

CHROM. 5718

## THE USE OF PRECOLUMN REACTIONS FOR THE SELECTIVE REMOVAL OF COMPOUNDS BEARING ALCOHOL OR CARBONYL FUNCTIONAL GROUPS IN THE GAS CHROMATOGRAPHIC ANALYSIS OF MIXTURES

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(Received August 16th, 1971)

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### SUMMARY

The use in conjunction with gas chromatography of 3-nitrophthalic anhydride and semicarbazide as reagents for selectively subtracting alcohols and carbonyl compounds, respectively, from mixtures containing these compounds was investigated. Each reagent, when deposited on a diatomaceous earth support and heated as a short plug in the injection block of a gas chromatograph, either removed or markedly reduced the peaks due to a large number of compounds bearing these functional groups. These short precolumns were amenable to operation with glass porous layer open tubular analytical columns under temperature-programmed conditions for compounds covering a moderate range of boiling points without seriously affecting the performance of the columns. The performance of the above reagents was compared with a number of other compounds cited in the literature as specific abstractors, *viz.*, boric acid for alcohols, free fatty acid phase and *o*-dianisidine for aldehydes and benzidine for aldehydes and ketones. These compounds were tested both as short-heated precolumns and also when coated on lengths of porous layer open tubular columns in the manner of normal liquid phases.

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### INTRODUCTION

The ability of gas chromatography (GC) to achieve remarkable separations of the components present in complex mixtures of organic compounds has been well matched in recent years by the development of sophisticated instrumental techniques to assign identities to the separated compounds. The use of selective detectors, spectroscopic techniques such as IR, NMR and particularly MS in conjunction with retention parameters frequently permits the determination of the structure of an unknown component even without isolation.

The development and application of the more classical chemical methods for use with GC systems to obtain structural information has been rather slow by comparison with advances in the instrumental methods. Yet, chemical reactions may often be combined with GC to advantage as an aid to identification, especially for compounds present in submicrogram amounts. For example, certain components in a mixture may be allowed to react with a specific reagent before injection onto the

chromatograph, or the separated components emerging from a column may be allowed to react with suitable reagents to produce a characteristic precipitate or colour change. The conversion of a compound within the GC system to a recognisable derivative can be achieved in many ways, *e.g.*, by hydrogenation, hydrogenolysis, dehydrogenation, hydrolysis, pyrolysis or carbon-skeleton chromatography. In another variation of what is termed "reaction GC", a functional group is allowed to react with a reagent contained within the system to yield a volatile fragment or derivative which may be identified by retention time. Alternatively, a functional group may be allowed to react with a reagent to produce a non-volatile derivative, the peak due to the former then being absent from the resulting chromatogram. Comprehensive reviews describing the current "state of the art" in the whole range of chemical modification methods allied to GC for structure determination appear in two recent texts<sup>1,2</sup>.

The reaction of a functional group with a reagent included in the GC system, either before or directly after the column, to selectively remove a compound by conversion to a non-volatile derivative is perhaps one of the simplest applications of chemical modification. The technique may be used with microgram or smaller amounts, and the absence or reduction of a peak in a mixture after passing over a suitable reagent, when combined with retention data, can lead to positive identification in suitable cases. Combined GC-MS analysis of complex mixtures obtained from natural products, represented, for example, by "flavour" concentrates of volatile compounds isolated from foodstuffs, may be simplified by the judicious application of suitable subtractive techniques. The analysis of such mixtures is generally carried out under temperature-programmed conditions, using high-temperature liquid phases such as Carbowax 20M, Apiezon and the silicone gums; the selectivities of these phases at the lower ends of their respective temperature ranges are rather poor. Even with columns of moderately high efficiencies multiple peaks are frequently encountered which in a linked GC-MS arrangement produce mixed mass spectra, and these are often difficult to interpret. Food flavour volatiles are generally rich in relatively low-boiling alcohols and carbonyl compounds, both saturated and unsaturated, and a ready method for selectively removing such functional groups has considerable analytical potential in the analysis of these mixtures.

Examples of subtractive processes for oxygenated compounds include the removal of alcohols with boric acid<sup>3,4</sup>, acids with zinc oxide<sup>5,3</sup>, aldehydes and ketones with benzidine<sup>3</sup> and hydroxylamine<sup>6</sup>, aldehydes with free fatty acid phase (FFAP)<sup>7,3</sup> and *o*-dianisidine<sup>3</sup> and epoxides with phosphoric acid<sup>3</sup>. The application of precolumn subtractive methods has to date been mainly concerned with the investigation of single components or simple mixtures, using packed analytical columns and isothermal conditions. Apart from the work of BEROZA and his co-workers<sup>3</sup>, little has been published on the effect of subtracting agents on other kinds of functional groups present in mixtures. Even less information is available on the behaviour of the reagents under temperature-programmed conditions as well as on their general compatibility with small diameter analytical columns.

