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GLASS CAPILLARY REACTION LOOPS FOR DETECTION OF ALCOHOLS, ALDEHYDES AND KETONES BY SUBTRACTION

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SUMMARY

Reaction loops coated with *o*-dianisidine, FFAP, 2,4-dinitrophenylhydrazine and benzidine for subtraction of carbonyl compounds, and a reaction loop coated with boric acid for subtraction of alcohols, were prepared in a 0.35-mm I.D. glass capillary. The efficiency and selectivity of the loops were tested with model compounds. The temperature dependence of the subtraction was investigated by varying the carrier gas flow-rate and temperature programming rate. Of the columns tested, *o*-dianisidine selectively subtracted aldehydes, while benzidine subtracted aldehydes and most ketones. FFAP did not subtract all aldehydes and the utility of the 2,4-dinitrophenylhydrazine loop was restricted by the decomposition of the subtracting agent. The boric acid loop selectively subtracted primary and secondary alcohols. A functional group can be detected in a few nanograms of a component in a mixture.

INTRODUCTION

Several reaction gas chromatographic methods for structure elucidation were announced during the 1960s¹. Subtraction loops, which are short columns containing an appropriate reagent deposited on a diatomaceous earth support, were developed for use in connection with packed²⁻¹⁴ and porous layer open-tubular (PLOT) columns¹⁵. A subtraction loop where the subtracting agent is deposited as an ordinary liquid phase on a length of PLOT column has also been described¹⁵. Subtraction loops are placed before^{4,5,7,10,12,13} or after^{3,7,15} the analytical column or between two analytical columns². Alternatively, the subtracting agent on a diatomaceous earth support is placed in the injection port^{8,9,15} or in a special reactor^{6,14}. *o*-Dianisidine^{7,9,14,15} and FFAP (free fatty acid phase)^{4,7,11,15} loops have been found to subtract aldehydes and hydroxylamine⁶, benzidine^{7,9,14,15}, sodium hydrogen sulphite⁹, semicarbazide¹⁵ and 3,4,5-trimethoxybenzylhydrazine¹³ loops both aldehydes and ketones. Boric acid^{2,3,7,8,12,14,15} and 3-nitrophthalic anhydride¹⁵ loops have been used to subtract primary and secondary alcohols, phosphoric acid loops to subtract epoxides^{6,7} and nitrogen bases⁹, and zinc oxide loops to subtract carboxylic acids^{5,7}. With packed columns it is possible to detect the functional group from 0.1-1 μg of component in a mixture^{7,8}.

The use of high-resolution glass capillary columns with appropriate injectors

and detectors is very advantageous in the analysis of natural products found in trace amounts, such as insect pheromones. In the identification of components of a mixture present at the level of a few nanograms, mass spectra and retention indices provide the only information on structure obtainable without isolation of the components concerned. This paper describes the preparation of subtraction loops where the subtracting agent is deposited on the walls of the glass capillary. The loops are used with small-diameter wall-coated glass capillary columns for detection of carbonyl and hydroxyl groups. Their efficiency and selectivity were studied with model compounds using temperature programming.

EXPERIMENTAL

Use was made of a Carlo Erba Fractovap 2350 gas chromatograph with a Grob-type injector and a flame-ionization detector. Hydrogen was used as the carrier gas. The analytical column (40 m \times 0.25 mm I.D.) was coated with OV-101. The subtractor columns were prepared as follows: a 0.35-mm I.D. glass capillary pre-coated with a barium carbonate layer as described by Grob and Grob¹⁶ was coated by the dynamic method with the subtracting agent in an appropriate solvent. The coating was repeated as indicated in Table I. The solutions of subtracting agents were a saturated solution of FFAP in dichloromethane, 10% benzidine in dichloromethane, 10% *o*-dianisidine in dichloromethane, 10% 2,4-dinitrophenylhydrazine in dimethylformamide and 10% boric acid in dimethylformamide. A 3-m length of subtractor column was connected by a PTFE tube to the back end of the analytical column. Before the subtraction experiments the loops were heated at 170 or 180°C for 20 min. The splitless injection technique was used. A sample containing 100 or 10 ng of each component was injected at a column temperature of 40°C. After an isothermal period of 2 min the temperature was raised as indicated in Tables I-III.

