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PREPARATION OF NOVEL, CURABLE CAPILLARY GAS CHROMATOGRAPHIC SYSTEMS AND THEIR APPLICATION TO THE ANALYSIS OF UNDERIVATISED BARBITURATES AND OTHER CONTROLLED DRUGS OF FORENSIC INTEREST

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SUMMARY

Three novel stationary phases have been prepared for the analysis of underivatised barbiturates by incorporating different monomers, N,N′-di(but-3-enyl)amylobarbital, N,N′-di(pent-4-enyl)amylobarbital and N,N′-di(hex-5-enyl)amylobarbital into a standard SE-54 matrix. The chromatographic behaviour of these columns is compared with a standard immobilised SE-54 column. They are shown to be less polar than the previously reported N,N′-diallylamylobarbital incorporated column. Of the three new phases, the one incorporating the monomer N,N′-di(but-3-enyl)-amylobarbital shows the best performance for the separation of a mixture containing 22 barbiturates but all are selective, very efficient, inert and can be used up to at least 300°C. The versatility of these new phases is demonstrated further by the analysis of other drugs of abuse. They offer unique selectivities not previously available.

INTRODUCTION

Recent developments of capillary column technology for gas chromatography (GC) have been reviewed by several research workers[1–3]. Polar, immobilised phases such as SE-54 into which have been incorporated N,N′-diallylamylobarbital (IV) have recently been prepared in our laboratory[4]. These phases are efficient, selective, thermally stable and exhibit very good tailing behaviour. We now report the preparation of three new, crosslinkable selective stationary phases by incorporating different monomers, N,N′-di(but-3-enyl)amylobarbital (I), N,N′-di(pent-4-enyl)amylobarbital (II) or N,N′-di(hex-5-enyl)amylobarbital (III), into an SE-54 matrix. Their chromatographic performance was evaluated by the use of Grob test mixtures, while the selectivity towards the separation of underivatised barbiturates was tested with a standard barbiturate mixture. The versatility of these columns can be demonstrated further by the analysis of other controlled drugs, especially the benzodiazepines.

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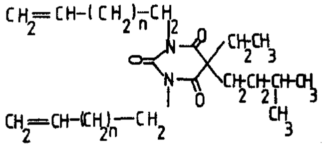

I, n = 1; IIm, n = 2; III, n = 3; IV, n = 0.

EXPERIMENTAL

Characterisation of barbiturate monomers

Unless otherwise stated, NMR spectra were recorded in deuterated chloroform solution using a Perkin-Elmer R32 NMR spectrometer, and are given in parts-per-million (δ) downfield from an internal tetramethylsilane (TMS) standard. The abbreviations s, d, t, q and m refer to singlet, doublet, triplet, quarted and multiplet peaks, respectively.

Infrared (IR) spectra were recorded on a Perkin Elmer 157 prism spectrophotometer. The abbreviations s, m and w refer to strong, medium and weakly absorbing peaks, respectively.

Synthesis of N,N'-di(but-3-enyl)amylobarbital (I)

To a stirred solution of 2 g of amylobarbital (May and Baker, Dagenham, U.K.) in 10 ml of acetone (distilled) in a round-bottomed flask were added 3 ml of (*ca.* 2.5 equiv.) triethylamine (freshly distilled). To this were added dropwise 4 g, (*ca.* 3 equiv.) of 4-bromo-1-butene (Aldrich). The reaction mixture was stirred overnight at room temperature. The white precipitate formed was dissolved in 50 ml of water and the reaction mixture extracted into diethyl ether (3 times 50 ml), washed with 50 ml of 1 *M* sodium hydroxyde, 50 ml of water, dried (anhydrous sodium sulphate) and filtered. The solvent was removed under vacuum to obtain a pale yellow oil. After purification by column chromatography [silica gel 60; acetone–chloroform (3:7, v/v)], a colourless oil [1.6 g, 56%, R_F 0.56; acetone–chloroform (3:7, v/v)] was obtained.

IR (film): 3050 cm^{-1} (m, =C–H stretch), 1700 cm^{-1} (s, C=O), 1000 and 840 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3) δ 1.80–2.20 (m, 4H, CH_2–C=C), δ 3.80–4.20 (t, 4H, CH_2–N), δ 4.80–5.30 (m, 4H, CH_2=C), δ 5.40–6.10 (m, 2H, CH=C).

$C_{19}H_{30}N_2O_3$ requires: C = 68.26, H = 8.98, N = 8.38; found: C = 67.90, H = 9.15, N = 8.65.

