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Functional Microanalysis by Chromato-spectrometric Methods

Ya.L.Kostyukovskii and D.B.Melamed

Chemical reactions involving the most important functional groups and yielding easily detectable compounds are discussed. The possible development of a system of functional microanalysis of organic compounds on the basis of chemical reactions, thin-layer and column liquid chromatography, and spectrometric methods is considered.
The bibliography contains 296 references.

CONTENTS

I. Introduction 199
II. Foundations of reactive chromato-spectrometric analysis 199
III. Methods of identifying organic compounds through functional groups 200
IV. Conclusions 208

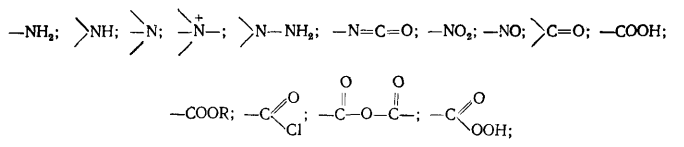
I. INTRODUCTION

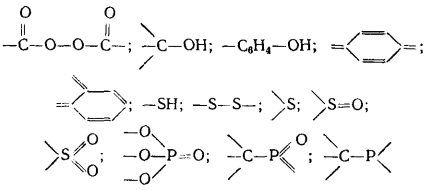
Analytical reactive chromatography includes chemical methods of preparing derivatives of the substances to be analysed, chromatographic separation, and detection of the derivatives. Reactive methods have greatly widened the scope of traditional chromatographic methods, and are now widely used in the analysis of organic compounds in the environment, in industrial and agricultural practice, in medicine, pharmacology, etc. Reactive chromatography is especially valuable for the determination of trace amounts of various substances in complex mixtures. It has also proved very successful as a method of functional analysis.

In recent years there have been two main trends in the development of organic functional analysis: the establishment of micro-methods of determining functional groups[1-3] and the application of these methods in conjunction with chromatographic separation.

Gas-chromatographic methods of functional analysis have been developed and reported most extensively.[4-6] On the other hand existing information on the combination of thin-layer chromatography (TLC) and column liquid chromatography with various methods of detection (in particular, spectrometric) has not yet been systematically reviewed, and the procedures have not been discussed as potential methods of functional analysis. The prospects of these methods are wider than those of gas-chromatographic methods, because they are applicable in principle not only to volatile compounds but also to involatile (and even to relatively unstable) compounds. The combination of chemical reactions (to produce easily detectable derivatives) and of the high resolving power offered by TLC and by column liquid chromatography, including detection by spectrometric methods, should provide an adequate foundation for a system of functional analysis of organic compounds in amounts of a few nanograms.

In this review we shall examine the chemical reactions used to prepare derivatives of the most important functional groups found in organic compounds:





and we shall generalise the most important results of recent work on the determination of the derivatives by a combination of TLC (or column liquid chromatography) and mass spectrometry, u.v. and visible spectrometry, and spectrofluorimetry.

II. FOUNDATIONS OF REACTIVE CHROMATO-SPECTROMETRIC ANALYSIS

Many chemical reactions have been used to convert the substances to be analysed into easily separable and detectable derivatives. Most of these studies have been directed towards reactive column chromatography. Their main outcome has been the identification of the optimum conditions for the synthesis of derivatives of organic compounds of various classes. The chemical reactions used for the functional analysis in conjunction with chromato-spectrometric methods can be applied either before the chromatographic separation (in order to convert the functional groups of the substances to be analysed into more easily separable and detectable derivatives), or after the separation (in order to reveal the functional groups).

The chosen chemical reactions should be selective towards the various functional groups, but in practice this requirement is not always met. Therefore in the analysis of complex samples a more reliable identification is obtained by using more than one reaction.

Two main schemes can be used for the analysis: 1) preparation of the derivatives, followed by chromatographic separation and spectrometric determination, and 2) chromatographic separation followed by chemical reaction and spectrometric determination. Depending on the chosen path, different requirements are specified for the reactions. In the analysis by the first scheme the derivatives are prepared by using reagents which give stable, easily separable, and

easily detectable products in almost quantitative yield.[7-10] Chromatospectrometric methods are often used with derivatives which absorb strongly in the u.v. and visible regions of the spectrum. Mass spectrometry, a powerful method of identification, can be used in principle to determine any derivative, but in practice the method is most successful with substances which form a large number of molecular ions and of characteristic fission ions.[11-15,19-21]

The derivatives are usually prepared outside the chromatographic assembly, but in some cases they have been prepared directly on the plate (for TLC) or in the liquid chromatograph, using fast reactions. Derivatives of multifunctional compounds are prepared by reactions at one or more groups: preferably the latter, since this improves the selectivity of the chromatospectrometric method. The preparation of derivatives from the substances to be analysed before carrying out the separation by liquid chromatography is often very effective, because it assists not only the separation and detection but also the interpretation of the results, thus greatly enhancing the reliability of the analysis.

In the second scheme the substance to be analysed is chromatographed first, and then the components are identified on plates or in eluates after being converted into easily detectable compounds by various chemical reactions. The choice of reactions for making visible the compounds on the TLC plates is relatively simple, but more stringent requirements apply to the reagents used in liquid chromatographs: the reaction must be fast, especially if used in a continuous process, and the excess of reagent should not interfere with the detection.[7,9,10] Many new models of liquid chromatographs are fitted with reactors for the preparation of easily detectable compounds.[7,9,10,16] The separation before the preparation of derivatives should be done under chromatographic conditions compatible with the properties of the functional groups of the substances to be analysed. Thus ion-exchange chromatography or reversed phase chromatography with ion pairing[7,10,17,18] are often used with acidic or basic compounds.

The most reliable method of identifying the separated components or their derivatives is mass spectrometry.[11,19-21] After separation of the mixture by TLC or column liquid chromatography the eluates of the various fractions are concentrated before being injected into the mass spectrometer. The more advanced systems offer a combination of a gas chromatograph, a mass spectrometer, and a computer. Some recently developed systems have even wider analytical capabilities, and include a liquid chromatograph, a mass spectrometer, and a computer.[11,20,22] However, so far these systems have been used only in development work, or to confirm the results of other analytical methods. More often the functional groups of the separated components are identified by u.v. spectrophotometry or spectrofluorimetry, or (in the case of TLC) by visual methods or with the help of a densitometer or other similar device.[7,9,10,18,23] In complex systems detection at two or more wavelengths has been recommended[24,25] as an alternative to recording the full absorption or fluorescence spectrum.

Chromato-spectrometric methods offer a very high sensitivity: the detection limit is 10^{-9}–10^{-12} g for mass spectrometers and fluorimeters (10^{-20} g if a laser is used), 10^{-8}–10^{-9} g for u.v. spectrophotometers, and 10^{-6}–10^{-7} g for visual assessment of the colour.

The quantitative determination of the identified substances is carried out by conventional chromatographic and spectrometric methods.[7,9,11,18,23,24,26,27]

The success of chromato-spectrometric methods of analysis is largely determined by the care taken in sample preparation, which is a difficult and critical stage of the process, especially in the determination of trace amounts of compounds in real samples from the environment, biological materials, food products, etc. This is because of the need to extract the trace impurities from a complex mixture in which the concentration of the wanted components is many times smaller than that of the other components.