RESULTS AND DISCUSSION

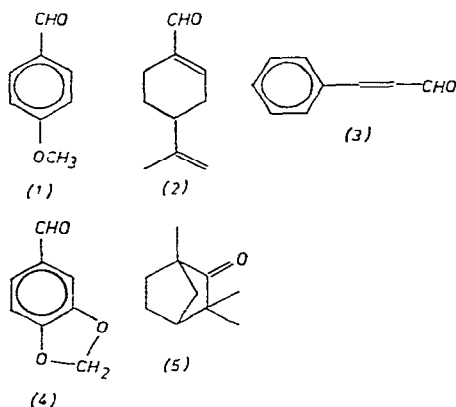
The efficiency and selectivity of subtractor columns were studied by injecting mixtures A (carbonyl compounds), B (hydroxy compounds) or C (ethers, esters, monoterpenes) together with *n*-alkanes (mixture D) in one set of experiments into the column, the back end of which was connected to the subtractor column, and in the other the column was used without the subtractor column (Tables I-III). When the carrier gas flow-rate and the temperature programming rate were such that the components of mixtures A and B separated properly, subtraction experiments were made by injecting a sample containing mixtures A, B and D. The temperature dependence of the subtraction was investigated by varying the carrier gas flow-rate (pressure) and temperature programming rate as indicated in Tables I-III. The expected elution temperatures were calculated for model compounds to determine the temperature limits for subtraction.

Reaction loops for subtraction of aldehydes

o-Dianisidine on a diatomaceous earth support has been found to subtract aldehydes selectively, including α -substituted, aliphatic unsaturated and aromatic aldehydes, over the temperature range 50-175°C^{7,9,14,15}. In addition to the aldehydes,

however, some epoxides are retained in the *o*-dianisidine loop⁷. A PLOT coil coated with *o*-dianisidine has been found to be effective for the subtraction of straight-chain saturated aldehydes¹⁵. A reactor column of sufficient length packed with 20% FFAP on a diatomaceous earth support subtracts most aldehydes within the temperature range 100–200°C^{7,11}. However, most α -unsaturated aldehydes are retained only partially^{7,11}. A short pre-column and a PLOT coil coated with FFAP have been found to subtract aldehydes only poorly.

In this study, the *o*-dianisidine loop subtracted aldehydes selectively, including aldehydes with an alicyclic or aromatic ring or double bond at the α -position [anisaldehyde (1), perillaldehyde (2), cinnamaldehyde (3), piperonal (4)] within the temperature range 64–144°C (Table I). The ketones, alcohols, esters, ethers, hydrocarbons and the epoxide (linalool oxide) tested passed through unaffected. Some broadening was observed in the peaks of geraniol (7) and 1-decanol. When used in connection with an apolar analytical column, the *o*-dianisidine loop increased the retention times of polar compounds by 1–10%. These results are in accordance with those of investigations made with *o*-dianisidine on a support^{7,9,14,15}, with the exception that an epoxide was not subtracted here.



The FFAP loop subtracted most aldehydes within the temperature range 65–115°C (Table I). Higher temperatures were not studied. The aldehydes with an alicyclic ring and a double bond [perillaldehyde (2)] or an aromatic ring [piperonal (4)] in the α -position were not subtracted. Heptanal was subtracted only partially. The ketones, alcohols, *n*-alkanes and the epoxide tested were not retained. The capillary coated with FFAP in this study showed a subtracting ability similar to that of FFAP on a support^{7,11}.

The 2,4-dinitrophenylhydrazine loop subtracted aldehydes only. *n*-Aldehydes were subtracted within the temperature range 97–147°C (Table I). For subtraction of perillaldehyde (2) an elution temperature of 170°C was required. Ketones and *n*-alkanes were not affected. Owing to the decomposition of the subtracting agent, the 2,4-dinitrophenylhydrazine loop soon lost its subtracting ability; when the experiment with the highest elution temperatures was repeated, most aldehydes were subtracted only partially. Mixtures B and C were not tested.

TABLE I

RESULTS OF SUBTRACTION EXPERIMENTS WITH THE SUBTRACTOR COLUMNS COATED WITH *o*-DIANISIDINE, FFAP AND 2,4-DNPH

— = Subtracted completely. —+ = Subtracted partially. + = Passed through subtractor column unaffected. b = Peak broadening observed. The temperature indicated is the expected elution temperature.