In this paper the utility as specific abstractors is described for two reagents well known in qualitative organic analysis for characterising alcohols and carbonyl compounds, respectively, *viz.* 3-nitrophthalic anhydride and semicarbazide. The anhydride when deposited on a diatomaceous earth support and operated as a short

precolumn in the heated injection block of a chromatograph removes or markedly reduces peaks due to a large number of alcohols, while the semicarbazide used in a similar manner subtracts aldehydes and many ketones. The dimensions and operating conditions of both precolumns, which were used in conjunction with glass porous layer open tubular (PLOT) analytical columns, were arranged so that inclusion of the abstractors in the system had the minimum of adverse effects on the performance of the latter. Both reagents could be satisfactorily used under temperature-programmed conditions to at least 150°.

Some comparisons of the performance of the above reagents in respect of overall effectiveness as subtracting agents, as well as compatibility with narrow-bore columns, was made with certain other specific abstractors, *viz.* boric acid for alcohols, FFAP and *o*-dianisidine for aldehydes, and benzidine for aldehydes and ketones. These substances were operated as short precolumns in exactly the same manner as 3-nitrophthalic anhydride and semicarbazide. In addition, the effectiveness of boric acid, *o*-dianisidine, and benzidine when deposited in the manner of ordinary liquid phases on lengths of PLOT columns was explored.

## EXPERIMENTAL

### *Apparatus and materials*

A Pye Series 104 temperature-programmed gas chromatograph equipped with a heated flame detector and a heated injection head was used throughout. The injection heater assembly consists of a cylindrical insulation plug (30 × 60 mm) inserted through the roof of the oven with a counter-bored hole in which the heating element, wound on a Sandango former, is located. The cylindrical former and element are enclosed by mica washers and a mica shroud to prevent direct heat from the element affecting the plug. The heater dimensions are such that the centre of the heating element is 7 cm below the top of the injection head. The injection heater was calibrated for a range of temperature settings with the oven at 70°, by means of a thermocouple inserted through the injection head into a glass tube (6.25 mm O.D.) packed with Celite 545. The position of maximum temperature for any setting, as well as the magnitude of temperature gradients within the tube, were recorded and used as guides in determining the dimensions of the precolumn tube and length of packing likely to achieve the best results.

### *Precolumn tubes*

Glass precolumn tubes which fitted directly to the standard compression coupling of the injection head and in which the packing could be conveniently heated were prepared in the following manner. A 70-mm length of pyrex tubing (6.25 mm O.D. × 3.25 mm I.D.) was joined over a gas/oxygen flame to a length of thick capillary tube (5.5 mm O.D. × 0.925 mm I.D.), care being taken not to alter the internal diameter of either tube at the junction. Two methods may be used to connect the precolumn to the analytical column. One of these, which has been described recently for joining glass capillary injection blocks to PLOT columns<sup>8</sup>, involves drawing out the thick capillary tube over a bunsen flame to give a narrow end to which a piece of thin-wall polytetrafluoro-ethylene (PTFE) tubing and a hypodermic needle is then connected. A more robust method was developed later and is

now described. The thick capillary was cut off at a distance of about 20 mm from the junction with the wider diameter tube. The adaptor was removed from a 40 mm long, 21 gauge hypodermic needle and the rounded end of the needle was inserted a distance of about 10 mm into the thick capillary on the precolumn tube. A paste made from some Morgans 591 refractory cement\* and a little sodium silicate was applied around the needle and the end of the glass tube, and allowed to partially set for 1 h. A further layer of cement was then applied and after being allowed to stand overnight was baked at 150°. A film of Araldite\*\* resin was applied over the cement junction by rotating the tube in a current of hot air until the resin had set hard. The precolumn tube was then connected via the hypodermic needle to the PLOT column through a piece of thin-walled PTFE tubing (bore 0.75 mm). The junction between the latter and the column was also reinforced with a little Araldite resin. This system provided leak-free joints under carrier gas pressures of 1.2 kg/cm<sup>2</sup> and operating temperatures up to 220°. Although the layer of Araldite over the cement turned dark brown above 200°, the system remained free of leaks after a 3-h test period at 220°.

#### *Preparation of packings for the precolumn abstractors*

**3-Nitrophthalic anhydride.** The reagent, after recrystallisation from chloroform (m.p. 162–165°), was deposited on 120–150 mesh acid-washed Celite 545 (40 % w/w) by evaporation from dry acetone solution.

**Semicarbazide.** The packing, containing 40 % w/w of semicarbazide on the support, was prepared by evaporation at reduced pressure of an aqueous slurry of semicarbazide hydrochloride on to 120–150 mesh Celite 545 to which enough ammonium carbonate was added to liberate the free base. The semicarbazide packing is susceptible to atmospheric oxidation and therefore was stored out of contact with air in a tightly capped tube until required.

**Boric acid.** The reagent was prepared by mixing 1 part of powdered boric acid with 20 parts by weight of 120–150 mesh Celite 545 which had been coated with Carbowax 20M (5 % w/w).

