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THE CHARACTERISATION OF ORGANIC FUNCTIONAL GROUPS IN GAS CHROMATOGRAPHIC ELUATES AT THE MICROGRAM AND SUBMICROGRAM LEVELS BY MEANS OF COLOUR REACTIONS

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SUMMARY

A procedure is described whereby unknown compounds eluted from a gas chromatograph may be trapped and subsequently identified by colorimetric functional group analysis at concentrations considerably lower than has previously been achieved. The basis of the method involves the trapping of gas chromatographic peaks on short lengths of glass porous layer open tubing containing a layer of activated aluminium oxide, followed by colour tests on the trapped bands. Detection limits, generally in the range 0.1–1 μg, have been determined for a range of compounds representing the more common functional moieties and the technique has also been applied to the analysis of a mixture.

INTRODUCTION

The identification of unknown components eluted from gas chromatographic (GC) columns by spectroscopic means such as IR, UV, NMR and especially MS for submicrogram quantities has been extensively practised in recent years. By contrast, relatively few workers have attempted to exploit the more classical methods of functional group analysis for the qualitative examination of GC eluates. Yet, although retention data alone are frequently of little use for identification purposes due to the fact that members of different homologous series may have identical retention characteristics, it has been shown by WALSH AND MERRITT[1] and by MERRITT[2] that, provided the functionality of an eluting component can be established, its identity may frequently be determined from the relationship between retention volume and carbon number. They carried out colour tests by bubbling the peaks eluted from a chromatographic column via a stream splitting device through several test solutions, each for a different functional group. Limits of detection in the range 20–100 μg were reported for a series of compounds containing the more common functional moieties. By employing a similar bubbling technique, others[3] have critically evaluated a range of colour tests to find the one most suitable for this kind of classification with particular reference to aldehydes[4] and ketones[5], and colour tests by this method have found application, mainly for confirmatory purposes, in the analysis of solvent mixtures[6].

Trapping onto a wetted support layer moving at the same speed as the chart recorder has also been used with colour reactions to monitor continuously the GC effluent[7].

One probable reason why colour reactions have not been more extensively exploited for qualitative analysis of GC peaks is the relatively high detection limits reported so far for most functional groups. In most problems which involve the analysis of complex mixtures of organic compounds by GC, as represented, for example, by concentrates of volatile compounds isolated from foodstuffs in aroma research, information is generally required on the nature of components present at the microgram level or lower. Sensitive colorimetric methods for detection of functional groups could advantageously complement data from other sources, *e.g.*, by assisting in the identification of the components of unseparated peaks in the combined GC–MS analysis of such mixtures. In the absence of combined GC–MS facilities, such methods would be invaluable.

In this paper a procedure is described which allows the detection of a range of the more common functional groups down to levels of 0.1–1 μg, and even as low as 50 ng for certain aldehydes, and aromatic compounds. The method involves the trapping of peaks emerging from the chromatographic column on layers of activated alumina in short lengths of glass porous layer open tubes (PLOT). The compounds are trapped in very sharp bands at the start of each tube, to which appropriate colour developing reagents are then applied. The trapping technique, which is especially suitable for use with the low carrier gas flow rates employed with PLOT analytical columns, also offers the advantage of being simple, and easy to manipulate, so that peaks eluted rapidly one after another can be successively adsorbed on fresh lengths of PLOT tubing. A number of colour tests may be applied to a single peak if desired by means of a simple splitting device which distributes the effluent gas equally between a number of alumina traps.

The general framework of colour tests previously reported[1] has been adopted with a few exceptions. It has been necessary in most cases to modify the reaction conditions, especially in respect of the concentrations of colour developing reagents, in order to adapt the tests to PLOT tubing, and to exploit the high sensitivities attainable by virtue of the compounds being trapped in sharp narrow bands. The limits of detection for a series of compounds representing the more common functional groups have been determined, and the advantages as well as the limitations of the relevant colour tests are discussed in some detail. The application of the technique to the qualitative analysis of a multicomponent mixture is also described.