A detailed discussion of methods of preparation and preliminary examination of samples for analysis is beyond the scope of the present review. Some relevant data are collected in Refs. 7 and 28–32. We shall only mention some of the more important details which we consider essential to a successful chromato-spectrometric analysis. An important aid to the extraction of substances for analysis is the use of mixed solvents of different polarity, and also of mixtures of counterions, which facilitate the removal of polar organic compounds. Reversed phase, ion-exchange, and gel permeation chromatography methods are also of interest.[7,9,11,28-30] Various forms of micro-distillation (especially under reduced pressure) from media at a suitable pH can also be used in the separation of volatile substances.[7,9,29,30,32] Extreme purification is usually laborious, and can lead to loss of material, for which allowance should be made when choosing a suitable method of analysis. By using chromato-spectrometric methods, involving a preliminary conversion of the compounds into their derivatives, the extract purification procedure can often be greatly simplified.[8-10,27,30] The reproducibility of the process can be improved by simultaneous analyses of experimental and of control samples prepared from the experimental samples by removing the compounds for analysis.[30,33]

A very important precaution is a preliminary study of the effect of acids, alkalis, oxidisers, and temperature on the substances for analysis, and also an elemental analysis and a determination of the spectral characteristics.[3,13,34] Often the compounds are available only in small quantities, insufficient for a detailed preliminary study. However, it is essential to obtain as much information as possible on the nature of the substances for analysis during the preparation and preliminary examination of the sample.

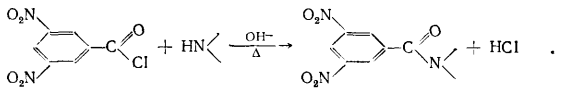
III. METHODS OF IDENTIFYING ORGANIC COMPOUNDS THROUGH FUNCTIONAL GROUPS

The success of the chromato-spectrometric methods of functional analysis depends on the quality of the chromatographic separation. In the determination of low-molecular-weight compounds in complex mixtures better results are obtained by chromatographing the derivatives. For example, in mass spectrometry the analysis of the derivatives is more effective than the analysis of the compounds themselves.[11-13,15,20,21] Many reagents for the various functional groups have been proposed: they all contain a fairly large group of aromatic or heterocyclic fragments which enhance the absorption of the molecule in visible and u.v. light or produce fluorescence. The "screened" functional groups can be identified after suitable chemical treatment (for example, hydrolysis). Compounds which are efficiently separated by TLC and column liquid chromatography methods are often analysed directly.

1. The $-NH_2$ and $>NH$ Groups

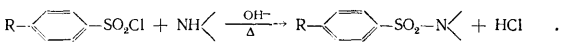
Many reagents and reactions for preparing derivatives of primary and secondary amines have been suggested. Acylation reactions are most frequently used, often at high temperatures and in the presence of catalysts (inorganic bases, tertiary amines, crown ethers, etc.). The most commonly used acylating agents are the acid halides of aromatic carboxylic acids and sulphonic acids.

Derivatives which absorb strongly in the u.v. are obtained by using 4-nitro-, 3,5-dinitro, 4-methoxy-, 4-dimethylamino-, 4-dimethylamino-3,5,-dinitrobenzoyl chlorides, and also 4-biphenyl-, 4-(4′-nitrophenylazo)benzene-, 4-(4′-dimethylaminophenylazo)benzenecarbonyl chlorides, etc.[7,8,10,14,35] The reactions follow the general scheme



Usually the yield is almost quantitative and the resulting amides are stable, easily extracted by organic solvents, and have an absorption coefficient in the u.v. region of about 14 000.[8,10,14] Mixtures of amides are easily separated by TLC or by column liquid chromatography. Many stationary phases and solvent systems have been used for these separations. Visual detection, and detection by mass spectrometry, can be used as well as u.v. spectrometry.[8,9,14,18,21,23,26,29,35,36] The sensitivity of detection of di- and polynitrobenzamides can be enhanced by treatment with alkali and acetone (Yanovskii reaction), which gives strongly coloured compounds.[38]

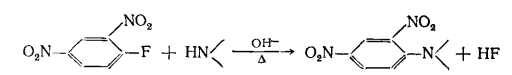
Derivatives which absorb strongly in the u.v. are obtained from the acid halides of aromatic sulphonic acids: 4-methyl-, 4-iodo-, 4-dimethylamino-, 4-(phenylazo)-, 4-(4′-*N*,*N*-dimethylaminophenylazo)benzenesulphonyl chloride, etc.[8,10,14,21] The reaction scheme is



Substituted sulphonamides are formed also by the reaction with trinitrobenzenesulphonic acid.[37,39] As a rule the sulphonamides absorb less strongly than the corresponding benzamides in the u.v.[10]

Compounds containing $-NH_2$ and NH groups form derivatives when treated with 1-dialkyl(C_1–C_5)aminonaphthalene-5-sulphonyl chlorides, *N*-methyl-2-anilinonaphthalene-6-sulphonyl chloride (mansyl chloride), 2-*p*-chlorosulphophenyl-3-phenylindone (disyl chloride), 4-(1-oxy-3-phenylinden-2-yl)benzenesulphonyl chloride, and 1,3-naphthoylenebenzimidazole-6-sulphonyl chloride.[7,10,14,27,40] Mixtures of sulphonamides are separated by TLC and column liquid chromatography methods, using a variety of stationary phases and eluents, and are detected by fluorescence, visual colour, u.v. absorption, and mass-spectrometrically.[14,18,26,27,36,41–43] Reactions with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) are widely used to identify primary and secondary amines, aminoacids (AA), aminophenols, and phenols.[10,14,27,44] We have shown that dansyl chloride can also be used to determine unsaturated amines.[44] The main advantage of dansylamides over other sulphonamides is the ease of separation and identification of the derivatives. Recommended conditions for the determination of dansylamides are given in Refs.12, 14, 18, 21, 27, 42, 43, and 45.

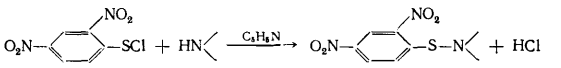
Other compounds including a relatively mobile halogen atom are also used to prepare derivatives, in particular 2,4-dinitrofluorobenzene, 2,4-dinitrochlorobenzene, 2,4-dinitro-5-fluoroaniline, 7-fluoro- and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride), and others.[8,10,14,15,33,46,47] 2,4-Dinitrofluorobenzene has been most widely used in practice, because it forms derivatives which absorb strongly in the u.v. and are easily separated under a variety of chromatographic conditions:



The analytical results have been confirmed by mass spectrometry.[8,14,26,37,48]

NBD chloride, which forms fluorescent NBD amides also absorbing strongly in the visible and u.v. spectra,[8,14,33,49,50] has been used almost as widely. Various amines and aminoacids give coloured derivatives (yellow, orange, or red) which can be used as an additional identifying criterion.[33,49,50] NBD–amine mixtures are easily separated by TLC and column liquid chromatography, and identified by fluorescence, colour, light absorption in the u.v., and mass spectrometry.[15,26,33,51–55] We have shown that NBD chloride is superior to dansyl chloride in a number of characteristics, including selectivity of the determination and stability of the derivatives.[27,55]

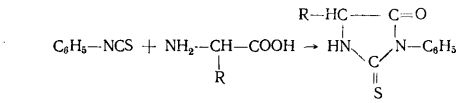
Another compound with a mobile halogen atom is 2,4-dinitrobenzoylsulphenyl chloride, which forms derivatives which absorb in the u.v.:



These derivatives are separated by TLC, for example on silica gel or Siluphol in the benzene–cyclohexane–chloroform–ethanol system.[35,36]

Other reagents have been suggested for preparing derivatives from $-NH_2$ and $>$NH groups, but they are used far less often.[8,57,58] They include 9-fluorenylmethylchloroformate, which gives fluorescent compounds, and *N*-(chloromethyl)phthalimide and *N*-(chloromethyl)isatin, which give products with a strong u.v. absorption.

