytical methods.

4. Determination of *S*-, *N*-, and *Si*-Alkyl Groups

The rupture of C–N, C–S, and C–Si bonds is many times more difficult than the rupture of the C–O bond. Therefore much more severe conditions than those described above are used for the determination of the corresponding alkyls. At the same time the different reactivities of the alkyls bound to S, N, and Si atoms can be used for their separate determination. Thus a method has been proposed for the quantitative determination of alkoxy- and *N*-alkyl groups present together[153]. In the first stage the alkoxy-groups are split off and determined under conditions analogous to those already described, after which the reactor is heated to 300°C (20 min) and then to 350°C (10 min). Under these conditions, the N–C bonds are broken and the resulting alkyl iodides are determined gas-chromatographically with intermediate concentration at a low-temperature.

The dissociation of C–S bonds in organic sulphides can be achieved with the aid of a suspension of Raney nickel in ethyl, n-butyl, or cyclohexyl alcohol[154]. The reaction is carried out in a simple microreactor (Fig. 4) consisting of a test-tube with two syringes for the injection of the reactants and for the removal of the gaseous reaction products. The corresponding hydrocarbons formed from the alkyl groups are separated and identified in a gas chromatograph connected to the reaction cell.

A similar reaction system was used also to determine alkyl groups linked to a silicon atom[155,156]. In this case the reaction with concentrated sulphuric acid in the presence of a vanadium catalyst was employed. Heating of the organosilicon compound being analysed to 175°C for 15 to 20 min in the above medium leads to the elimination of alkyl groups linked to silicon in the form of the corresponding hydrocarbons (methane from methyl groups, benzene from phenyl groups, etc.), the gas-chromatographic separation and identification of which presents no difficulty. Ethyl and phenyl groups can also be determined quantitatively by their thermal dissociation from the silicon atom with formation of ethane and benzene[157]. The reaction is carried out in the presence of phosphorus pentoxide in water, with the temperature raised from 20° to 500–580°C (depending on the group being determined).

A method has also been described for the determination of alkyl groups in aromatic compounds by their catalytic abstraction in a stream of hydrogen to yield saturated hydrocarbons[158–159].

It was shown recently[158] that the alkoxy-groups in organosilicon compounds can be identified by determining the products of the reaction of the substances analysed with boron tribromide. The alkoxy-groups are split off in the form of alcohols (which are then converted into unidentified compounds).

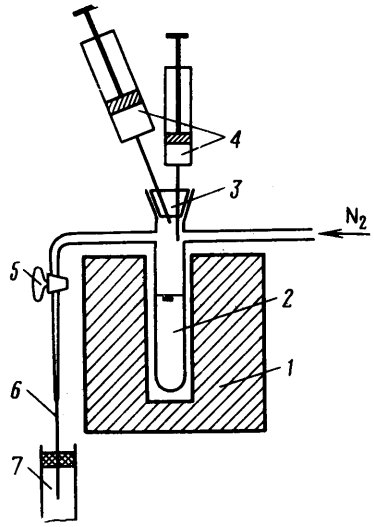


Figure 4. Schematic illustration of the microreactor[154]: *1*) heated block; *2*) glass reactor; *3*) rubber stopper; *4*) syringes; *5*) shut-off tap; *6*) needle for injection into the column; *7*) upper part of chromatographic column.

5. Determination of Acyl Groups

Virtually all the methods developed so far are based on the transesterification of the initial specimen with sulphuric acid, a mixture of methanol with hydrochloric or toluene-*p*-sulphonic acid, and a mixture of toluene-*p*-sulphonic acid and its methyl ester[160–165]. In the first gas-chromatographic method described[160] the simultaneous qualitative and quantitative determination of formyl and acetyl groups on a single sample was already achieved. The sample of the test substance, toluene-*p*-sulphonic acid, and methanol were introduced into the reactor and the reaction was carried out at 70–80°C for 30 min. The reaction products were then collected in a cooled trap and an aliquot part of the distillate was analysed. Methyl formate and methyl acetate were separated from one another and from methanol in a column with Carbowax-1500.