**Others.** FFAP (20 % w/w), *o*-dianisidine (10 % w/w) and benzidine (20 % w/w) were each deposited on the Celite by evaporation from solutions in chloroform. Each packing was conditioned under nitrogen before use, 3-nitrophthalic anhydride for 1 h at 225°, semicarbazide for 20 min at 115°, boric acid for 1 h at 150°, and FFAP, *o*-dianisidine and benzidine each for 30 min at 160°. For the evaluation work, a 25-mm length (about 140 mg) of packing was placed in each precolumn tube between two small plugs of glass wool. When connected to the heated injection block of the chromatograph, the centre of the injection heater coincided with the centre of the length of packing. The space above the packing to the top of the tube was filled with a short length of loosely fitting capillary tube drawn from a piece of 9 mm O.D. × 3 mm I.D. tubing to an approximate diameter of 2.5 mm. This capillary provided a convenient guide for directing the hypodermic syringe needle on to the top of the packing during injection of samples. A schematic representation of the precolumn, heater and connection system is shown in Fig. 1.

A number of 2-m long lengths of PLOT coils were coated with a 5 % w/v aqueous solution of boric acid, or with 10 % w/v solutions in chloroform of FFAP

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\*\* Ciba (A.R.L.) Ltd., Duxford, Cambridge, Great Britain.

*o*-dianisidine and benzidine, by forcing the solutions through the coils from a syringe, followed by removal of excess liquid under a small pressure of nitrogen. The coils for the carbonyl compounds were conditioned under nitrogen at 170° for 30 min, while the boric acid after complete removal of the water at 110° was then coated with a 0.5 % w/v solution of Carbowax 20M in dichloromethane and subsequently conditioned for 1 h at 150°.

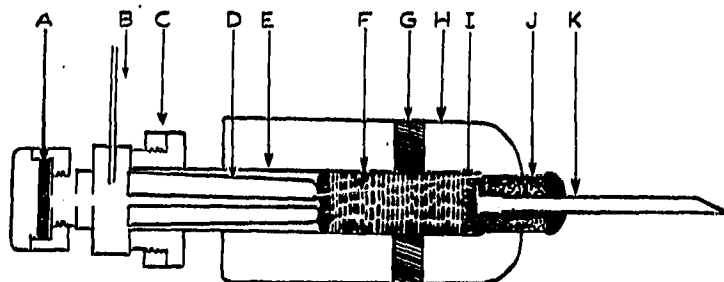


Fig. 1. Schematic representation of a heated precolumn system. (A) = Septum; B = carrier gas inlet; C = compression nut; D = loose-fitting capillary tube; E = glass tube (6.25 mm O.D.  $\times$  3.25 mm I.D.); F = precolumn packing; G = injection heater; H = cylindrical insulation plug; I = glass wool; J = glass capillary tube (5.5 mm O.D.  $\times$  0.925 mm I.D.); K = 21-gauge hypodermic needle cemented to glass capillary tube.

All analyses, except those involving free aliphatic acids, were carried out on a 37 m  $\times$  0.5 mm I.D. glass PLOT column, coated with a 0.5 % w/v solution of Carbowax 20M and prepared by a method recently described<sup>9</sup>. Low-boiling free aliphatic acids were analysed on a 12-m PLOT column coated with Carbowax 20M terminated with terephthalic acid (TPA) and deactivated before coating, by injecting several 0.5- $\mu$ l charges of hexamethyldisilazane (HMDS) at 110°. Peaks for the free acids exhibited no tailing.

#### *Subtraction experiments*

The compounds were generally injected as ether solutions in concentrations of about 1-2 % v/v. The average weight was 10  $\mu$ g. Normal alkanes having retention times close to compounds under investigation were included as internal standards in all runs and the amount of compound subtracted was determined by comparing its peak height with that of an alkane, both in the presence and in the absence of the precolumn. In temperature-programmed runs, the precolumn heater was switched off immediately after injection of the sample so as to minimize bleeding of the reagent onto the column. The analyses of the lower-boiling compounds were carried out isothermally at 75°, while the column was programmed at 2°/min from 70° for runs with the higher-boiling materials. This procedure was used so that the occurrence of any low-molecular-weight breakdown products resulting from anomalous behaviour of a compound in the precolumn could be observed during the analysis.

## RESULTS AND DISCUSSION

### *Subtraction of alcohols with 3-nitrophthalic anhydride*

A 25-mm long precolumn containing 40 % by weight of this reagent completely subtracts a large number of alcohols and drastically reduces the peaks due to several others. The esters formed have high boiling points and are mostly retained in the

TABLE I

SUBTRACTION OF LOW-BOILING ALIPHATIC ALCOHOLS WITH A 3-NITROPHTHALIC ANHYDRIDE PRE-COLUMN AT VARIOUS TEMPERATURES

Compound	% Removed			
	Average temperature 125° (max. 142°)	Average temperature 147° (max. 170°)	Average temperature 170° (max. 200°)	Average temperature 190° (max. 225°)
Ethanol	90	99	100	100
Isopropanol	62	77	84	92
<i>n</i> -Butanol	97	100	100	100
Isobutanol	87	99	100	100
2-Butanol	55	79	83	92
2-Methylbutanol	97	100	100	100
3-Pentanol	50	71	76	86
2-Pentanol	67	83	87	96