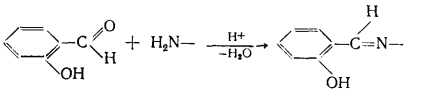
The large group of substituted iso- and isothiocyanates forms derivatives with amines. They include phenyl- and 4-(6-methylbenzothiazol-2-yl)phenyl isothiocyanates, phenyl-, naphthyl-, and 4-phenylazobenzoyl thiocyanates, etc.[7,8,18,21,34,35] These are most commonly used to identify aminoacids by forming thiohydantoins:



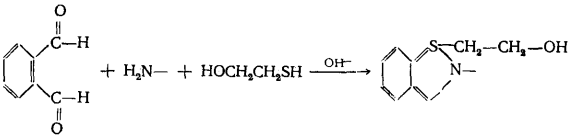
These compounds are readily separated by TLC and column liquid chromatography methods, and determined by u.v. absorption. Analytical results have been confirmed mass spectrometrically.[10,26,59–61]

Amino groups can also be identified by the formation of fluorescent compounds with fluorescein-, 9-acridyl-, and 4-dimethylamino-1-naphthalyl isothiocyanates or with 4-(6-methylbenzothiazole-2-yl)phenyl isocyanate or 2-(4-isothiocyanatophenyl)-3-phenylindone.[7,10,14,27,62]

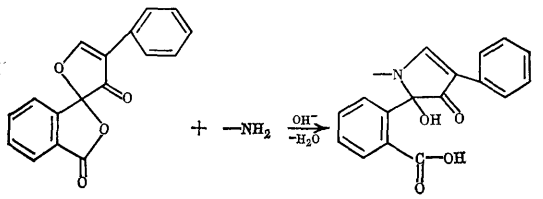
Amines (especially the primary) react vigorously with reagents containing a carbonyl group, for example with 4-dimethylamino- and 2,4-dinitrobenzaldehydes, or with salicylaldehyde:[8,35,38]



The resulting azomethynes react with diphenylboric anhydride to give complexes easily separated by TLC.[10,63] *o*-Phthalic aldehyde (*o*-PA) has been used frequently: in the presence of some thio-compounds (for example, monothioglycol) it forms fluorescent cyclic compounds which can be separated chromatographically:[10,27,51,64–67]



The identification of primary amino groups and AA with 4-phenyl-*spiro* (furan-2,3H-1-phthalan)-3,3'-dione (fluram)[10,14,27,35,68,69] is also of interest:



The resulting, strongly fluorescent compounds are easily separated by TLC or column liquid chromatography.[10,14,27,35,68,69]

Fluram and *o*-PA can be used as reagents for the derivatisation of secondary amines after conversion into primary amines, for example by treatment with NaOCl and 2,2'-thiodiglycol.†

Chromato-spectrometric methods of functional analysis can be applied to compounds in which $-NH_2$ or $>NH$ groups can be produced by appropriate chemical reactions. They include pesticides, therapeutic products, and toxic and carcinogenic compounds.[7,9,10,18,27,30,54] For example, methods of converting carcinogenic nitrosamines into the corresponding secondary amines and identifying the latter as dansyl amides or NBD amides have been developed.[33,42,49,55] These derivatives have been used in the detection of nitrosamines in the environment by combined chromatographic and mass spectrometric analysis.[15,45,55,70]

Chromatographic separation before derivatisation is preferred in the functional analysis of AA and aromatic or heterocyclic amines. TLC of aminoacids is usually carried out on silica gel, aluminium oxide, cellulose, polyamides, or ion-exchange resins (for example, Fixion or Dowex) in various systems containing phenols, organic acids, and ammonia.[23,26,50,71-76] Some aliphatic amines and AA are well separated by high-performance TLC methods on chemically bonded phases of the RP-18 type.[73,77,78]

Aromatic amines are separated on a thin layer of silica gel, alumina, polyamide, cellulose, and also on ion-exchange resins and on silica gel impregnated with phenols, oxalates, acetates, sulphates, chlorides, and surface-active substances, using different solvent systems.[23,26,71-74,79,80] A study of the dependence of the chromatographic mobility of amines on their structure has been reported.[26,79]

Various reagents have been suggested for the visualisation of compounds containing $-NH_2$ and $>NH$ groups, both universal (iodine, $KMnO_4$, $K_2Cr_2O_7$, phosphomolybdic and phosphotungstic acids, H_2SO_4, $SbCl_3$, $SbCl_5$, pH indicators, etc.) and relatively specific in action. The latter include solutions of ninhydrin, fluram, *o*-PA, NBD chloride, $Ca(OCl)_2$ in conjunction with KI, a mixture of acetylacetone and formaldehyde, etc.[14,26,27,53,73-75,78]

Aromatic amines can also be determined by the following reactions: coupling with diazotised sulphanilic acid, 4-nitroaniline, etc., diazotisation and coupling with phenols (say), or treatment with 4-dimethylaminobenzoic and cinnamaldehyde. U.v. spectrophotometry is used for detection.[18,26,38,71,76,81] Some aromatic and heterocyclic amines show spontaneous fluorescence.[53,82]

Ion-exchange liquid chromatography has been used most commonly in the separation of AA, but also (on a few occasions) for aliphatic amines. The great importance of AA in biology has stimulated the development of automatic and semi-automatic AA analysers, which can be used also to determine some other amino compounds (mono-, di-, and polyamines#). The most commonly used stationary phases include Amberlites, Apinexes, Biorex, Sephadexes, Partisil SCX, etc.; various buffer systems are used as eluents. The eluates are treated with ninhydrin, *o*-PA, fluram, NBD chloride, or other developers, and their colour is noted visually or measured spectrophotometrically or spectrofluorimetrically.[9,10,27,83-85] The high-performance liquid chromatography method (usually in the reversed-phase mode) is now often used in the analysis of AA and aliphatic amines on Lichrosorb RP-18, Permaphase ODS, Sorbax C_8 or ODS, μ-Bondapak C_{18}, and other stationary phases with aqueous-alcoholic mixtures often containing counter-ions: the addition of counter-ions has been shown to improve the separation of AA.[10,17,18,76,85-90] The separated compounds are usually derivatised with ninhydrin or *o*-PA, and the resulting derivatives are determined using u.v. or fluorescence detectors.§

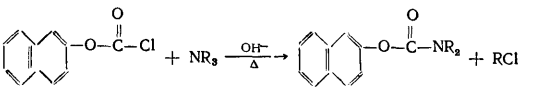
Extensive studies of the separation of aromatic amines by high-performance liquid chromatography have been reported. Various stationary phases and eluents have been proposed, and u.v. detectors have been used to identify the amines.[7,10,81,91-94] Mass spectrometric methods have also been used for detecting aromatic amines after their separation.[13,20,95,96]

Amides are usually analysed after being converted into the corresponding amines. In principle the formation of hydroxamic acids could be used to detect the amides after separation.[23,38]

The very large number of chemical reactions proposed for the derivatisation of primary and secondary amines allows an easy choice of the best variant for the separation of particular systems. It is often convenient to prepare fluorescent derivatives and separate them by TLC: a simple method, which offers a high sensitivity in the identification of these functional groups.

2. The N and $>\overset{+}{N}$ GROUPS

The chromato-spectrometric analysis of these groups has not yet been adequately developed. Some tertiary amines have been determined through their derivatives formed by the reaction with pentafluorobenzyl- or 2-naphthylchloroformate:[10,97]



A likely intermediate step in this reaction is the dealkylation of the amines: if this is the case, the reaction can be used also to prepare a series of derivatives from secondary amines.

Tertiary amines are separated by TLC on silica gel, aluminium oxide, or other sorbents. The solvent systems usually contain ammonia and dichloroethane. The separated compounds are made visible by the Dragendorf reagent, K_2PtCl_6, chloranil, tetranitromethane, tetracyanoquinonedimethane, tetracyanoethylene, etc.[2,3,14,26,98,99] With tetracyanoethylene the reaction is



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†see A.Himuro, Analyt.Chim.Acta, 1983, **147**, 317.

#see N.Sauem and E.Ronald, J.Chromatogr., 1983, **256**, 313.

§see R.Cunico and T.Schlabach, J.Chromatogr., 1983, **266**, 461; R.Simpson, J.Chromatogr., 1983, **261**, 407.