EXPERIMENTAL

Apparatus and materials

A Pye Series 104 temperature-programmed flame ionisation chromatograph was used throughout. It was equipped with a heated injection head as well as a heated fraction collecting exit port, mounted on the side of the oven, which may be used to heat a stainless-steel capillary line in the transfer of the column effluent to the alumina traps. The effluent was split by means of a three-way 1.55 -mm O.D. stainless-steel coupling* between the flame ionisation detector (FID) and a 12-cm length

* Simplifix Couplings Ltd., Maidenhead, Berks. (Great Britain).

of stainless-steel capillary line (1.55-mm O.D. × 0.65-mm I.D.), which led through the wall of the oven and terminated in the external three-way stream splitting arrangement shown in Fig. 1. The stream splitting device was constructed from a short length (20 mm) of 6.25-mm diameter stainless-steel rod, which was drilled out to produce four convergent outlets of approximately 1.8-mm diameter, arranged spatially as shown in the figure. Three pieces of capillary tubing (1.55-mm O.D. × 0.65-mm I.D.) × 10 mm were silver soldered into three of the outlets to provide connection points for the PLOT alumina traps, while the transfer-capillary line from the column was soldered to the fourth outlet. The diameter of the stainless-steel block was then machined to a diameter of about 3.5 mm over a distance of 6 mm from the entry of the transfer capillary line. This enabled the outlet splitter to be slotted tightly into the outlet heater block and provided excellent thermal contact for the splitter. A removable polytetrafluoroethylene (PTFE) ferrule was used to connect the transfer capillary line to the Simplifix connector within the oven; this enabled the outlet splitter to be easily connected to and withdrawn from the system as desired.

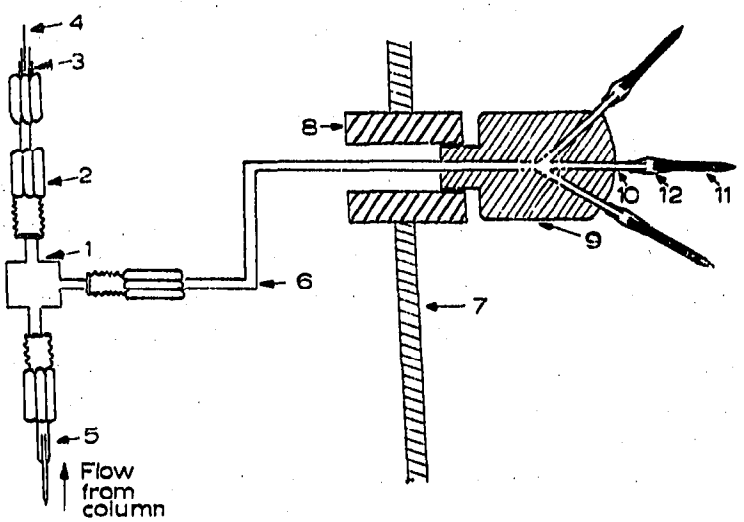

Fig. 1. Schematic representation of internal column effluent splitter connected to external three-way stream splitter and PLOT alumina traps. 1 = 1.55-mm O.D. Simplifix three-way connector; 2 = connecting nut; 3 = connection to FID; 4 = restricting wire; 5 = hypodermic connection to column; 6 = transfer capillary line (1.55-mm O.D. × 0.65-mm I.D.); 7 = oven wall; 8 = outlet heater; 9 = drilled stainless-steel stream splitter block (6.25-mm diameter); 10 = 10-mm length of stainless steel capillary tubing (1.55-mm O.D. × 0.65-mm I.D.); 11 = PLOT capillary trap; 12 = silicone rubber (bore 0.5 mm) connecting tube.

Preparation of PLOT alumina traps

Straight lengths of glass PLOT tubing (0.5-mm O.D. × 2 m) containing an even, stable layer of alumina were prepared using a glass capillary drawing machine according to a method described recently for the preparation of glass PLOT analytical columns[8]. The latter contain a layer of Celite tightly bound to the capillary wall using fused powdered B-37 glass* as the binding agent. Aluminium oxide (B.D.H. laboratory chromatographic reagent) was treated with concentrated hydro-

* Supplied by: Glass Tubes and Components Ltd., Lemington, Newcastle-upon-Tyne, Great Britain.

chloric acid to remove heavy metals present. It was then washed with distilled water until the washings were neutral, and dried at 100°. The material was sieved and the 150–200 mesh fraction mixed with finely powdered B-37 glass sieved to smaller than 200 mesh, in the proportion by weight of 4 parts alumina to 1 part glass. The mixture was heated at 200° for 2 h prior to preparation of the tubing so as to remove all traces of water. After preparation the ends of each length of tube were sealed off in a microburner flame to prevent contamination of the tubing and to maintain the alumina in an activated state.