Parameter	<i>o</i> -Dianisidine	FFAP	2,4-DNPH
No. of times coating performed	2	4	3
Carrier gas pressure (kg/cm ²)	0.7	0.7	0.9
Programming rate (°C/min)	4	4	4
	8	4	10
<i>Mixture A:</i>			
2-Heptanone	+	+	+
Heptanal	—(63°C)	—	—+(65°C)
2,5-Hexanedione	+	+	+(56°C)
Fenchone	+	+	+
Nonanal	—	—	—
Decanal	—	—(121°C)	—(103°C)
Carvone	+	+	+
Anisaldehyde	—	—	+
Perillaldehyde	—	—	+
Cinnamaldehyde	—	—	—(115°C)
Piperonal	—(122°C)	—(146°C)	+
Benzophenone	+	+	+
			+(115°C)
			+(115°C)
			—(170°C)
			+
<i>Mixture B:</i>			
Cyclohexanol	+	+	+
Linalool oxide	+	+	+
1-Octanol	+	+	+
Linalool	+	+	+
Borneol	+	+	+
Menthol	+	+	+
α -Terpineol	+	+	+
Geraniol	+b	+b	+
1-Decanol	+b	+b	+
Thymol	+	+	+
<i>Mixture C:</i>			
Dibutyl ether		+	
Anisole		+	
Limonene		+	
Methyl benzoate		+	
Bornyl acetate		+	
<i>Mixture D:</i>			
Octane	+	+	+
Tridecane	+	+	+
Pentadecane	+	+	+
Heptadecaene	+	+	+

Benzidine loop for subtraction of aldehydes and ketones

Benzidine (20%) on a diatomaceous earth support has been found to subtract aldehydes, most ketones and epoxides over the temperature range of 100–170°C⁷. Sterically hindered ketones are subtracted only partially⁷. The selectivity of benzidine

on a support is also restricted owing to the partial subtraction of ethers, esters and alcohols⁷. A PLOT coil coated with benzidine has been found to be ineffective in subtracting aldehydes and ketones¹⁵.

In this investigation, the reaction loop coated once with 10% benzidine solution subtracted aldehydes selectively between 63 and 120°C (Table II). Perillaldehyde

TABLE II

RESULTS OF SUBTRACTION EXPERIMENTS WITH THE SUBTRACTOR COLUMNS COATED WITH BENZIDINE

Symbols as in Table I.

Parameter	Value				
No. of times coating performed	1	5	5	5	5
Carrier gas pressure (kg/cm ²)	0.7	0.7	0.7	0.35	0.35
Programming rate (°C/min)	4	4	8	10	12
<i>Mixture A:</i>					
2-Heptanone	+	+	+	+	+(130°C)
Heptanal	-(63°C)	-(63°C)	-	-	-
2,5-Hexanedione	+	+	+(81°C)	-(126°C)	-
Fenchone	+	+(86°C)	+	+	+(171°C)
Nonanal	-	-	-	-	-
Decanal	-	-	-	-	-(180°C)
Carvone	+	+(105°C)	-(126°C)	-	-
Anisaldehyde	-	-(107°C)	-	-	-
Perillaldehyde	-+	-	-	-	-
Cinnamaldehyde	-	-	-	-	-
Piperonal	-(120°C)	-(120°C)	-	-	-
Benzophenone	+(158°C)	-(158°C)	-	-	-
<i>Mixture B:</i>					
Cyclohexanol	+	+		+	
Linalool oxide	+	+		+	
1-Octanol	+	+		+	
Linalool	+	+		+	
Borneol	+	+		+	
Menthol	+	+		+	
α -Terpineol	+	+		+	
Geraniol	+	+		+	
1-Decanol	+	+		+	
Thymol	+	+		+	
<i>Mixture C:</i>					
Dibutyl ether				+	
Anisole				+	
Limonene				+	
Methyl benzoate				+	
Bornyl acetate				+	
<i>Mixture D:</i>					
Octane	+	+	+	+	+
Tridecane	+	+	+	+	+
Pentadecane	+	+	+	+	+
Heptadecane	+	+	+	+	+

(2) was subtracted only partially, however. Ketones and mixtures B and D passed through unaffected.