The separation of tertiary amines by high-performance liquid chromatography relies on the use of mobile phases containing counter-ions, for example 9,10-dimethoxyanthracene-2-sulphonate or acridone-2-sulphonate.[9,10,18,100,101]

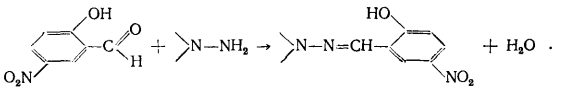
Compounds containing the $\geqslant\overset{+}{N}-$ group are separated by TLC on silica gel, aluminium oxide, or cellulose, with mobile phases usually containing hydrochloric, acetic, citric, or other acids. Visualisation is carried out by treating the plate with Dragendorf's reagent or with K_2PtCl_6.[3,23,102-104]

Quaternary ammonium compounds are chromatographed on ion-exchange resins, for example Partisil SCX, Amberlite CG-50, or Zeokart 226, with eluents containing counter-ions of the naphthalene- or anthracene-2-sulphonate type.[9,85,105-107]

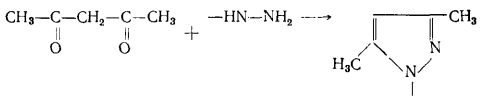
Efficient separation of these substances, including long-chain compounds, is achieved by the high-performance liquid chromatography method on Nucleosil-5 $N(CH_3)_2$, using the hexane–chloroform–ethyl acetate system as the solvent, or on Nucleosil CN with an aqueous solution of methanol containing *p*-toluenesulphonic acid.¶

3. The $>N-NH_2$ Group

Compounds containing this group (alkyl- and arylhydrazines, acid hydrazides, etc.) can be analysed chromatospectrometrically by preparing relatively stable and easily separable compounds. Thus, hydrazines react with 5-nitro-2-hydroxybenzaldehyde to give[8,10,21,38]



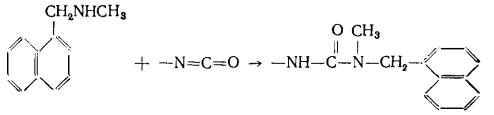
Alkylhydrazines react with acetylacetone to give pyrazolone derivatives:[8,10,21]



The fast reaction of the $>N-NH_2$ group with carbonyl compounds can be used to identify this group by using aldehydes and ketones. The hydrazines are usually separated (before derivatisation) by high-performance liquid chromatography on phases of the μ-Bondapak C_{18} or Aminex type, with various eluents.[18,108,109]

4. The $-N=C=O$ Group

As was said above, isocyanates are used to form derivatives with amines: the same reaction can be used to identify the isocyanates. 9-(*N*-methylaminomethyl)anthracene or *N*-methyl-1-naphthalenemethylamine are most often used:[110,111]



Other reagents for isocyanates include *N*-4-nitrobenzyl-*N*-alkylamines, aminophenols, 1-(2-pyridyl)- and 1-(2-methoxyphenyl)piperazines, which form derivatives with a strong u.v. absorption.[112-116]

Aromatic isocyanates react with alcohols by the scheme



The derivatives are easily separated chromatographically, and identified by u.v. spectrometry.[110,112-116]

The $-N=C=O$ group can be detected after separation (for example, by high-performance liquid chromatography on μ-Bondapak C_{18} or Lichrosorb RP-18, using mobile phases of aqueous acetonitrile or methanol): detection in this case is by u.v. or i.r. spectroscopy.[2,10,21]

Isocyanates (and also isothiocyanates) can also be identified chromato-spectrometrically after hydrolysis to the corresponding amines.

5. The $-NO_2$ and $-NO$ Groups

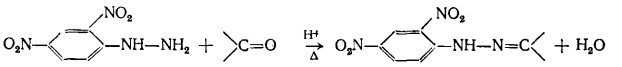
Chromato-spectrometric methods relying on a preliminary separation by TLC or high-performance TLC on RP-2 or RP-18 silica gel, acetylcellulose, etc. have been applied to the analysis of nitrocompounds. The solvent systems usually include methanol, ethanol, and acetonitrile.[26,117,120] The presence of the NO_2 group causes a fairly strong u.v. absorption, which can be used for detecting the nitro compounds on chromatographic plates or in eluates.

Aromatic di- and polynitro compounds can be made visible by the Yanovskii reaction, or by treating them with *N*,*N*-diethylaniline or Na_2SO_4.[117-119] Nitro compounds are separated, for example, by reversed phase liquid chromatography on Lichrosorb RP-18 or RP-8, using aqueous solutions of alcohol or of acetonitrile as the mobile phase. U.v. detectors are used for their identification.[91,120,121] Analytical results have been confirmed mass-spectrometrically.[122,123]

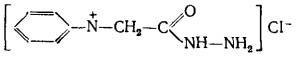
A few studies of the identification of the NO group by chromato-spectrometric methods have been reported. Various *N*-nitrosamines have been determined (after denitrosation) through the resulting nitrite ion and amino group.[33,42,55] Nitroso compounds can be converted into nitro compounds or into hydrazines by oxidising or reducing treatment respectively.

6. The $>C=O$ Group

Most of the work on the preparation of derivatives of carbonyl compounds has been on the reaction with hydrazines (in particular, phenylhydrazine and its derivatives):[8-10,21,35,124]



Similar reactions are obtained with azobenzene-4-carboxyhydrazine and with the Girard reagent:[10,35]



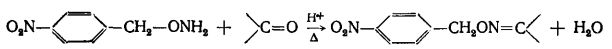
By using dansyl-, mansyl-, and NBD-hydrazines, 2-phenylacetyl-1,3-indandione-1-hydrazine, isonicotinylhydrazine, or other compounds, strongly fluorescent hydrazones have been obtained.[7,10,21,54,125,126] Silica gel, aluminium oxide, and cellulose can be used as sorbents for the TLC of hydrazones.[3,26,127,128] These compounds are detected by their colour, by fluorescence, or by u.v. absorption.[26,124,126-128] Hydrazones have also been separated by high-performance liquid chromatography and identified by u.v. or fluorescence detection.[10,

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¶see M.Kudon, J.Chromatogr., 1983, **261**, 293.

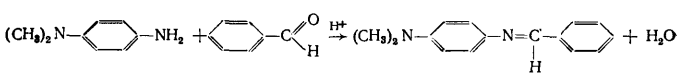
[126 129–133] Good results were obtained, for example, by using Sorbax ODS and a mixture of acetonitrile and water.†

Derivatives of carbonyl compounds which absorb in the u.v. are obtained by condensation with substituted hydroxylamines and semicarbazides, for example with 4-nitrobenzyloxyamine:[10, 13, 21]



The conditions for the chromatographic separation of oximes and semicarbazones have been discussed.[9, 21, 26, 35, 134, 135] The mass spectra of many hydrazones and oximes give a convenient identification of these compounds.[10, 21, 126, 134, 136]

Aldehydes react easily with primary amines, for example with *N*,*N*-dimethyl amino-1,4-, 1,3-, and 1,2-phenylenediamine, ethylenediamine, aminopiperidine, etc.[8, 35, 124, 137] These reagents are useful in practice for the functional analysis of aldehydes because the resulting Schiff's bases are strongly coloured and absorb in the u.v. region of the spectrum. Aromatic aldehydes react as follows:



α-Dicarbonyl compounds and α-keto-acids form fluorescent quinoxazolones with 1,2-phenylenediamine.[8, 138–140] Both the Schiff's bases and the quinoxazolones are easily separated by chromatographic methods.[35, 137–140] Aldehydes (unlike ketones) react with 5,5-dimethylhydroresorcinol (dimedon) to give coloured compounds which can be separated by TLC.[13, 35, 141, 142] The α-oxomethylene group is identified by the formation of fluorescent compounds by the reaction with *N*-methylnicotinamide chloride. The products are separated by thin-layer or column chromatography.[9, 10, 143] Aliphatic aldehydes form fluorescent lutidines by reacting with acetylacetone and primary amines.[26, 27, 53, 144]

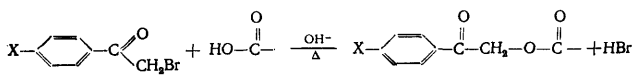
Substances containing a carbonyl group can be separated before derivatisation by TLC on silica gel, alumina, or cellulose, usually with a system of solvents of widely different polarities. Visualisation by universal or by relatively specific reagents is possible (for example, *o*-aminobiphenyl or other aromatic amines).[26, 32, 135, 145]

Good results have been obtained in the separation of carbonyl compounds by normal or reversed phase liquid chromatography on various stationary phases. Detection was by u.v. absorption or, after treating the eluent with *o*-phenylphenol, *N*-methylnicotinamide chloride, etc., by a fluorescence detector.[9, 131, 135, 143, 146, 147] The results have been confirmed mass spectrometrically.[11, 13, 136, 148]

Substances containing the >C=O group are often determined by derivatisation followed by detection in the u.v. or visually (by colour). Fluorescent derivatives are very seldom used in spite of the much higher sensitivity which they offer. This may be due to the difficulty of preparing the necessary reagents.