Trapping procedure

Short lengths of 30–40 mm of tubing were used as traps. One end of each trap was sealed off in the flame of a microburner and the other end, after gentle annealing in a flame to remove sharp edges, was connected to each of the outlet tubes of the three-way stream splitter, by means of a short length (15 mm) of narrow bore (0.5 mm) silicone rubber tubing. Except when it was intended to perform a number of colour tests on a given peak, two of the traps remained permanently blanked off, while the third was continually replaced by a fresh tube as each peak of interest emerged. This procedure was followed in all work where limits of detection were being measured.

The method of trapping peaks was as follows. With an unsealed alumina trap in position, the flow from the column was split in the ratio of 9:1 in favour of the trap by restricting the narrow capillary tube leading to the FID with a length of thin stainless-steel wire (see Fig. 1). By replacing the open capillary with a blanked off tube, the entire column effluent was diverted to the FID. The sample was injected onto the column under these conditions, and as the leading edge of the peak of interest just appeared on the recorder chart, the sealed end of the capillary trap was quickly snapped off with a small pliers thereby diverting 90% of the component to the trap. Once the peak had passed, another sealed trap was inserted in readiness for the next peak to be trapped. In order to avoid leaks after repeated changing of traps, it was found desirable to routinely replace the silicone rubber connecting tube after about ten changes of trap.

In evaluating the effectiveness of the trapping system and the detection limits attainable, one of three different glass PLOT columns was chosen, depending on the nature and volatility of the compounds under examination. For low-boiling oxygen- or sulphur-containing compounds, a 43-m column coated with a 0.5% w/v solution of Carbowax 1540 in methylene chloride was used. Medium- and higher-boiling substances were chromatographed on a 23-m column coated with 0.8% w/v Carbowax 20M in methylene chloride, while free aliphatic amines were analysed on a 23-m column coated with a 2% w/v ethanolic solution of potassium hydroxide followed by a 2% solution of Carbowax 1540. Aliphatic amines at low concentrations exhibited no tailing on this column. Nitrogen carrier gas was used throughout with flow-rates in the range 3–5 ml/min.

Solutions of the compounds being tested were made up at appropriate concentrations in diethyl ether or pentane and 0.5-μl quantities were injected.

Test reagents

The selection of colour tests was based on sensitivity, but was determined in part

by the physical nature of the PLOT traps. Thus, from information available in standard microanalytical texts[9,10], most tests involving white precipitates or weak colours, which would not show up clearly on the traps, were excluded, as were tests involving application of more than two solutions or prolonged heating for development of the colour.

Solutions were applied to the end of the PLOT trap using the tip of a very fine glass rod, the amount of reagent being easily controlled in this manner, and in general, application of the minimum amount of reagent ensured the least degree of spreading in the tube and consequently maximum sensitivity for the test.

Reagents were prepared as follows:

Carbonyl compounds. (i) 2,4-Dinitrophenylhydrazine (DNP) solution (for aldehydes and ketones)[9,10] — DNP (0.2 g) in methanol (10 ml) plus sulphuric acid (0.8 ml). The solution is stable, but care should be taken that its concentration does not change by evaporation of methanol. (ii) Schiff's reagent (for aldehydes only)[9,10] — basic Fuchsin (0.1 g) dissolved in 1 % w/v aqueous HCl (100 ml) plus sodium metabisulphite (1 g)*.

Alcohols. (i) Nitrochromic acid test[9] — A 5 % aqueous solution of potassium dichromate (1 ml) diluted with 7.5 *N* nitric acid (12 ml). (ii) Ceric nitrate test[9,10] — Ammonium ceric nitrate (0.4 g) dissolved in 1 *N* nitric acid (2 ml).

Esters. Ferric hydroxamate test[9,10] — Solution A: 2 *N* hydroxylamine hydrochloride in anhydrous methanol (5 ml) plus 2 *N* sodium ethoxide (10 ml)*. The supernatant liquid was used for the test. Solution B: hydrated ferric chloride (0.1 g) in 2 *N* HCl (10 ml).

Amines. (i) Ninhydrin test (for primary and secondary amines) — Ninhydrin (0.5 g) dissolved in acetone (10 ml). The solution is stable if kept at 0°. (ii) Sodium nitroprusside test (for secondary amines)[9,10] — Solution A: sodium nitroprusside (0.4 g) dissolved in 20 % aqueous acetaldehyde (10 ml)*. Solution B: sodium carbonate (1.2 g) dissolved in distilled water (10 ml). (iii) Quinhydrone test (for primary and secondary amines)[9] — A saturated solution of quinhydrone in methanol was mixed with an equal volume of absolute ethanol*.