The gas-chromatographic separation of acyl groups up to butyryl and isobutyryl groups was described later[164,165]. The transesterification was carried out in a mixture of toluene-*p*-sulphonic acid and its methyl ester at 225–230°C for 20 min and the substances evolved were chromatographed on compound columns with poly(ethylene glycol adipate) and a T-1 emulsifier by passing the carrier gas through the reactor. In this analytical method the accuracy, reproducibility, and sensitivity are naturally higher than in the analysis of only part of the solution. It was noted[164] that the yield of methyl acetate in the transesterification reaction is 90% and therefore an appropriate correction factor must be applied in the calculation.

Alkaline hydrolysis[162] with subsequent gas-chromatographic determination of acetic acid can also be used to identify the acetyl group.

6. Determination of Hydroxy-, Carboxy-, and Carbonyl Groups.

The quantitative determination of hydroxy-groups can be achieved by estimating the active hydrogen[166]. The methane evolved in the reaction between a hydroxyl-containing polymer and a Grignard reagent is determined with the aid of a column containing a 5 Å molecular sieve connected directly to a column filled with 10% of *N*-methylpyrrolidinone on alumina, in which methane is separated from the solvent. The authors showed that the gas-chromatographic method permits the determination of hydroxy-groups at concentrations so low that chemical methods are inapplicable.

A more specific method for the determination of primary and secondary alcohols is based on the reaction of the specimen being analysed with acrylonitrile and the determination of the excess of the latter by chromatography on a 3 m column filled with Chromosorb containing 10% of poly(ethylene glycol)-3000.[167]

The microdetermination of carboxy-groups can be achieved by decarboxylating organic acids by pyrolysis in helium in the presence of copper carbonate and quinoline, the specimen being heated for 45 min in a reactor at 225°C.[168] The carbon dioxide evolved is determined gas-chromatographically. To increase the productivity of the method, it is recommended that a system of three reactors, connected in parallel, be used.

The reaction of α-aminoacids with ninhydrin serves as the basis of a method for the determination of carboxy-groups in aminoacids from the amount of carbon dioxide evolved[169]. The reaction is carried out in a system consisting of an attachment to a standard chromatograph. The sample of the substance (3–5 mg), a buffer mixture at pH 4.7, and about 50 mg of ninhydrin are placed in the cooled reaction vessel, which is flushed with the carrier gas, whereupon the latter is disconnected and the system is heated to 100°C. When the reaction is complete (after 3 min), the reaction system is again connected to the chromatograph and the carbon dioxide evolved is determined on a column with AG-3 activated charcoal. The duration of analysis is 10–15 min and the standard deviation of the determination is ±0.39%.

The method for the determination of carboxy-groups by the pyrolysis of the specimen at 1000–1100°C in sulphur vapour is very interesting[170]. The carboxy-groups are then quantitatively split off in the form of carbon disulphide, which is determined using silica gel.

The pyrolytic method has been successfully applied also in the determination of carbonyl groups[171]. The analysis is based on the fact that in the pyrolysis of different ketones and methyl esters of aromatic acids dissolved in carbon tetrachloride in an atmosphere of argon, carbon monoxide is formed in an amount equivalent to the content of the group being determined. The pyrolysis is carried out in a reactor filled with crushed quartz at 800–860°C.

7. Determination of Sulphur-containing Groups

It is recommended that alkane- and arene-thiols be determined quantitatively by reaction with acrylonitrile and

subsequent determination of the excess of the latter which failed to react[167]. The reaction is carried out in a pyknometer for 1–2 min and the solution is analysed gas-chromatographically.

In the determination of sulphate and sulphite groups in organic substances, reductive hydrolysis in phosphoric acid in the presence of a tin(II) salt for 15 min at 300°C can be used[172]. A column with silica gel is employed to separate the hydrogen sulphide evolved from other gases.