7. The –COOH and –COOR Groups

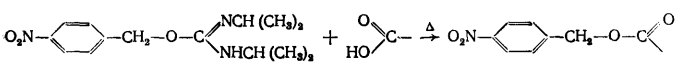
Derivatives of carboxylic acids are often prepared from phenacyl halides, for example 4-bromo-, 4-methoxy-, 4-nitro-, or 4-phenylazo-*N*,*N*-dimethylaminobenzeneazophenacyl bromides:



†see S.Slavin, J.Liq.Chromatogr., 1983, 6, 425.

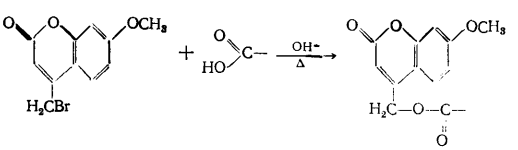
Phenacyl esters absorb strongly in the u.v. (absorption coefficient ~18 000),[7–9, 10, 35, 135, 149, 150] and naphthacyl esters, formed from compounds with a carboxylate group by the action of 2-naphthacyl bromide or 1-bromoacetylpyrene, are fluorescent.[10, 151, 152] TLC of these derivatives is carried out on silica gel (Lichrosorb RP-18) in various solvent systems (benzene–ethyl acetate, dimethylformamide–water, etc.).[26, 135, 150, 153] Separation is by liquid chromatography, for example in the reversed phase mode on μ-Bondapak C_{18}, μ-Porasil C_{18}, Lichrosorb RP-18, or Radialpak AC_{18} by the gradient elution method. The compounds are detected by mass spectrometry or by using fluorescence or u.v. detectors.[151, 152, 154–160]

An interesting method of identification of carboxylic acid groups by u.v. spectrophotometry relies on converting the acids (including long-chain acids) into benzyl or nitrobenzyl esters. The reaction is carried out by using the appropriate bromides, or 4-nitrobenzyl-3-*p*-tolyltriazine, or *p*-nitrobenzyl-*N*,*N*'-diisopropylisourea:[7, 8, 10, 135, 149]



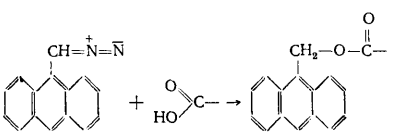
These esters are stable and easily separated by TLC or column chromatography on a variety of phases.[9, 18, 26, 135, 149]

Fluorescent esters, which are of interest for the identification of the carboxyl group, can be prepared by the reaction with chloromethylisatin or chloromethylanthracene, *N*-(chloromethyl)- or *N*-(chloromethyl-4-nitro)phthalimides, 4-bromomethyl-7,8-benzocoumarin, and 4-bromethyl-7-acetoxy- or 4-bromomethyl-7-methoxy coumarins.[8, 54, 58, 138, 161–166]



The conditions for separating these esters by various methods have been reported,[54, 162–168] including liquid chromatography on Partisil C_{18} or Nucleosil $10C_{18}$ with elution gradients in an aqueous methanol solution.

Fluorescent derivatives of acids are obtained also by the action of diphenyl-, 1-naphthyl-, 1-(4-biphenyl)-, or 9-anthryldiazomethanes:



The resulting compounds are separated by liquid chromatography (for example, in the reversed phase), using fluorescence or u.v. detection methods.[7, 169–172]

9-(Hydroxymethyl)anthracene, which forms fluorescent esters easily separable by chromatography, has been suggested[173] as a reagent for the carboxyl group.

The ability of carboxylic acids to react with amines (in particular, 1-naphthylamine, *p*-aminophenol, and 9,10-diaminophenanthrene) to give amides has also been used in practice.[174, 175] Separation was by liquid chromatography on μ-Bondapak C_{18} or Nucleosil C_{18}, with an aqueous solution of methanol as the eluent and fluorescence or u.v. detection. Compounds containing the –COOH and –COOR groups form

hydroxamic acids with hydroxylamine, which are easily separated and identified by their colorimetric reactions with tervalent iron.[1,2,26,38]

Derivatives obtained from polyfunctional compounds containing a carboxylic group have been discussed in many papers. Thus α-ketoacids react with 2,4-dinitrophenylhydrazine,[7-9,26,153,176] o-phenylenediamine,[8,10,138-140,177] and *N*-methylnicotinamide chloride.[9,10,143] Some hydroxyacids produce fluorescent compounds with resorcinol and 2-naphthol.[27,53,138]

Many publications discuss the chromatographic separation of substances containing −COOH and −COOR groups before derivatisation. Low-molecular-weight acids are separated by TLC on silica gel, alumina, polyamide, cellulose, starch, and other sorbents by using a system of solvents usually including alcohols, ethers, and bases (ammonia, diethylamine, etc.).[26,80,153,178-182] Long-chain acids are usually separated on silica gel impregnated with undecane or dodecane by using a system of solvents containing acetonitrile, dimethylformamide, and formic or acetic acid.[26,80,153] The thin-layer separation of esters of saturated carboxylic acids is carried out similarly.[26,153,180,183,184] Saturated acids are easily separated from unsaturated acids by TLC on silica gel impregnated with Ag^+.[18,26,182]

Silica gel or aluminium oxide with various solvent systems, often including methanol, formic and acetic acid, ammonia, etc. are used for the separation of aromatic acids.[26,80,185] The *p*-, *m*, and *o*-substituted aromatic acids are separated by TLC by using polyamide with aqueous solutions of α-cyclodextrins.[186]

Di- and polyacids are often separated on unmodified silica gel, but better results are obtained on silica gel or kieselguhr impregnated with poly(ethylene glycols), using solvent systems which include alcohols, tetrahydrofuran, formic or acetic acid, and ammonia.[3,26,153,188]

Many reagents have been proposed for determining the acids separated by TLC, but the universal reagents (iodine, pH indicators, phosphomolybdic acid, etc.) are still the most widely used.[26,153,178,180,184,185] Di- and polyacids form coloured complexes with Cu^{2+} salts, and fluorescent derivatives with dicyclohexylcarbodiimide.[187,188]

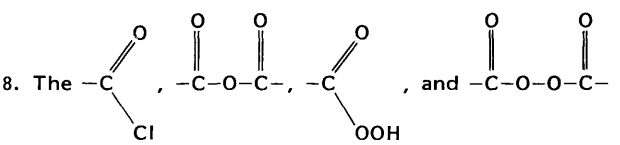
These acids are separated by reversed-phase (column) liquid chromatography, using counter-ions.[7,17,146,157,182,189-194] Other forms of chromatography have also been used (in particular, ion-exchange chromatography), and various analytical procedures have been recommended.[16,31,182,195-203]

The esters of saturated acids are usually separated by reversed-phase liquid chromatography, for example on Lichrosorb RP-18 or Bondapak C_{18}, using aqueous solutions of methanol or acetonitrile as the mobile phase.[9,18,182,194,204]

Unsaturated acids are chromatographed on Corasil P, Porasil A, Partisil 10 or 20, Spherisorb 5W, and other sorbents, containing 2–5% Ag^+. Silver nitrate is added also to the mobile phase by using aqueous organic solvents.[9,18,205-207]

The best conditions for the column liquid chromatography of polycarboxylic acids have been identified.[18,91,157,182,197,203] The visualisation of the acids after separation is usually straightforward: it relies on the absorption of the acids in the i.r. and near-u.v. regions of the spectrum, the absorption at the shorter wavelengths being the more effective for aromatic acids.[18,91,189,190,199,202,205,206,208] Some acids have been determined by treating the eluates with o-nitrophenol, Ce^{4+} salts, etc.[7-10,18,182] Complex samples have been successfully analysed by chromato-mass spectrometry, using column liquid chromatography for the separation stage.[11,13,136,148,209,210]

Thus both schemes of chromato-spectrometric analysis can be used to determine substances containing −COOH groups, depending on the properties of the sample.