precolumn. The reactivity of the reagent increases with rise in temperature of the precolumn. Table I shows some data obtained for a number of low-boiling saturated aliphatic alcohols at a series of temperatures, the percentage of each compound removed being a measure of the ease of reaction of that compound with the reagent. Table II summarises the behaviour of a wider range of alcohols, as well as a number of non-alcoholic compounds at the highest average temperature 190° (maximum temperature at centre of packing, 225°) used for the experiments. At this temperature, all primary alcohols tested, both saturated and unsaturated were totally removed, while most secondary alcohols were reduced by 90% or more. Within a given homologous series the degree of reactivity increased slightly with increase of molecular weight especially for the lower members (compare the figures for ethanol and *n*-butanol in Table I). For secondary alcohols the reactivity was observed to decrease the greater the distance between the hydroxyl group and the end of the carbon chain, *e.g.*, at any temperature about 10% more of 3-pentanol passed the precolumn than of the isomeric 2-pentanol (Table I). In all cases the peak shape and also the retention time of the portion of a peak which passed the precolumn remained unchanged.

Tertiary alcohols did not react with 3-nitrophthalic anhydride, but underwent partial dehydration to the corresponding olefins. Dimethylformamide was used as solvent instead of ether (which obscured the olefin peaks) when examining the behaviour of *tert.*-butyl and amyl alcohols. At a precolumn average temperature of 170° (max. 200°) *tert.*-butyl and amyl alcohols were dehydrated to the extent of about 65% and 35%, respectively, while at an average temperature of 190°, the former was totally dehydrated and *tert.*-amyl was affected to the extent of 70-80%. The dehydration of tertiary alcohols occurs not only on this reagent but also on a boric acid precolumn, although with the latter the degree of dehydration appears to be independent of temperature<sup>3</sup>.

Unsaturated alcohols having an allylic double bond, *e.g.* 2-alken-4-ols, have been reported to undergo dehydration to the conjugated diene in the presence of boric acid<sup>3</sup>. With the anhydride precolumn at the maximum operating temperature the peak due to 2-octen-4-ol was totally removed and two small peaks with close retention times were eluted earlier in the chromatogram. Together they represented

only about 10–15 % of the amount injected, so the compound appears to have undergone mainly normal subtraction. Two labile terpene alcohols,  $\alpha$ -terpineol and 4-terpinenol, were found to undergo extensive dehydration to the monoterpenes, the latter having much shorter retention times than the alcohols, so it is clear that considerable care must be exercised when applying subtractive methods with mixtures containing labile compounds of this type.

TABLE II

SUBTRACTION OF COMPOUNDS WITH A 3-NITROPHTHALIC ANHYDRIDE PRECOLUMN AT AN AVERAGE TEMPERATURE OF 190° (MAX. 225°)

<i>Compounds completely subtracted or markedly reduced</i>	<i>Compounds partially subtracted or not at all</i>
C <sub>1</sub> –C <sub>10</sub> <i>n</i> -Alkanols <sup>a</sup>	2-Methylbut-3-en-2-ol
3-Buten-1-ol <sup>a</sup>	<i>tert.</i> -butyl alcohol <sup>d</sup>
1-Buten-3-ol <sup>b</sup>	<i>tert.</i> -Amyl alcohol <sup>d</sup>
4-Penten-1-ol <sup>a</sup>	$\alpha$ -Terpineol <sup>d</sup>
1-Penten-4-ol <sup>b</sup>	4-Terpinenol <sup>d</sup>
1-Hepten-4-ol <sup>b</sup>	1-Nonene
3-Hexen-1-ol <sup>a</sup>	2-Methylbutanal
4-Heptanol <sup>b</sup>	2-Ethylbutanal
Isohexanol <sup>a</sup>	<i>n</i> -Hexanal
2-Octanol <sup>a</sup>	2-Hexenal
3-Octanol <sup>a</sup>	Benzaldehyde
4-Octanol <sup>b</sup>	Furfural
1-Octen-3-ol <sup>b</sup>	Methyl isoamyl ketone
1,2-Butanediol <sup>a</sup>	3-Octanone
Benzyl alcohol <sup>a</sup>	1-Octen-3-one
Phenylethanol <sup>a</sup>	<i>n</i> -Butyl propionate
Furfuryl alcohol <sup>a</sup>	Isoamyl butyrate
Cyclohexanol <sup>a</sup>	Acetic acid
Linalyl acetate <sup>a</sup>	Butyric acid
Phenol <sup>c</sup>	2-Methylbutyric acid
Methyl salicylate <sup>c</sup>	(—)-Carvone

<sup>a</sup> Compound completely removed.

<sup>b</sup> Reduced by 90% or more.

<sup>c</sup> At least 50% removed.

<sup>d</sup> Extensively dehydrated.

Most other oxygen-containing functional groups passed the precolumn unchanged or with only slight reductions (see Table II). Peak height reductions of 20 % or less were not considered significant, since this reduction was sometimes accompanied by slight broadening of the peak, the area remaining essentially unchanged. However, there were exceptions, *e.g.*, linalyl acetate was totally removed and compounds with aromatic hydroxyl groups, *i.e.* phenol and methyl salicylate, were abstracted to the extent of at least 50 % relative to pentadecane. Free aliphatic acids were totally unaffected.