Sulphur compounds. (i) Sodium nitroprusside test (general test for mercaptans, sulphides and disulphides)[9] — Solution A: 5 % w/v potassium cyanide in 0.2 *N* sodium hydroxide (1 ml) plus 95 % ethanol (5 ml). Solution B: aqueous sodium nitroprusside (1 % w/v). (ii) Isatin test (for mercaptans and disulphides)[9] —Isatin (0.1 g) in sulphuric acid (10 ml). Stable for several days in a tightly capped bottle. (iii) Phosphate–molybdate test (for mercaptans)[11] — Solution A: Dodeca-molybdophosphoric acid (1 g) dissolved in distilled water (10 ml). Solution B: 15 % aqueous sodium hydroxide (1 ml) plus absolute ethanol (6 ml). (iv) Lead acetate test (for mercaptans)[9] — A saturated solution of lead acetate in methanol.

Aromatic nuclei. Le Rosen test[9,10] — Formaldehyde (1 ml) + concentrated sulphuric acid (9 ml). Stable for a few hours only.

RESULTS AND DISCUSSION

The adsorption of compounds eluted from a chromatographic column onto PLOT tubing containing a layer of activated alumina under ambient conditions as

* Freshly prepared reagent *must* be used at all times.

described above proved to be a highly effective method for trapping most compounds covering a large range of functional classes. The most satisfactory traps were those containing an even, tightly held layer of alumina, possessing uniform and minimal pressure drops across the lengths used (10 mm). Such traps were produced from 150–200 mesh alumina containing 20 % w/v of powdered B-37 Pyrex glass powder as a binding agent. Very even layers were obtained with traps having a lesser amount (10 % w/v) of glass binder, but the packing was easily dislodged by the carrier gas flow, and pressure drops varied considerably over equivalent lengths. Higher proportions (30 % w/v) of glass produced tightly held but uneven layers.

With carrier gas flow-rates of up to 5 ml/min, most compounds were trapped in a narrow band over the first 2–3 mm of packing. The only exceptions were certain low-molecular-weight sulphur compounds, which were found to trap over a length of PLOT tubing rather than as sharp bands, and this much reduced the sensitivity of the colour tests. However, sharp trapping could be obtained for these compounds, provided the near end of the trap was cooled, and this was readily achieved by winding a thin strip of copper foil around the capillary tubing and allowing its ends to dip into a flask of liquid nitrogen.

Limits of detection for colour reactions after trapping

The limits of detection by means of colour reactions were measured for representative members of functional classes, comprising ketones, aldehydes, alcohols, esters, amines, sulphur-containing compounds and some aromatic derivatives. The

TABLE I

LIMITS OF DETECTION FOR COLOUR REACTIONS OF CARBONYL COMPOUNDS

Compound	*Minimum detectable amount (μg) 2,4-DNP test*	*Colour produced 2,4-DNP test*	*Minimum detectable amount (μg) Schiff's reagent (purple coloration)*
2-Pentanone	0.1–0.15	bright orange	
2-Hexanone	0.1–0.15	orange yellow	
2-Heptanone	0.2–0.3	deep yellow	
2-Octanone	0.4–0.6	yellow	
2-Nonanone	0.4–0.6	yellow	
2-Decanone	0.4–0.6	yellow	
2-Undecanone	0.4–0.6	yellow	
2-Butanal	1.0–1.5	yellow	0.6 –0.9
n-Hexanal to *n*-undecanal	1.0–1.5		0.07–0.15
2-Methylbutanal			0.15–0.25
3-Methylbutanal			0.15–0.25
2-Ethylhexanal			0.07–0.15
Acrolein			0.6 –0.9
2-Hexenal			0.3 –0.45
2-Nonenal			0.1 –0.15
Cinnamaldehyde			0.1 –0.15
Benzaldehyde	0.1–0.15	orange red	0.3 –0.45[a]
4-Tolualdehyde			0.1 –0.15
Furfural			0.1 –0.3[a]
4-Methylacetophenone	0.1–0.15	red	

[a] Pink coloration, forms slowly.

results obtained are summarized in Tables I–VI. Blanks were run in all experiments involving the estimation of detection limits, *i.e.*, the region of the chromatogram just before and after each trapped component was itself trapped and tested with the appropriate reagent(s). Aspects of the behaviour of individual functional groups with their respective colour developing reagents are described in some detail below.