8. The 

Groups

Anhydrides and acid chlorides are determined by the methods used for acids after converting them into the corresponding derivatives or into the acids. Some of these compounds form phenylmethane dyes when treated with *p*-dimethylaminobenzaldehyde and *N*,*N*-dimethylaniline on silica gel.[211]

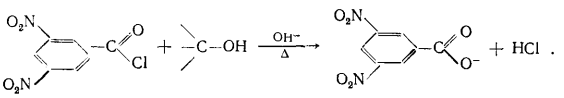
Hydroperoxides and peroxides can be identified after reduction to the acids. Chromato-spectrometric methods of analysis of these compounds have been proposed, even without derivatisation, by using normal or reversed-phase TLC in conjunction with detection by u.v. absorption.[18,26,212-215]

For example, the hydroperoxides of the linoleic acid isomers are well separated on Spherisorb Si 60 with the ethanol–hexane system.# The sensitivity of the analysis can be increased by adding sodium iodide or aromatic mono- and diamines to the eluent.[9,215,216]

The column liquid chromatography method has been applied[18] to hydroperoxides after converting them into ethers.

9. The ≥C–OH Group

The most extensive studies have been carried out on the reactions of alcohols with 4-methoxy-, 4-methylthio-, and 3,5-dinitrobenzoyl chlorides, by the scheme[2,7-10,32,35,135,217]

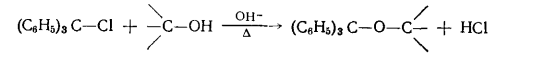


The resulting esters are more stable than the corresponding amides.

Coloured and strongly u.v.-absorbing esters (absorption coefficient ~14 000), suitable for the identification of the alcohols, are obtained by treating the sample with 4-(4′-nitrophenylazo)benzene- and 4-(4′-dimethylamino phenylazo)-benzenecarbonyl chlorides; separation is by TLC, usually on silica gel, and also on starch, cellulose, or talc, with various solvent systems.[26,32,218] Reversed-phase liquid chromatography on μ-Bondapak C_{18}, Lichrosorb RP-18, etc. has also been applied successfully to the separation of esters: elution was with aqueous solutions of alcohols, and detection by mass spectrometry or u.v. spectrophotometry.[7-10,135,219]

Sulphonyl chlorides (except 4-iodobenzenesulphonyl chloride and disyl chloride) are not often used to prepare alcohol derivatives. Disyl chloride forms strongly fluorescent esters.[9,21,40,220,221]

Alcohols also react with other compounds containing a relatively mobile halogen atom, for example with 2,4-dinitrofluorobenzene, 2,4-dinitrobenzenesulphene chloride, and triphenylchloromethane (trityl chloride).[2,35,135,222] In the latter case the reaction is

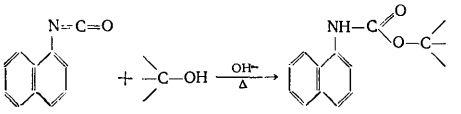


see J.Koskas, J.Chromatogr., 1983, **258**, 2880.

We note that ethers of this type, unlike the corresponding amine derivatives, are insoluble in acids.[10]

Fluorescent derivatives, suitable for identification, are formed by primary alcohols with 1-ethoxy-4-(dichloro-5-triazinyl)naphthalene, which also contains mobile halogen.[7,10]

Stable esters which absorb u.v. radiation or fluoresce according to the choice of reagent have been obtained by the reaction of alcohols with isocyanates. The preparation of these derivatives with phenyl-, *o*-nitrophenyl-, *p*-toluenesulpho-, 1-naphthylanthranyl-, and 4-(6-methylbenzothiazol-2-yl)phenylisocyanates has been reported:[2,13,35,62,223]



These esters can be separated by TLC on silica gel, MNSilG, Lichrosorb RP-18, and other sorbents, with different solvent systems.[26,62,223,224] Satisfactory separation is obtained by reversed-phase column liquid chromatography on Lichrosorb RP-18 and silica gel C_{18} with gradient elution by mixtures of acetonitrile and methanol with water; determination is by fluorescence or u.v. detection.[9,35,62,223] The separation of these esters by normal-phase chromatography has also been reported.[9,18,225] An interesting analytical scheme[3,226] relies on the reaction of C_1–C_6 alcohols with hydrogen sulphide and potassium hydroxide to give alkyl xanthates, which can be separated in a thin layer of microcellulose with a butanol–water–ammonia eluent and identified by u.v. spectrophotometry.

Compounds containing the –CH(OH)–CH(OH)– group are identified by the formation of coloured cyclic derivatives with aldehydes and fluorescent derivatives with naphthalene- and phenanthreneboric acids.[177,227]

Many aliphatic alcohols are separated chromatographically by TLC before derivatisation. The sorbents for the separation of the lower alcohols are silica gel, aluminium oxide, or cellulose, and for the higher alcohols silica gel or kieselguhr impregnated with paraffin oil. In the latter case aqueous-organic solvent systems are used.

Glycols and polyhydric alcohols are chromatographed on silica gel impregnated with ammonium borate or on magnesium silicate, aluminium oxide, etc.[3,26,153,178,228] Visualisation of these alcohols after separation by TLC or column liquid chromatography is usually by universal reagents.[3,26,135,178,228] Selective reagents have also been used, including sodium metavanadate and 8-hydroxyquinoline, which form coloured derivatives.[2,26,135]

Substances containing the $\geq$C–OH group have been separated by reversed-phase column liquid chromatography on Partisil-5 ODS, Sorbax ODS, etc., with aqueous-organic eluents, and identified by using u.v. detectors.[16,18,138,147,229,230] Equally good results were obtained by normal-phase, ion-exchange, and other forms of chromatography.[231,232] Sephadex LH-20 has been used to separate short-chain from long-chain alcohols.[219,231]

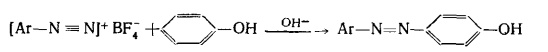
Polyhydric alcohols have been separated on Partisil A, Aminex A27, and other sorbents with various eluents, and identified by u.v. absorption or visually (by colour) after reaction with acidified $K_2Cr_2O_7$ solutions.[3,9,18,31] The determination of alcohols in eluates by a luminescence method with hydrogen peroxide and cobalt luminal has been reported.[233] In complex systems the presence of alcohols has been confirmed by mass spectrometry.[11,13,148]

Thus in the analysis of alcohols it is usually preferable to develop a colour or a derivative which absorbs in the u.v. before carrying out the chromatographic separation.