An example of the analysis of a miscellaneous mixture comprising saturated and unsaturated alcohols and aldehydes, ketones and esters under temperature-programmed conditions, both in the absence and in the presence of the anhydride precolumn is shown in (a) and (b), respectively, in Fig. 2. It can be seen that the performance of the analytical column is scarcely affected by inclusion of the short

anhydride precolumn. There is some loss in the separation of two of the early peaks, *viz.* 2-methylbutanal (2) and methyl ethyl ketone (3), while benzaldehyde (15) is broadened slightly. It was possible to reduce these effects by decreasing the precolumn length to about 12 mm, but this was accompanied by some loss in the efficiency of alcohol subtraction. Likewise, reduction of the loading of the reagent on the support to 20 % w/w resulted in a substantial reduction in its effectiveness. Operation of the

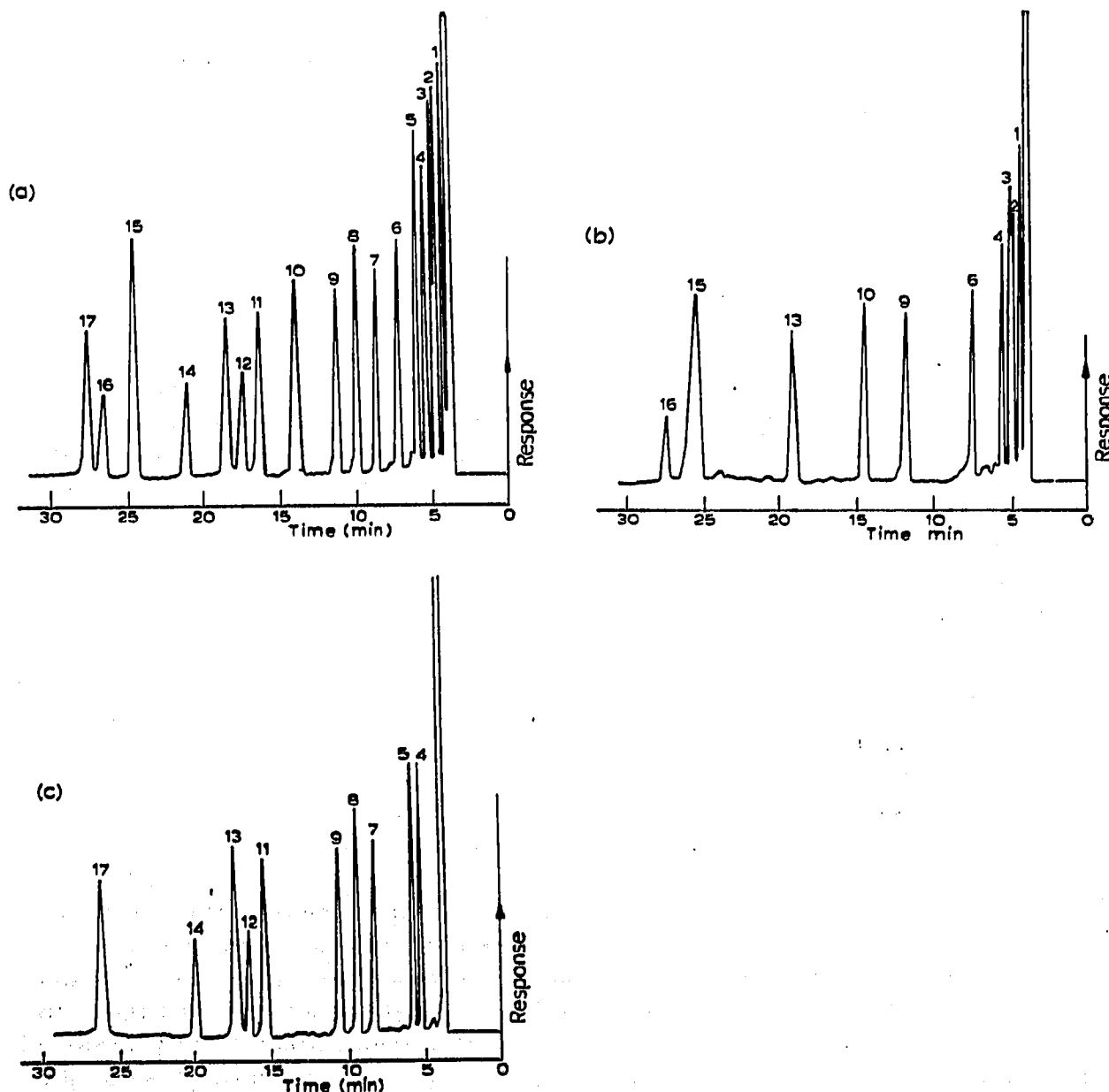


Fig. 2. Temperature-programmed analysis of a miscellaneous mixture with (a) no precolumn, (b) a 25-mm precolumn of 3-nitrophthalic anhydride (40% w/w) operated at an average temperature of 190°, and (c) a 25-mm precolumn of semicarbazide (40% w/w) operated at an average temperature of 115°. Column: 37 m × 0.5 mm I.D. PLOT column, coated with a 0.5% w/v solution of Carbowax 20M. Flow rate: 4 ml/min nitrogen, programmed from 70° at 2°/min from injection. Peak identity: 5 μg each in ether of (1) isobutanol, (2) 2-methylbutanal, (3) methyl ethyl ketone, (4) *n*-propyl acetate, (5) *n*-propanol, (6) *n*-hexanal, (7) 3-buten-1-ol, (8) 2-methylbutanol, (9) *n*-butyl butyrate, (10) 2-octanone, (11) *n*-hexanal, (12) *cis*-3-hexen-1-ol, (13) tridecane, (14) 1-octen-3-ol, (15) benzaldehyde, (16) 2-nonenal, and (17) *n*-octanol.



precolumn at around 200° or over resulted after a time in a certain amount of reagent bleed into the narrow capillary portion of the precolumn tube and this caused some peak broadening, especially for higher-boiling compounds. When this occurred it was necessary to transfer the packing to a fresh tube. Some contamination of the early coils of the PLOT column also occurred in time, especially during temperature-programmed runs, but this could be rapidly