Carbonyl compounds

Application of a small quantity of the yellow DNP test reagent to the tip of a PLOT trap gave a yellow to deep orange coloration with an aldehyde or ketone, the minimum detectable amounts (Table I) being between 0.1 and 0.5 μg for ketones and about 1 μg for aldehydes. Since a number of aldehydes give yellow colours not significantly different from the background of the test reagent, it is particularly important that the test solution is not too deeply yellow in colour, *i.e.*, it is of the strength given above. The intensity of colour of the precipitate can provide useful information as to the identity of the unknown carbonyl compound. Conjugation of the carbonyl group with double bonds or benzene rings shifts the absorption maximum to the visible region, the hydrazone precipitate appearing redder in colour; 2-hexenal, 2-nonenal, benzaldehyde and *p*-methylacetophenone all produced precipitates of a very distinctively red/orange colour.

The high sensitivity (less than 0.1 μg for some compounds) of the Schiff test for aldehydes is apparent from the data in Table I. For most compounds the deep purple coloration was seen within 10 sec of applying the reagent. The importance of running blanks and of only using the reagent freshly prepared as described above was particularly evident for this sensitive test. The reagent which contains an excess of sulphurous acid loses sulphur dioxide if exposed to the atmosphere and becomes considerably more sensitive. This is normally manifested by the appearance of a distinct purple coloration shortly after applying a drop of the reagent to a blank PLOT tube.

Alcohols

The most sensitive but by no means most specific test for alcohols at the present time is the nitrochromic acid test. When this test was carried out in a test tube, a colour change from pale yellow to faint blue was observed. However, at low concentrations of alcohols, the blue colour was too weak to be seen in the PLOT traps, and the observed colour change was from faint yellow to colourless.

Using potassium dichromate as the reagent, aldehydes as well as alcohols gave a positive reaction, as will any other readily oxidisable compound. However, FEARON AND MITCHELL[12] have reported that potassium chromate, which constitutes the true nitrochromic acid test, is specific to primary and secondary alcohols, being negative to tertiary alcohols, aldehydes other than formaldehyde, and aliphatic ketones. Although the reaction was slightly slower, positive results for aldehydes (C_4–C_8) were obtained in the present work, the colour change again being from yellow to colourless. The explanation for these contradictory findings is not clear, but it is in any case difficult to see why there should be a major difference in the behaviour of the two salts.

A selection of alcohols examined by the potassium dichromate–nitric acid reagent gave detection limits of about 1 μg (Table II). The colour change occurred

more slowly for the higher-molecular-weight alcohols, but the change was still detectable within 1–2 min of applying the reagent. With blanks, the yellow colour remained without fading for several hours. This test must be used with great caution in the analysis of unknown mixtures, and may only be regarded as positive if the absence of readily oxidisable compounds in a given peak has been confirmed by other means, *e.g.*, by a negative Schiff test for aldehydes.

TABLE II

LIMITS OF DETECTION FOR COLOUR REACTIONS OF ALCOHOLS

Compound	*Minumum detectable amount (μg) Nitrochromic acid test (yellow to colourless)*
n-Propanol	0.4–0.6
n-Butanol	0.6–0.8
n-Butanol, 2-pentanol, 4-heptanol, 2-octanol, *n*-hexanal	0.8–1.2

The conventional ceric nitrate test for aliphatic alcohols, which involves a colour change from yellow to a deeper yellow/amber, was not really suited to the present work, since it possesses a low intrinsic sensitivity. Detection limits of about 25 μg were found for a number of alcohols and this is somewhat better than limits of about 400 μg reported by FEIGL[10] and of around 100 μg (C_1–C_8 alcohols) by WALSH AND MERRITT[1].

A clear need still exists for a sensitive specific colour test for alcohols, especially one which is adaptable to PLOT traps; a number of potentially useful tests are still under investigation.