10. The $-C_6H_4-OH$ Groups

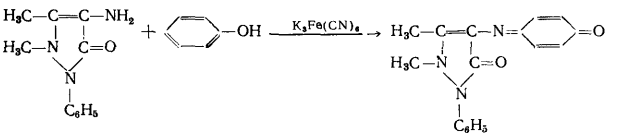
One of the most widely used methods of preparing derivatives of phenols and of alcohols is the reaction with substituted benzoyl chlorides, especially 4-nitro- and 3,5-dinitrobenzoyl chlorides.[2,9,10,18,32,35] Derivatives obtained by the reaction with the acid chlorides of sulphonic acids are also used to identify the phenyl groups. Thus 4-iodo- and 4-azobenzenesulphonyl chlorides give products which absorb in the u.v., while fluorescent products are obtained with dansyl and disyl chlorides.[7,9,27,35] Dansyl chloride is used most often, giving dansyl esters under conditions similar to those used in the preparation of dansylamides but at a higher pH. Polysubstituted derivatives are obtained from polyphenols and from aminophenols.[7,21,27,54,234] The products are separated either immediately after the reaction or after extraction and concentration by TLC or by high-performance TLC or column liquid chromatography, and are determined by u.v. absorption, fluorescence, or mass spectrometrically.[7,10,20,54,234] Chromato-spectrometric methods based on dansyl chloride are now widely applied in the analysis of various contaminants, therapeutic products, pesticides, and other compounds containing phenolic hydroxide or reacting to give phenols.[10,18,27,54,68]

Fluorescent or u.v.-absorbing derivatives recommended for determining phenols include the compounds with 2,4-dinitrofluoro- (or chloro-) benzene, *p*-(5-fluoro-2,4-dinitrophenylazo)-*N*,*N*-dimethylaniline, 1-ethoxy-4-(dichloro-5-triazinyl)-naphthalene, and others.[7–9,35,235] More specific derivatives, with u.v. absorption and colour, are formed by the reaction with diazonium salts:[2,3,38,236,237]



Diazonium salts prepared from 4-nitrobenzoic or sulphanilic acid or from 4-aminobenzonitrile are most often used in this reaction. The azophenols are efficiently separated by TLC on silica gel or by reversed- or normal-phase column chromatography (for example, on Lichrosorb RP-18 with an aqueous solution of methanol): detection is visual, by colour.[7,32,236–238]

Coloured quinoneimines are formed by the reaction of phenols with 4-aminoantipyrine:[2,3,32,38]



The separation of the quinoneimines is straightforward: either by TLC on silica gel or by reversed-phase of normal-phase column chromatography with photometric detection.[26,32,233,239] For example, complex mixtures of phenols can be separated as quinoneimines on μ-Bondapak C_{18} and on μ-Bondapak CN with an aqueous solution of methanol or a mixture of hexane and tetrahydrofuran as the mobile phase§.

The most interesting of the substances less frequently used for the determination of phenols is 1-nitroso-2-naphthol, which reacts with some substituted phenols to form fluorescent compounds easily separated chromatographically.[3,27,53]

The separation of phenol derivatives prepared directly on a chromatographic plate by treatment with a solution of Fast Blue BB has been reported.[240]

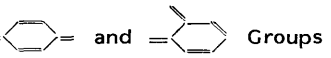
§ see G.Blo, J.Chromatogr., 1983, 257, 69.

Many papers describe separations of phenols before derivatisation. The TLC of these materials is carried out on silica gel, RP-18 silica gel, or silica gel impregnated with polyamines or with vaseline, surface-active substances, or $AgNO_3$, and also on polyamide, cellulose, volcanic tuff, Dowex 50W, etc. Mixtures of organic solvents, buffer systems, and solutions of α-cyclodextrin are used as the mobile phase.[26,72,241-246]

Polybasic phenols are separated on silica gel or aluminium oxide with eluents which usually include chloroform and methanol.[26,247-249] After separation the phenols are made visible by treatment with universal reagents.[26,54,135,244,247] Coloured or fluorescent compounds are formed by treating the phenols with solutions of Ce^{4+}, Fe^{3+}, or Th^{4+} salts.[3,38,135,241] Diazonium and antipyrine salts (with $K_3Fe(CN)_6$) are more specific for phenols.[2,3,7,26,246] Polybasic phenols can be detected by their reaction with monochloroamines.[2,3,38,248,249]

Phenols are more commonly separated before derivatisation by reversed-phase column liquid chromatography on chemically bonded stationary phases: μ-Bondapak C_{18}, Ultraspher C_{18}, Sorbax ODS, etc., with aqueous solutions of acetonitrile or methanol (often containing buffer systems, acetic acid, or counter-ions) as the eluent.[250-258] The phenols can also be separated by normal-phase chromatography on μ-Bondapak NH_2 or CN, μ-Porasil, Lichrosorb Si 60, Zipax CN, Sorbax Sil, and Sephasorb HP; the mobile phase is a mixture of organic solvents, including hexane, heptane, chloroform, isopropanol, etc.[18,23,252,257,258] The chromatographic separation is improved by impregnating the stationary phase with solutions of Fe(III) or of 8-hydroxyquinoline.[260] The phenols are identified by mass spectrometry, u.v. and i.r. spectrophotometry, or spectrofluorimetry.[9,13,20,239,252,256-258]

As in the case of acids, both schemes of chromato-spectrometric analysis can be used successfully for the determination of phenols.

11. The  Groups

Quinones can be detected by oxidation, using their reaction with 2,4-dinitrophenylhydrazine, aromatic amines, polybasic phenols, and cyanoacetic ester,[1,3,10,38] but these methods are seldom compatible with separation, possibly because of the ease of reduction to the corresponding phenols.

The reaction of benzo- and naphthaquinones with benzoylsulphinic acid produces coloured and fluorescent dihydroxydiphenyl- or dihydroxyphenylnaphthylsulphones, which can be separated by TLC on silica gel or Siluphol.[247,261]

Silica gel, silica gel impregnated with $AgNO_3$, polyamide, or other sorbents can be used to separate quinones before derivatisation.[26,262] Analysis by column liquid chromatography (usually in the reversed-phase mode) is carried out on Porasil C_{18} or on Permophase ODS, with aqueous-organic eluents and u.v. detection.[9,18,239,263]

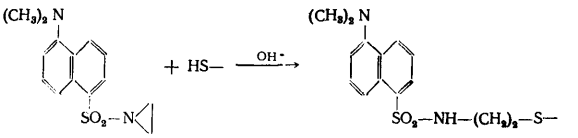
12. The −SH and −S−S− Groups

So far only a few derivatives compatible with the chromato-spectrometric determination of −SH compounds (or of compounds in which the −S−S− group has been reduced to −SH) have been reported.

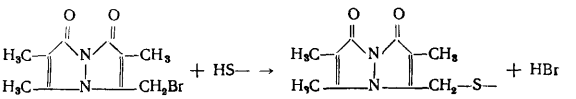
The interaction of thiols with S,S'-dithio-bis-(2-nitro)-benzoic acid at pH 8 produces coloured disulphides which can be separated, for example, by reversed-phase column liquid chromatography on Lichrosorb RP-18 with a methanolic phosphate buffer (pH 8.0); identification is by u.v. detection.[9,264]

2,4-Dinitrothioethers (produced by the reaction of thiols with 2,4-dinitrochlorobenzene) are easily separated by high-performance liquid chromatography on Lichrosorb RP-18 with an aqueous solution of acetonitrile. ¶

Thiols react with dansyl aziridene to give stable derivatives which are easily separated chromatographically:[8,265]



Monobromobimane reacts with thiols as follows:



The resulting compounds are fluorescent, and easily separable by chromatographic or electrophoretic methods.[266-268]

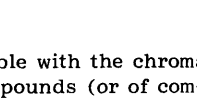
Fluorescent derivatives suitable for the analysis of thiols are obtained also from the reaction with 9-acridyl-, *N*-(*p*-2-benzimidazyl)phenyl-, and *N*-(7-dimethylamino-4-methylcoumarinyl)maleimides.[138,269,270] A new reagent for the preparation of fluorescent derivatives has recently been proposed: ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate, which reacts with the −SH group at ~60 °C and pH ~9.5. Like NBD chloride, to which it is structurally similar, this preparation is relatively stable and is itself non-fluorescent. The analytical conditions for the column liquid chromatography of these compounds are discussed in Ref. 271.