eliminated by reversing the flow in the column and then baking out for a short period at the maximum temperature of the run. The temperature profile within the precolumn was such that the maximum temperature occurred at the centre of the length of packing and then decreased by an approximately equal amount over the portions above and below this point. The latter gradient was of considerable use in that it helped to minimize the bleed of reagent from the centre of the packing into the capillary section of the precolumn tube.

#### *Subtraction of alcohols with boric acid*

This reagent when used as a short precolumn in the same manner as 3-nitrophthalic anhydride removed the peaks due to 10- $\mu$ g quantities of most alcohols. The subtracted compounds eventually bled off as very diffuse peaks, presumably as borate esters, with delays of up to an order of magnitude or even more in retention time. The latter presented little difficulty under isothermal operation but caused marked drifts and irregularities of the baseline under temperature-programming conditions especially at the higher attenuation settings. An example of the analysis at 75° of a mixture containing between 5–10  $\mu$ g each of 2-methylbutanal, 2-ethylbutanal, *n*-hexanal, 1-penten-4-ol, methyl isoamyl ketone, undecane and 2-hexenal both with and without the boric acid precolumn (at 120°) is shown in Figs. 3a and b,

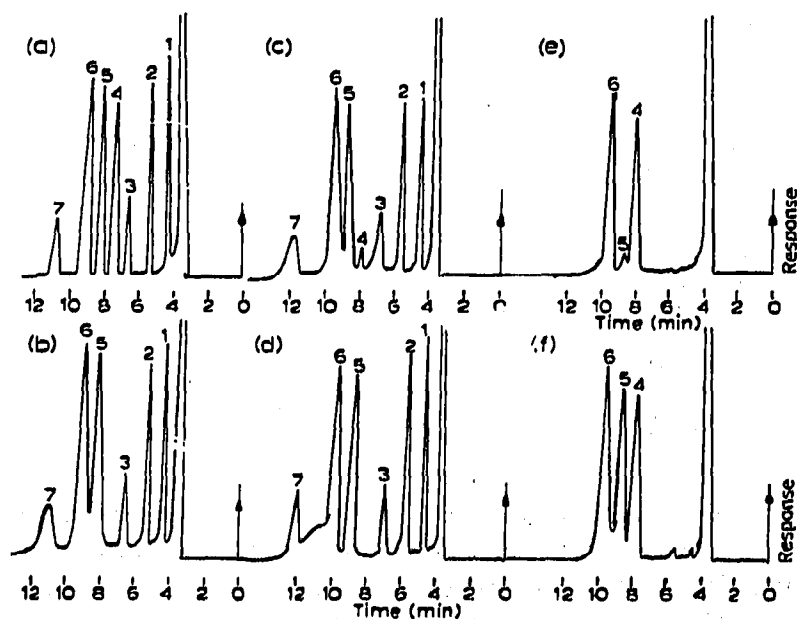


Fig. 3. Analysis of a simple mixture illustrating the effects of various subtracting agents. (a) No reagent, (b) a 25-mm boric acid precolumn, (c) a 25-mm 3-nitrophthalic anhydride (40% w/w), (d) a 2-m PLOT coil coated with 5% w/v aqueous boric acid solution (e) a 25-mm semicarbazide (40% w/w) precolumn, and (f) a 25-mm *o*-dianisidine (10% w/w) precolumn. Column: as in Fig. 2; temperature: 75°. Flow rate: 4 ml/min nitrogen. Peak identity: (1) 2-methylbutanal, (2) 2-ethylbutanal, (3) *n*-hexanal, (4) 1-penten-4-ol, (5) methyl isoamyl ketone, (6) undecane, and (7) 2-hexenal.

respectively. The degree of peak broadening and the loss in separation between methyl isoamyl ketone was about the same as that obtained when the same mixture was analysed on the 3-nitrophthalic anhydride precolumn (Fig. 3c).