It has been suggested that arenesulphonic acids and their salts be determined by alkaline fusion[173] and pyrolysis[174]. In the first case the process is carried out in apparatus (Fig. 5) consisting of a quartz tube part of which is inserted in a cylindrical furnace. Several milligrams of potassium hydroxide and sodium alkoxide are added to the test sample in a platinum boat. The fusion is carried out for 20 min, after which the boat is removed from the hot zone and placed under an outlet with a rubber membrane through which a saturated aqueous solution of maleic acid is injected into the boat from a syringe. The maleic acid displaced phenols from the melt. The boat is then again moved to the heated space and the volatile products are determined chromatographically.

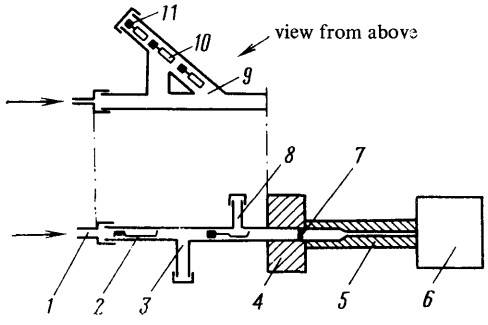


Figure 5. Attachment of alkaline fusion[173]: *1*) inlet for carrier gas; *2*) retraction device; *3*) pocket for the ejection of boats after analysis; *4*) furnace; *5*) heated connection to the chromatograph; *6*) gas chromatograph; *7*) device limiting the travel of boats; *8*) outlet with rubber membrane; *9*) outlet for boats with samples; *10*) platinum boat; *11*) device for moving boats.

Sulphur dioxide in an amount equivalent to the content of the sulpho-groups is evolved by the pyrolysis of arenesulphonic acids and their salts at 710–790°C in the presence of added silicic acid[174]. The pyrolysis products are separated on a column with Porapak Q, which also permits the simultaneous determination of the initial hydrocarbon formed in an amount up to 50% (Fig. 6). The method is particularly interesting, because in certain cases the addition of a hydrogenating agent (for example, a carbohydrazide) to the specimen undergoing pyrolysis increases the yield of the initial hydrocarbon to the theoretical value (thus the yield of benzene from benzenesulphonic acid was 98%), which in principle makes it possible to identify the specimen fully on the basis of a single sample.

8. Determination of Vinyl and Alkyl Groups

The vinyl groups in organosilicon polymers can be determined by the pyrolysis of the initial specimen and subsequent gas-chromatographic determination of the relative amounts of ethylene and methane in the volatile pyrolysis products[175].

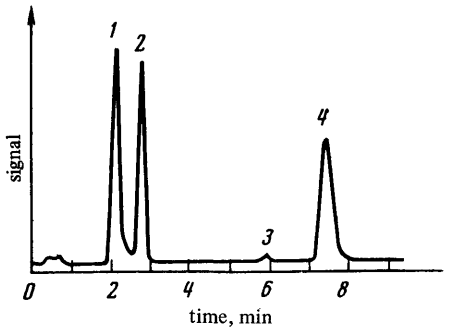


Figure 6. Chromatogram of the pyrolysis products of toluene-*p*-sulphonic acid[174]: *1*) water; *2*) sulphur dioxide; *3*) benzene; *4*) toluene.

The elimination of terminal vinyl groups in the form of ethylene when the specimen of the organosilicon polymer is treated with concentrated sulphuric acid also served as the basis for the development of an analytical method[176]. The sample is placed in a reactor, 450 μl of 90% sulphuric acid is poured in, the solution is heated in a stream of helium from 65° to 250°C (at a rate of 10 deg min^{-1}), and the decomposition products are trapped at low temperatures. When the reaction is complete (after approximately 1.5 h), the trapped volatile products are determined chromatographically. It was shown that vinyl groups are quantitatively converted into ethylene, ethyl groups into ethane, and methyl groups into methane.

Methods have been described for the identification of vinyl groups in the form of ethylene after alkaline hydrolysis[158] and alkaline fusion[150].