TABLE III

LIMITS OF DETECTION FOR COLOUR REACTIONS OF ESTERS

Compound	*Minimum detectable amount (μg) Ferric hydroxamate test (violet to purple colour)*
Ethyl acetate, methyl salicylate, *n*-propyl acetate	0.3–0.45
n-Butyl acetate, *n*-amyl acetate, 2-ethylhexyl acetate, *n*-octyl acetate, phenylethyl acetate, diethyl malonate	0.4–0.6
n-Butyl valerate, ethyl benzoate, diethyl oxalate	0.6–0.9
Ethyl propionate, butyl propionate, ethyl butyrate, butyl butyrate	0.7–1.0
Isopentyl butyrate, methyl hexanoate, ethyl hexanoate, isopentyl propionate	0.8–1.2

Esters

The limits of detection for a number of aliphatic and aromatic esters using a modified form of the conventional ferric hydroxamate test are presented in Table III. The test was carried out by applying a small quantity of solution A (hydroxylamine hydrochloride–sodium ethoxide) to the trapped ester, allowing 1 ½–2 min for reaction and then applying a small quantity of solution B (ferric chloride–hydrochloric acid), a positive test being the immediate formation of a violet to purple coloration in the tube. At concentrations close to the detection limit, the coloration was more pink in nature.

The conventional hydroxamate test, which employs hydroxylamine in alcoholic potassium hydroxide for solution A, was unsatisfactory for use with PLOT alumina traps, since the addition of solution B always resulted in the formation of dark brown ferric hydroxide, which interfered with the ester coloration and considerably raised the detection limits. In the modified test using ethoxide rather than hydroxide, the amount of ferric hydroxide formed was drastically reduced, resulting in an improvement in the detection limit by approximately one order of magnitude. Since the brown coloration (which is dispersed by a slight excess of solution B) was not totally eliminated by using ethoxide, it was most important to run blanks under identical conditions and at the same time as a test.

TABLE IV

LIMITS OF DETECTION FOR COLOUR REACTIONS OF AMINES

Test	*Compound*	*Minimum detectable amount (μg)*	*Colour*
Ninhydrin	Isopropylamine	0.4 –0.6	blue-grey
	n-Butylamine	0.2 –0.3	violet
	2-Butylamine	0.4 –0.6	violet
	Allylamine	0.4 –0.6	brown-violet
	Diethylamine	0.2 –0.3	blue-violet
	Di-isobutylamine	1.0 –1.5	grey-violet
Sodium nitroprusside (2° amines)	Diethylamine	0.4 –0.6	blue
	Di-isobutylamine	0.2 –0.3	blue
	Piperidine[a]	0.4 –0.6	blue-grey
	Pyrrolidine[a]	0.07–0.15	blue
Quinhydrone (1° amines)	Isopropylamine	0.4 –0.6	red-brown
	n-Butylamine	0.4 –0.6	red-brown
	2-Butylamine	0.4 –0.6	red-orange
	Isobutylamine	0.5 –0.75	red-orange
	Allylamine	0.4 –0.6	orange

[a] Limits obtained by direct injection of sample onto PLOT trap.

Amines

Three sensitive tests for volatile aliphatic amines which could be easily applied to PLOT alumina traps were selected and evaluated. The results are presented in Table IV.

Primary and secondary amines produced blue/violet coloration on heating the trap at 100° for 2–3 min after applying a drop of the ninhydrin reagent. Sodium

nitroprusside may be used to distinguish between primary and secondary amines by means of a two-solution reaction. In the presence of secondary amines, addition of a drop of solution A results in the production of a blue colour, the intensity of which increases markedly upon addition of a drop of solution B. At amine concentrations close to the limit of detection the blue colour cannot be detected until after the addition of solution B. To obtain maximum sensitivity for this test, higher concentrations than those recommended by FEIGL[10] were used for both reagents.

The detection of primary amines in the presence of secondary amines is readily achieved by means of the red-brown colour (against a yellow background) produced on addition of a drop of quinhydrone solution. There was no interference from secondary and tertiary amines at levels up to 5 μg in the PLOT traps, although colour changes with quinhydrone in test tube reactions have been reported[9].

TABLE V

LIMITS OF DETECTION FOR COLOUR REACTIONS OF SULPHUR COMPOUNDS

Compound	*Minimum detectable amount (μg)*			
	Sodium nitroprusside test	*Isatin test*	*Phosphate–molybdate test*	*Lead acetate test*
n-Propyl mercaptan	0.15–0.3	0.6–0.9	0.3 –0.45	6
tert.-Butyl mercaptan	3–4	neg.[a]	1.0 –1.5	15
n-Octyl mercaptan	0.07–0.15	0.2–0.3	0.15–0.3	0.8
Dimethyl disulphide	0.2 –0.3	0.1–0.5	neg.[a]	neg.[a]
Ethyl sulphide	8	neg.[a]	neg.[a]	neg.[a]
n-Propyl sulphide	neg.[a]	neg.[a]	neg.[a]	neg.[a]
Allyl sulphide	neg.[a]	neg.[a]	neg.[a]	neg.[a]

[a] neg. means the test gave a negative result.