Boric acid coated on a 2-m length of PLOT column as described earlier and connected to the detector end of the analytical column was also effective in subtracting alcohols, although in this case the retarded and diffuse derivatives were eluted more rapidly than in the precolumn mode of operation. This is demonstrated in Fig. 3d where the alcohol peak (4) is removed and a small diffuse peak is eluted on the tail of the undecane peak (6). It is also apparent from Fig. 3d that the performance of the analytical column is impaired to a lesser extent when boric acid is used as an extension of the analytical column rather than as a short packed precolumn.

#### *Subtraction of carbonyl compounds with semicarbazide*

The well-known reaction for characterization of carbonyl compounds by formation of their semicarbazones was investigated as a subtractive method in combination with GC. A 25-mm precolumn containing 40 % by weight of the reagent efficiently subtracted a wide variety of saturated and unsaturated aldehydes including  $\alpha$ -substituted compounds, aromatic aldehydes, cyclopentanone, and most methyl ketones. Other ketones were only partially subtracted. A list of compounds investigated is given in Table III. Esters, ethers, alcohols, olefins and paraffins were

TABLE III

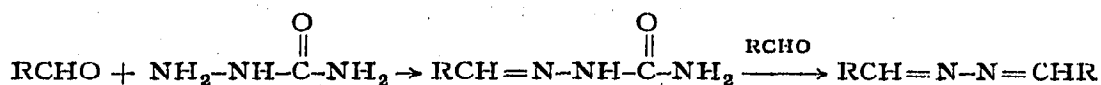
SUBTRACTION OF CARBONYL COMPOUNDS WITH A 25-MM SEMICARBAZIDE PRECOLUMN AT AN AVERAGE TEMPERATURE OF 115°

<i>Compound</i>	<i>% Subtracted</i>	<i>Compound</i>	<i>% Subtracted</i>
Isobutanal	100	Diacetyl	100
2-Methylbutanal	100	Methyl vinyl ketone	100
2-Ethylbutanal	100	Acetone	100
<i>n</i> -Hexanal	100	Methyl ethyl ketone	100
2-Hexenal	100	2-Pentanone	95
<i>n</i> -Heptanal	100	2-Hexanone	92
<i>n</i> -Octanal	100	Methyl isoamyl ketone	95
2-Octenal	100	2-Heptanone	98
Benzaldehyde	100	2-Octanone	98
Phenylacetaldehyde	100	2-Methylhept-2-en-6-one	100
Citronellal	100	Ethyl isopropyl ketone	42
		3-Octanone	60
		Cyclopentanone	100

completely unaffected by the precolumn. The performance of the analytical column was not impaired by the presence of the precolumn, as is apparent from the temperature-programmed analysis of the miscellaneous mixture in Fig. 2c.

Two important factors limited the range of operating temperature for the semicarbazide precolumn. Firstly, at higher temperatures the reagent tends to undergo self-condensation to form the biurea derivative. For example, a precolumn held at 140–150° was initially very efficient as a subtracting agent, but after about an hour began to lose its effectiveness for removing, initially, ketones and subsequently aldehydes as well. The water produced in the condensation did not adversely affect

the performance of the chromatographic system as steady baselines were maintained even at high attenuation settings. A much more important effect, occurring at higher operating temperatures, was the formation of volatile byproducts from the reaction of carbonyl compounds with the reagent. These were identified by combined GC-MS as azines, *i.e.* compounds of the type  $\text{RCH}=\text{N}-\text{N}=\text{CHR}$  for aldehydes. The reactions giving rise to these compounds were not investigated in the present work but a number of possible routes may be suggested. For example, they may result from hydrolytic cleavage of a semicarbazone between the amide function and the hydrazino nitrogen atom followed by (or contemporaneously with) electrophilic attack by a second molecule of the carbonyl compound, *i.e.*



Alternatively they may result from breakdown of the semicarbazide itself under the conditions used, to produce some hydrazine which then condenses with two molecules of the carbonyl compound. Under isothermal conditions these compounds were often barely detectable, appearing usually as small broad humps on the baseline with retentions several times longer than the parent carbonyl. In temperature-programmed runs, however, they were eluted more rapidly as sharper small peaks and frequently caused anomalies by interfering with other minor components present in a sample.

Fortunately, it was possible to reduce the formation of azines to insignificant levels by maintaining the precolumn at a moderately low temperature. An average temperature of around  $115^\circ$  was optimal, being low enough to avoid formation of the azines and high enough to subtract the carbonyl compounds given in Table III. Precolumns with freshly prepared packing and conditioned under nitrogen for 20 min at  $115^\circ$  before use gave good reproducibility of behaviour in removing the compounds discussed. After about 2 h at the optimum operating temperature, methyl ketones began to pass the precolumn. A small amount of contamination of the analytical column occurred after several analyses, but was easily removed by reversing the flow of gas through the column in the manner described for the anhydride precolumn.

The reactivity of semicarbazide when coated on a 2-m length of PLOT column was very low for all carbonyl compounds below its melting point ( $96^\circ$ ) and it was thus of little general use as a subtracting agent in this mode.