Synthesis of 5-bromo-1-pentene

To a stirred solution of 10 g of 4-penten-1-ol (Aldrich) at −10°C in a three-necked round-bottomed flask was added 4.5 g (0.33 equiv.) of phosphorus tribromide (Aldrich) dropwise over a period of 5 min, moisture being kept out by means of a Drierite-filled tube. The reaction mixture was stirred overnight at room temperature. After pouring into 50 ml of ice-water, the reaction mixture was extracted into diethyl ehter (3 times 50 ml), washed with 50 ml of water, 10% (w/v) sodium carbonate solution (2 times 100 ml), 50 ml of water, dried (anhydrous sodium sulphate) and filtered. The solvent was removed under vacuum and the residue was distilled to give

5-bromo-1-pentene as a colourless liquid [7.2 g; 42%; R_F 0.30; diethyl ether–chloroform (2:8, v/v)].

IR (film): 3050 cm^{-1} (m, =C–H), 1640 cm^{-1} (m, C=C), 1000 and 920 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3): δ 1.00–2.40 (m, 4H, CH_2–CH_2), δ 3.20–3.50 (t, 2H, CH_2–Br), δ 4.80–5.20 (m, 2H, CH_2=C), δ 5.50–6.10 (m, 1 H, CH=C).

C_5H_9Br requires: C = 40.29, H = 6.04, Br = 53.65; found: C = 40.04, H = 6.30, Br = 53.35.

Synthesis of N,N'-di(pent-4-enyl)amylobarbital (II)

A solution of 2 g of amylobarbital in 10 ml of acetone, 3 ml of triethylamine and 4 g of 5-bromo-1-pentene was treated under the same conditions as described for the synthesis of I to give II as a colourless oil [1.6 g; 52%, R_F 0.56; diethyl ether–chloroform (3:7, v/v)].

IR (film): 3050 cm^{-1} (m, =C–H stretch), 1700 cm^{-1} (s, C=O), 1000 and 840 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3):δ 1.80–2.20 (m, 4H, CH_2–C=C), δ 3.80–4.20 (t, 4H, CH_2–N), δ 4.80–5.20 (m, 4H, CH_2=C), δ 5.50–6.20 (m, 2H, CH=C).

$C_{21}H_{34}N_2O_3$ requires: C = 69.61, H = 9.39, N = 7.73; found: C = 69.38, H = 9.62, N = 7.95.

Synthesis of 6-bromo-1-hexene

Amounts of 10 g of 5-hexen-1-ol (Aldrich) and 8.98 g (0.33 equiv.) of phosphorus tribromide (Aldrich) were treated under the same conditions as described for the synthesis of 5-bromo-1-pentene to give 6-bromo-1-hexene as a colourless liquid [6.3 g; 39%, R_F 0.33 diethyl ether–chloroform 2:8, v/v)].

IR (film): 3050 cm^{-1} (m, =C–H), 1640 cm^{-1} (m, C=C), 1000 and 920 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3): δ 1.40–2.20 (m, 6H, CH_2–CH_2–CH_2), δ 3.30–3.50 (t, 2H, CH_2–Br), δ 4.80–5.20 (m, 2H, CH_2=C), δ 5.50–6.10 (m, 1H, CH=C).

$C_6H_{11}Br$ requires: C = 44.20, H = 6.75, Br = 49.05, found: C = 43.95, H = 7.04, Br = 48.90.

Synthesis of N,N'-di(hex-5-enyl)amylobarbital (III)

A solution of 2 g of amylobarbital in 10 ml of acetone, 3 ml of triethylamine and 4 g of 6-bromo-1-hexene was treated under the same conditions as described for the synthesis of I to give III as a colourless oil [1.8 g; 54%, R_F 0.44; diethyl ether–chloroform (2:8, v/v)].

IR (film): 3050 cm^{-1} (m, =C–H stretch), 1700 cm^{-1} (s, C=O), 1000 and 940 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3): δ 1.80–2.20 (m, 4H, CH_2–C=C), δ 3.80–4.20 (t, 4H, CH_2–N), δ 4.80–5.20 (m, 4H, CH_2=C), δ 5.50–6.20 (m, 2H, CH=C).

$C_{23}H_{38}N_2O_3$ requires: C = 70.76, H = 9.74, N = 7.18; found: C = 70.55, H = 9.96, N = 7.42.