Sulphur compounds

The detection limits for a group of aliphatic sulphur compounds using four different colour tests are presented in Table V.

A positive sodium nitroprusside test, which involves the application of a drop of a potassium cyanide–sodium hydroxide mixture (solution A) followed 2–3 min later by a drop of nitroprusside (solution B), results in the production of a pink to red colour against a yellow background. The test is given as being positive for mercaptans, sulphides and disulphides. However, with the limited number of compounds available, the test was found to work well only for mercaptans and disulphides. Despite experiments with varying reagent concentrations and reaction conditions, sulphides, with the exception of ethyl sulphide, which gave a high limit, did not respond.

As mentioned previously, low-molecular-weight sulphur compounds were not trapped in a sharp band on the PLOT traps unless the collecting end of the trap was cooled. When applying the isatin test, it was possible to dispense with cooling by applying the reagent to the trap prior to connecting it to the splitter. The very distinctive colour change (red/yellow to bright green), which was positive for mer-

captans and disulphides but negative for sulphides, was then observed to take place at the tip of the trap as the compound eluted. Detection limits were determined in this manner.

Of the two specific tests for mercaptans which could be applied to PLOT traps, *viz.*, the lead acetate and phosphate–molybdate tests, the latter was much more sensitive. Application of a small amount of solution B, followed by solution A, resulted in the formation of a characteristic blue colour when mercaptans were present. Aldehydes were found to give a pale blue colour with the reagent, but only when present in very large excess (100 μg or more).

The lead acetate test (colourless to yellow) was affected to a greater degree than most other tests by the quantity of test reagent applied. It was found important to apply only a very small drop of reagent to the trap as any excess resulted in marked weakening of the colour response.

TABLE VI

LIMITS OF DETECTION FOR COLOUR REACTIONS OF AROMATIC NUCLEI

Compound	*Minimum detectable amount* (μg) (*Le Rosen test*)	*Colour*
Benzyl alcohol	0.07–0.15	brick-red
2-Phenyl ethanol	0.07–0.15	orange-red
Ethylphenyl acetate	0.15–0.25	yellow
Methyl salicylate	0.4–0.6	pink-red
Ethyl cinnamate	0.3–0.4	pink-orange
2-Phenethyl isothiocyanate	0.07–0.15	orange-red
Isopropyl benzene	0.07–0.15	orange
2-Butylbenzene	0.07–0.15	orange
p-Xylene	0.07–0.15	purple
Toluene	0.07–0.15	pink

Aromatic nuclei

The responses of a number of aromatic compounds to the Le Rosen test, which are given in Table VI, demonstrate the high sensitivity which is obtainable with this test. However, there are a number of severe limitations associated with the test, which reduce its general usefulness and necessitate great caution in the interpretation of the responses obtained. Firstly, all aromatic compounds do not react positively, *e.g.*, ethyl benzoate and benzaldehyde gave no coloration. In addition, a number of organic compounds give coloured solutions in concentrated sulphuric acid alone, and there is the possibility of other reactions occurring in the medium, *e.g.*, dehydration. Acetophenone, cinnamaldehyde and 4-tolualdehyde gave deep red colours with concentrated sulphuric acid alone. Hence, a positive test was taken as one where no coloration was produced with sulphuric acid alone, but where there was a coloration (usually red) with the test reagent.

It has been reported[1] that unsaturated aliphatic compounds also give a positive Le Rosen test. In the present work no response was detected with 1-nonene at levels below 20 μg.

Application of the technique to the analysis of mixtures

In working with mixtures it was found unnecessary to apply colour tests immediately after trapping a compound. When there was insufficient time to perform each test immediately after trapping, *e.g.*, where peaks of interest eluted rapidly, the traps were stored until the end of a chromatographic run (sometimes as long as 40 min). Thus, one operator can trap a large number of peaks and perform the colour tests later, thereby reducing the pressure of having to trap and test almost simultaneously during the course of a run.

Provided that sufficient of an unknown component is present in a mixture, a number of colour tests may be performed by splitting the eluting peak between a number of alumina PLOT traps. The three-way splitting device shown in Fig. 1 was useful for this purpose. Since the short PLOT tubes possess minimal pressure drops, the carrier gas-flow was split approximately equally between any three traps connected to the splitter, provided the lengths of the traps were kept roughly equal.