#### *Evaluation of other reagents for subtraction of carbonyl compounds*

The utility of FFAP and *o*-dianisidine for removal of aldehydes and benzidine for the removal of aldehydes and ketones using 15-cm long (6.25 mm O.D.) stainless-steel loops of packing in conjunction with packed analytical columns has been described in some detail by BIERL *et al.*<sup>3</sup> The performance of these reagents with PLOT analytical columns was investigated in the present work for two modes of operation of the reagent, *viz.* (a) as short (25-mm) lengths of packing heated in the injection port of the chromatograph, and (b) coated on 2-m lengths of PLOT column. The compounds shown in Fig. 3a were used as the test mixture and the following behaviour was observed.

**FFAP.** (a) As a precolumn (10% w/w), the reagent was operated at a number of temperatures up to  $200^\circ$ . The efficiency of aldehyde subtraction was extremely

poor at any temperature and all peaks were severely broadened. (b) FFAP was similarly ineffective when coated on a 2-m PLOT coil.

*o-Dianisidine.* (a) As a precolumn (10 % w/w), at a precolumn average temperature of 125° (max. 142°), aldehydes, including  $\alpha$ -substituted compounds at the 10- $\mu$ g level, were reduced by 95 % or more in peak height. Methyl ketones were slightly reduced, but never by more than 20 %. At higher temperatures, abstraction of ketones was more pronounced. Peaks due to other compounds were broadened only slightly more than with semicarbazide (compare chromatograms (e) and (f) in Fig. 3). (b) A 2-m PLOT coil, coated with a 10 % w/v solution of the reagent and conditioned for 15 min at 170°, gave peak reductions for most aldehydes, but was really only effective for straight-chain saturated aliphatic aldehydes. For example, 0.5- $\mu$ l sample of the mixture in Fig. 3a gave the following reduction in peak heights: 2-methylbutanal (40 %), 2-ethylbutanal (31 %), *n*-hexanal (100 %), methyl isoamyl ketone (2 %), and 2-hexenal (44 %). The reagent when used in this manner may be of potential use for detecting straight-chain aliphatic aldehydes in mixtures containing substituted derivatives. The substance also displayed moderately good bleed characteristics during temperature programming at 3°/min to 150°, the baseline drift recorded at an attenuation of  $\times 200$  being about 20 % f.s.d. over this range.

*Benzidine.* (a) As a precolumn (10 % w/w), this reagent, operated at an average precolumn temperature of 125° (max. 142°), showed subtractive properties similar to those of semicarbazide. Aldehydes, including  $\alpha$ -substituted compounds, were totally removed, while methyl ketones were reduced by upwards of 90 %. Other ketones were partially subtracted. Non-carbonyl compounds were largely unaffected, but peaks were generally broader than those obtained with semicarbazide, especially for the higher boiling compounds. For example, in the temperature-programmed analysis of the miscellaneous mixture of Fig. 2a, *cis*-3-hexen-1-ol (12) and tridecane (13) were only partially separated, while the width of *n*-octanol was almost twice that obtained for the corresponding peak with semicarbazide (Fig. 2c). (b) A 2-m PLOT coil coated with a 10 % w/v solution of benzidine was not especially useful as a subtracting agent for aldehydes and ketones. At temperatures below 100°, low-boiling aldehydes including  $\alpha$ -substituted compounds showed substantial reductions (70–100 %), but methyl ketones were only slightly affected (20 % reduction or less). Above 100° the reactivity with ketones increased with temperature. Baseline drift during temperature programming at 3°/min from 70° was about 80 % f.s.d. at 150° at an attenuation of  $\times 200$  and was quite unusable at higher temperatures.

## CONCLUSIONS

It is apparent from the work described that a number of reagents, two of which, 3-nitrophthalic anhydride and semicarbazide, had not been described previously can be used successfully in combination with PLOT analytical columns to selectively remove or reduce peaks of alcohols and carbonyl compounds in organic mixtures covering a moderate boiling point range. The reagents are most effectively utilized as short heated precolumns, although, as in the case of boric acid, deposition of the reagent on a length of PLOT column has certain advantages in applications where even a small loss in the separating power of the column is undesirable. Each reagent for a specific functional group has advantages and disadvantages over the other; e.g.

there was no interference from the 3-nitrophthalates of abstracted alcohols under temperature-programmed operation up to at least 150°, while considerable irregularities of the baseline were observed under similar conditions, when alcohols were removed with a boric acid precolumn. On the other hand, boric acid is reactive over a wide range of temperature, 50–200°, while the anhydride is reactive only at moderately high temperatures, which can lead to dehydration of some sensitive compounds. Similarly for carbonyl compounds, semicarbazide produces less peak broadening for non-carbonyl compounds than benzidine, but is somewhat limited in temperature range due to the formation of byproducts at high temperatures.

The potential usefulness of subtractive methods in combination with GC is obvious and merits further study. Since the reagents examined to date have been tested with only a relatively small range of compounds, caution should always be exercised in the interpretation of results obtained with mixtures of unknown constitution.

#### ACKNOWLEDGEMENT

Financial support from the Science Research Council for this work is gratefully acknowledged.

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