The loop coated five times with 10% benzidine solution subtracted aldehydes and most ketones (Table II). The *n*-aldehydes were subtracted between 63 and 180°C and the unsaturated aldehydes between 107 and 180°C. The lowest subtraction temperature detected for 2,5-hexanedione and carvone was 126°C and for benzophenone 158°C. 2-Heptanone and fenchone (5) were not retained in the reactor column up to 130 and 171°C, respectively. The non-subtractivity of fenchone due to steric hindrance in the formation of a Schiff base is in accordance with the results of Bierl *et al.*⁷. The elution temperature of 140°C used in the experiments of Bierl *et al.*⁷ to subtract 2-heptanone (Table II) was not reached here. The partial subtraction of other types of compounds was not observed in this study. The benzidine loop increased the retention times of polar compounds by 1–10%.

Boric acid loop for subtraction of primary and secondary alcohols

Boric acid (5%) on a column packing has been found to subtract saturated primary and secondary alcohols and α -unsaturated primary and secondary alcohols with terminal double bonds between 75 and 200°C^{2,3,7,8,12,15}. Tertiary alcohols do not form trialkyl borates. Boric acid, however, can at least partially dehydrate a tertiary alcohol or an unsaturated primary or secondary alcohol with an internal double bond^{3,7}. A lower concentration of boric acid (0.5%) on a column packing does not dehydrate tertiary alcohols and allows the differentiation of tertiary alcohols from primary and secondary alcohols but not from other compound classes^{8,12}. The higher concentration of boric acid additionally subtracts some epoxides and unsaturated aldehydes^{7,8}.

In this study, the boric acid loop selectively subtracted primary and secondary alcohols within the temperature range 56–116°C (Table III). Tertiary alcohols and phenols could be differentiated from primary and secondary alcohols by distinct peak broadening between 56 and 116°C. Characteristic to the tertiary alcohols was a broad peak with a sharp edge, probably formed by partial dehydration of the alcohol. For example, linalool (6) gave such a peak at elution temperatures of 82 and 110°C. When the subtraction experiment was undertaken at an elution temperature of 157°C, linalool (6) gave a sharp peak due to dehydration. In experiments not reported in Table III, geraniol (7) and 1-decanol were found to elute as a broad peak, when the elution temperature was higher than 140°C. Compounds of other types tested were unaffected, with the exception of 2,5-hexanedione, which eluted as a broad peak, probably owing to enolization. The effect of the boric acid loop on the retention times was

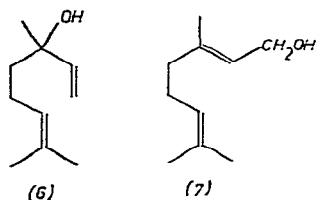


TABLE III

RESULTS OF SUBTRACTION EXPERIMENTS WITH THE SUBTRACTOR COLUMN COATED WITH BORIC ACID

Symbols as in Table I.

<i>Parameter</i>	<i>Value</i>		
Amount injected (ng)	100	100	10
No. of times coating performed	3	3	3
Carrier gas pressure (kg/cm ²)	0.9	0.7	0.7
Programming rate (°C/min)	4	4	4
<i>Mixture A:</i>			
2-Heptanone		+	+
Heptanal		+	+
2,5-Hexanedione		+b	+b
Fenchone		+	+
Nonanal		+	+
Decanal		+	+
Carvone		+	+
Anisaldehyde		+	+
Perrillaldehyde		+	+
Cinnamaldehyde		+	+
Piperonal		+	+
Benzophenone		+	+
<i>Mixture B:</i>			
Cyclohexanol	-(56°C)	-(61°C)	-
Linalool oxide	-	-	-
1-Octanol	-	-	-
Linalool	+b(82°C)	+b(110°C)	+b
Borneol	-	-	-
Menthol	-	-	-
α -Terpineol	+b	+b	+b
Geraniol	-	-	-
1-Decanol	-	-(116°C)	-
Thymol	+b(118°C)	+b(129°C)	+b
<i>Mixture C:</i>			
Dibutyl ether		+	
Anisole		+	
Limonene		+	
Methyl benzoate		+	
Bornyl acetate		+	
<i>Mixture D:</i>			
Octane	+	+	+
Tridecane	+	+	+
Pentadecane	+	+	+
Heptadecane	+	+	+

very small. To investigate the applicability of the subtraction loops for detection of functional groups from a few nanograms of a component in a mixture, 10 ng of the components of mixtures A, B and D were injected (Fig. 1, Table III). The results were identical with those of the experiment in which 100 ng of each component were in-