It has been suggested that alkyl groups be determined by the degradative decomposition of the test substance in a stream of hydrogen, which serves at the same time as the carrier gas, in the presence of a catalyst: a mixture of aluminium oxide silicate with quartz impregnated by tungsten and molybdenum oxides[177].

9. Determination of Unsaturated Bonds

Before the start of any determination of functional groups, it is important that the investigator knows about the presence and positions of unsaturated bonds for a correct choice of reaction conditions in order to avoid side processes, which frequently involve unsaturated bonds. We therefore feel it useful to consider, if only briefly, the main advances achieved in chromatographic methods in this field.

It is well known that the oxidation of substances containing multiple bonds by oxidising agents such as potassium permanganate and periodate, etc. results in the cleavage of the

molecule at the site of the bond with formation of two or more fragments (depending on the number of unsaturated bonds). Chromatography in all its versions is the most promising procedure for solving the problem of separation and identification of fragments which then arises and this has been reflected in a large number of published studies on chromatographic methods for the determination of the number and location of unsaturated bonds. It is noteworthy that in certain cases involving the formation of complex mixtures of oxidation products identification for the determination of the structure is frequently altogether impossible without a final chromatographic step.

The widely used methods involving oxidation with potassium permanganate in acetic acid[178], frequently resulting in too far reaching oxidation, and with the milder potassium permanganate–potassium periodate mixture[179] have been rapidly giving way recently to the ozonolysis method first proposed by Bon[180]. It is based on the formation of ozonides, their decomposition, and gas-chromatographic identification of the products.

Several types of apparatus, mainly of similar design[181–183], ensuring an ozone concentration of about 2.5–3.0%, have been developed. The usual sequence of steps is as follows[184–188]. The test substance (10–100 μg) in ethyl acetate or carbon disulphide (the use of a solvent for solids is not obligatory[186]) is placed in the reaction tube, the mixture is cooled to a temperature from -70° to 10–15°C (depending on the nature of the substance being analysed), and a stream of oxygen enriched with ozone is passed through the reactor until the initial concentration of ozone is restored at the outlet from the tube. The ozone is detected at the outlet with the aid of a solution of potassium iodide and starch in sulphuric acid. When the reaction is complete (after 0.5–5.0 min), nitrogen is bubbled through the solution and, after the decomposition of the resulting ozonides, the products are analysed gas-chromatographically. Depending on the structure and number of unsaturated bonds, the main products are formaldehyde, acetaldehyde, acetone (in the presence of isopropylidene groups), glyoxal (particularly from compounds with conjugated double bonds), carbon dioxide (in the presence of cumulative bonds), and other aldehydes and aldoesters.

The ozonides are decomposed either with the aid of a triphenylphosphine powder[185,189] or thermally[190,192]. A microreactor in which the specimen with a volume up to 5 μl may be ozonised, decomposed, and injected into the gas chromatograph without any transfer of the test substance and associated losses has been described for the latter case[186].

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In this review the authors deliberately did not consider the methods for the determination of the carbon skeleton of organic compounds, which constitute one of the branches of functional group analysis and significantly facilitate identification. The possibilities and achievements of gas chromatography in this field are very great and require special consideration. The determination of the carbon skeleton by hydrogenation has been described in detail in Beroza's review[192].

The survey of the principal achievements of gas chromatography in the qualitative identification of organic compounds and quantitative determination of functional groups leads to the conclusion that, by virtue of the sum total of its advantages, the application of gas chromatography has now resulted in radical changes in one of the most important branches of organic chemistry–functional group analysis, particularly in the study of multicomponent mixtures. We may note that the traditional chemical methods have not lost their value. On the contary, the greatest advances have been achieved precisely in those cases where gas chromatography is used in combination with traditional chemical methods.

It is noteworthy that the growth in the use of gas-chromatographic methods in analytical practice is now held back by the lack of a universal chromatographic apparatus with different reactors designed for functional group analysis with simultaneous employment of chemical and chromatographic procedures.

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