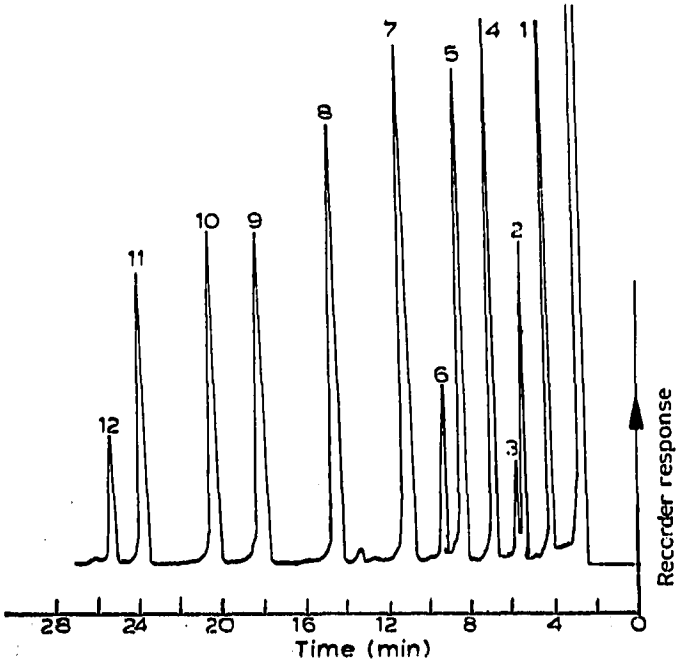

Fig. 2. Chromatogram of mixture used to evaluate the trapping-colour test scheme for analysis of functional groups. Column: 33-m × 0.5-mm I.D. glass PLOT column coated with 0.5% w/v Carbowax 20M; flow-rate, 3.5 ml/min; isothermal at 70° for 2 min, then programmed at 2°/min. Peak identity: 1 = 2-butyl acetate; 2 = *n*-hexanal; 3 = dimethyl disulphide; 4 = 1-penten-3-ol; 5 = 2-heptanone; 6 = 2-hexenal, 7 = 2-butylbenzene; 8 = 2 methyl-2-hepten-6-one; 9 = 2-ethylhexyl acetate; 10 = *n*-heptanol; 11 = 2-decanone; 12 = 2-nonenal (approximately 5 μg of each).

In order to evaluate the effectiveness of the splitting, trapping and colour development schemes for a multicomponent system, a synthetic mixture of twelve components, carrying between them five different functional groups and containing about 5 μg of each component, was prepared and chromatographed under the conditions given in Fig. 2. Two separate chromatographic runs were then carried out by an operator, to whom the constitution of the sample was unknown. Each emerging peak was split and trapped, and a systematic analysis performed to determine the

functionality of each component. The tests used were DNP, Schiff's reagent, nitrochromic acid, ferric hydroxamate, isatin and Le Rosen. The isatin test was used so as to obviate the need for cooling in the trapping of volatile sulphur compounds.

All the compounds responded positively for their respective functional groups. Peaks 2 and 3, which were eluted very rapidly, were trapped together and were shown to consist of an aldehyde and a sulphur compound. The red colour of the DNP test for peaks 5 and 11 correctly indicated the presence of a carbonyl group conjugated with a double bond system. The only anomaly was given by peak 7, which, in addition to giving a strong DNP test, also gave a weakly positive alcohol test.

CONCLUSIONS

The significant increase in the sensitivity of colour reactions for the qualitative analysis of GC eluates, resulting from the trapping of peaks as sharp narrow bands on a layer of alumina in a PLOT tube, represents a considerable extension of the utility of such reactions as an aid to the identification of unknown compounds. The technique is considered to be complementary to other methods of identification, and, where a test is insufficiently sensitive or intrinsically weak for any other reason, *e.g.*, the nitrochromic acid test for alcohols, the evidence may be readily strengthened by allied chemical methods, such as subtractive chromatography[13,14].

A sensitive and reasonably comprehensive classification scheme covering the more important functional groups has been presented which is rapid and reliable within the limitations clearly stated above. The scheme may be readily extended to cover functional groups not dealt with here, as well as to tests for specific compounds. Large numbers of such colour tests are described in the literature, many having very low detection limits, and capable of being developed for use with the method of trapping described in this paper.

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