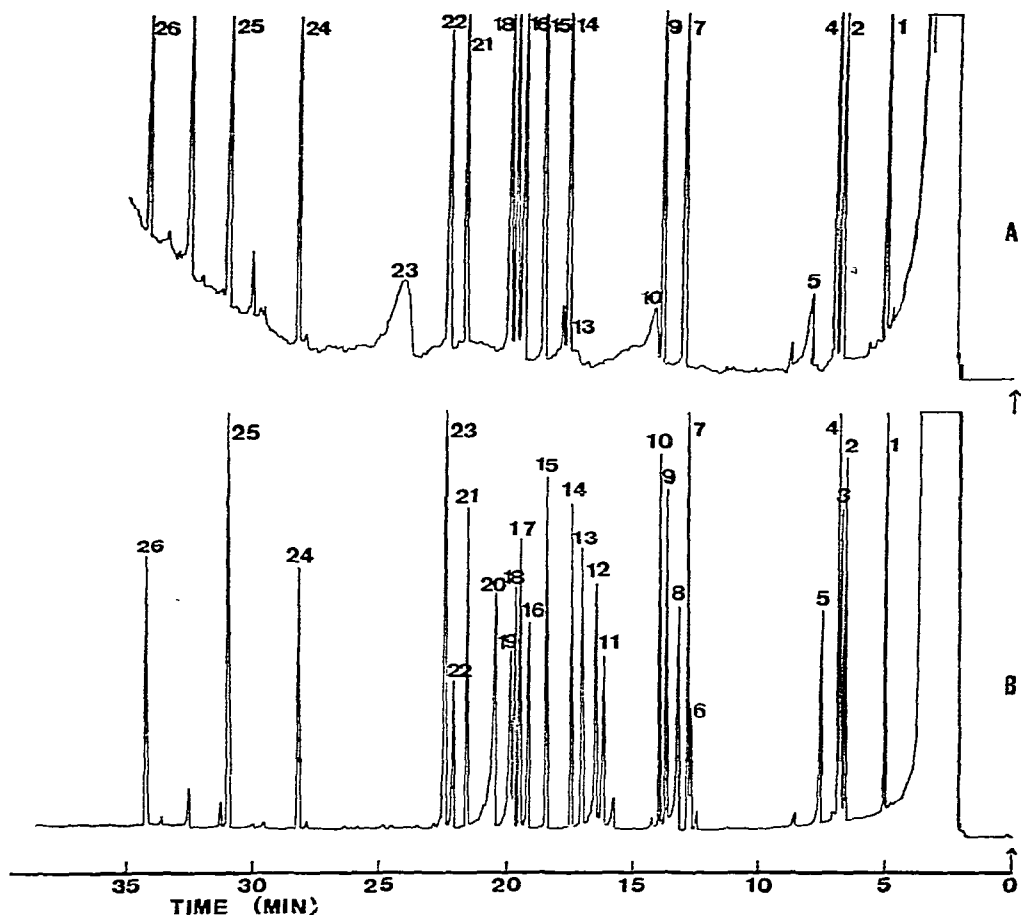


Fig. 1. Chromatograms of mixtures A, B and D (Table III) run with an OV-101 column, hydrogen as the carrier gas (0.7 kg/cm^2), 2 min at 40°C and then programmed 4°C/min to 180°C . A, Chromatogram run with H_3BO_3 subtractor column, 10 ng of each compound injected. B, Chromatogram run without subtractor column, 100 ng of each compound injected. Peaks: 1 = octane; 2 = 2-heptanone; 3 = cyclohexanol; 4 = heptanal; 5 = 2,5-hexanedione; 6 = linalool oxide; 7 = fenchone; 8 = 1-octanol; 9 = nonanal; 10 = linalool; 11 = borneol; 12 = menthol; 13 = α -terpineol; 14 = decanal; 15 = carvone; 16 = anisaldehyde; 17 = perillaldehyde; 18 = cinnamaldehyde; 19 = geraniol; 20 = 1-decanol; 21 = tridecane; 22 = piperonal; 23 = thymol; 24 = pentadecane; 25 = benzophenone; 26 = heptadecane.

jected. The present results are in accordance with those obtained with boric acid on a column packing^{2,3,7,8,12,15}, with the exception that compounds of other types were unaffected here. In addition, working at lower temperatures without a support allowed the differentiation of tertiary alcohols and phenols from primary and secondary alcohols, as well as from compounds of other types, on the basis of peak shape.

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