Many papers describe the separation of thiols and disulphides by TLC before derivatisation. Silica gel or mixtures of silica gel with palladium chloride, aluminium oxide, or cellulose are used as sorbents.[26,80,272-274]

Visualisation of the −SH and −S−S− groups on the plates or in the eluates is possible both with universal and with more specific reagents: the complex of dithiofluorescein with *o*-hydroxymercurybenzoic acid, mixtures of sodium azide and iodine, mixtures of 3,5-di-t-butyl-1,2-benzoquinone and ferric chloride, and mixtures of 6,6'-dihydroxy-2,2'-dinaphthyl disulphide, the Dragendorf reagent, hydrogen sulphide solutions, and potassium hydroxide in aqueous butanol.[26,274-277]

Substances containing the −SH and −S−S− groups can be separated by column liquid chromatography (especially the reversed-phase variant) on μ-Bondapak C_{18}, Spherisorb ODS, HC Pellosil ODS, and other sorbents, with aqueous-alcoholic solutions at controlled pH.[10,138,278,279] The electrochemical detector[10,278-280] is found to be useful in the identification of these compounds.

Reagents for detecting thiols (after separation) by the colour or the fluorescence of the resulting compounds are proposed also in Refs. 281–284. Thus a mixture of *o*-phthalic aldehyde and taurine or other primary amine is shown to produce fluorescent isoindoles.

13. The  Groups

Compounds containing these groups are analysed mainly by TLC methods, with separation before derivatisation, using both pure and impregnated silica gel or aluminium oxide and

¶ see H.Langenhove, J.Chromatogr., 1983, 257, 170.

various solvent systems.[26,80,272,273,285] Iodine, tetracyanoethylene, 2,3-dichloro-5,6-dicyano-1,3-benzoquinone, and other reagents are used for visualisation on the TLC plate.[26,80,273] If necessary, the analytical results are confirmed mass spectrometrically.[272,273,286]

14. The $-O-P(=O)(-O-)-O$ Group

Separation of alkyl and aryl phosphates before derivatisation is carried out by TLC on silica gel or cellulose with various eluents, including benzene, methanol, and chloroform, and visualisation is by hydrolysis to the PO_4^{3-} anion followed by reaction (for example) with ammonium molybdate or vanadate to give coloured compounds.[26,287,290]

The separation before derivatisation of compounds containing these groups by normal- and reversed-phase column liquid chromatography with u.v. detection has been reported.[10,291,292]

15. The $-C-P<$ and $-C-P(=O)<$ Groups

Only a few reports of the chromato-spectrometric identification of groups containing the C–P bond have been published. Direct separation of a series of phosphonic acids has been achieved by TLC, with visualisation by oxidation to the PO_4^{3-} anion and reaction with ammonium molybdate or other similar reagent. Column chromatography has also been used, with various detectors.[26,289,293,294]

Some results on the determination of these groups are quoted in discussions of analytical methods for organophosphorus insecticides and their degradation products.[10,18,138,295,296]

IV. CONCLUSIONS

The results examined in this review show that a large volume of experimental information on the functional microanalysis of organic compounds by a combination of chemical reactions, chromatographic separation, and spectrometric identification is now available. Chromato-spectrometric methods are of interest for the determination of very small amounts (10^{-9}–10^{-11} g) of organic substances of various types, and their potential performance in this respect is far superior to that of classical methods of functional analysis.

Relative assessments of the reagents used in chromato-spectrometric methods have not so far been compiled: this hinders the choice of the best reagent for the analysis of complex mixtures. We have therefore combined published data and experimental results obtained in our laboratory to produce a list of some of the characteristics of the most commonly used reagents, which are often known to be the optimum choice for specific chromato-spectrometric analyses (see Table). This tabulation should prove useful in the selection of reagents for the analysis of various types of material.

Reactive chromato-spectrometric methods have found practical applications in the micro-analysis of many organic substances for functional groups, but the scope of their exploitation is still far from reaching its full potential. An effort is needed to extend the area of practical application of chromato-spectrometric methods, preferably by simplifying the various stages and the automation procedures of the analysis.

Characteristics of some reagents used in chromato-spectrometric methods.

Reagent	Application: before separation	Application: after separation	Groups	Method of detection
3,5-Dinitro- or 4-nitrobenzoyl chloride	+	–	$-NH_2$; $>NH$, $>C-OH$; $-C_6H_4-OH$	Col., u.v., MS
Dansyl chloride	+	–	$-NH_2$; $>NH$; $-C_6H_4-OH$	U.v., fl., MS
2,4-Dinitrofluorobenzene	+	–	$-NH_2$; $>NH$; $-SH$; $>C-OH$; $-C_6H_4-OH$	Col., u.v., MS
NBD chloride	+	+	$-NH_2$; $>NH$	Col., u.v., fl MS
Phenyliso- or phenylisothiocyanate	+	–	$-NH_2$; $>NH$; $-C_6H_4-OH$; $>C-OH$	U.v., MS
o-Phthalic aldehyde	+	+	$-NH_2$	U.v., fl.
4-Phenyl-*spiro*(furan-2), 3H-1′-phthalan-3,3′-dione (fluram)	+	+	$-NH_2$	Fl., u.v.
Ninhydrin	–	+	$-NH_2$	Col., u.v.
Diazotised sulphanilic acid or 4-nitroaniline	+	+	$-C_6H_4-NH_2$; $-C_6H_4-OH$	Col., u.v.
Dragendorf reagent	–	+	$>N$; $>\overset{+}{N}-$	Col.
N-4-Nitrobenzyl-*N*-propylamine	+	–	$-N=C=O$	U.v.
Acetone and NaOH	–	+	$-C_6H_3(NO_2)_2$ and $-C_6H_2(NO_2)_3$	Col., u.v.
2,4-Dinitro- or 4-nitrophenylhydrazine	+	–	$>C=O$	Col., u.v., MS
Dansylhydrazine	+	–	$>C=O$	Fl., u.v., MS
4-Nitrobenzyloxyamine	+	–	$>C=O$	U.v., MS
N′-Methylnicotinamide chloride	+	+	$-CH_2-C(=O)<$	Fl.
Dimedon	+	–	$H-C(=O)<$	Col., fl.
N,*N*′-Dimethylamino-4-aniline	+	–	$H-C(=O)<$	Col.
4-Bromo- or 4-nitrophenacyl bromide	+	–	$-COOH$	U.v., MS
2-Naphthacyl bromide	+	–	$-COOH$	Fl., u.v., MS
p-Nitrobenzyl-*N*,*N*′-diisopropylisourea	+	–	$-COOH$	Col., u.v.
4-Bromomethyl-7-methoxycoumarin	+	–	$-COOH$	Fl., u.v.
1-Naphthyl- or 9-anthryldiazomethane	+	–	$-COOH$	Fl., u.v.
o-Phenylenedimaine	+	–	$-C(=O)-C(=O)-$	Fl., u.v.
Ce^{4+} salts	–	+	$-COOH$; $>C-OH$; $-C_6H_4-OH$	Fl.
Na vanadate and 8-hydroxyquinoline	–	+	$>C-OH$	Col.
4-Aminoantipyrine	+	+	$-C_6H_4-OH$	Col.
5,5′-Dithio-*bis*(2-nitro)benzoic acid	–	+	$-SH$	Col.
Tetracyanoethylene	–	+	$=S$; $=S=O$; $>S(=O)_2$; $>N$	Col., MS
Ammonium molybdate	–	+	$-O-P(=O)(-O-)-O$	Col.

Symbols denoting the methods: U.v., by absorption in the u.v. region of the spectrum; Fl., by fluorescence; MS, by mass spectrometry; Col., by visual colour.

Further work on the development of a rational system of functional micro-analysis of organic compounds by chromatospectrometric methods is needed. Substantial improvements in the sensitivity of the determination (in particular, by fluorimetric and mass spectrometric methods) should result from the use of analytical lasers.

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