Synthesis of N,N'-diallylamylobarbitaol (IV)

The monomer IV was synthesised by diallylation of 5-ethyl-5-isoamyl barbituric acid (May and Baker) with allyl bromide according to a procedure described[5].

Column preparation

Glass capillaries (0.23 mm I.D. and 0.80 mm O.D.) were drawn from Pyrex glass tubes (3.5 mm I.D. and 6.0 mm O.D.) on a Carlo Erba GCDM 60 glass-drawing machine. Cleaning of glass tubing, leaching followed by rinsing and dehydration were carried out as described earlier[5]. Persilylation was performed with divinyltetramethyldisilazane (DVTMDS)[4].

A solution for static coating was prepared by first dissolving SE-54 (0.29%, Phasesep) in pentane (AnalaR grade; BDH) and then adding 30% (w/w) of the synthesised unsaturated barbiturate monomer (I, II, III or IV) [2% (w/w) in methylene chloride]. Finally, 2.5% (w/w) dicumyl peroxide (DCUP) (2% in toluene) was added, with the exception of the column incorporating IV in which 1.5% (w/w) DCUP was used. Columns were statically coated as described earlier[5] and 2 m at both ends were kept free of coating. After static coating, the columns were flushed with dry nitrogen for 1 h and cured according to the method of Blomberg *et al.*[6] in which the capillary was sealed under vacuum and then placed in an oven at 140°C for 30 min. The oven was cooled rapidly and each column flushed with dry nitrogen for 3 h. The ends of the columns were straightened using a Carlo Erba GESM 102-20 automatic electrical end-straightening machine under a flow of dry nitrogen. The columns were conditioned at 300°C overnight and test run. Finally, each column was washed with 5 ml methylene chloride (AnalaR grade; BDH), dried in a stream of nitrogen and reconditioned at 300°C before re-testing.

Preparation of the human urine standard spiked with ten drugs

A urine sample was spiked with amphetamine, ephedrine, phendimetrazine, benzocaine, procaine, methaqualone, cocaine, codeine, ethyl morphine and morphine. The urine standard was prepared by a simple, one-step extraction with methylene chloride from the sample which was made alkaline with ammonium hydroxide, the organic layer dried, evaporated and reconstituted with ethyl acetate. A portion of the reconstituted material was injected onto the column.

Gas chromatography

Capillary GC was performed with a standard Hewlett Packard Model 5710A gas chromatograph modified for capillary GC analysis and equipped with a flame ionisation detector (air, 240 ml/min; hydrogen 30 ml/min). The carrier gas was nitrogen with injector and detector temperatures of 300°C. A split ratio of 50:1 was used. Nitrogen was used as the make-up gas (20 ml/min).

RESULTS AND DISCUSSION

Chromatograms of Grob test mixtures for SE-54 into which has been incorporated N,N′-di(but-3-enyl)amylobarbital (I), N,N′-di(pent-4-enyl)amylobarbital (II) and N,N′-di(hex-5-enyl)amylobarbital (III) show that the column incorporating monomer (I) is more polar than the column incorporating monomer II which in turn is more polar than the column incorporating III. This means that increasing the carbon chain length of the unsaturated substituents linked to the nitrogen atom of the incorporated monomer leads to a decrease in the polarity of the resultant phase. Columns incorporating monomers I and II are slightly basic in character whereas the

TABLE I

CHARACTERISTICS OF THE NEW STATIONARY PHASES PREPARED

*Stationary phase**	*TZ*	*k' for hexadecane at 140°C*	*HETP** (mm) for hexadecane at 140°C*	*CE*	*Decrease in k' of hexadecane at 140°C after rinsing with solvent (%)*
SE-54 + 30% I	27.3	5.75	0.23	85.2	3.5
SE-54 + 30% II	27.4	4.89	0.24	80.4	3.9
SE-54 + 30% III	27.4	5.65	0.22	89.2	4.0

* All columns are of the same dimensions: 15 m × 0.23 mm I.D.
** Height equivalent to a theoretical plate.

column incorporating monomer III is slightly acidic. All three phases show very good tailing behaviour and are very efficient as evidenced by their high Trennzahl (TZ) values (Table I). They also show very high coating efficiencies (CE) and can routinely be used up to at least 300°C. All phases were immobilised and extraction with methylene chloride led to a decrease in capacity factor (k') values of only *ca.* 3.5–4.0%. This reflected the observed minor differences in chromatographic performance before and after the dichloromethane washings of the prepared columns.

The separation of a mixture of 22 underivatised barbiturates commonly encountered in toxicological cases on a standard SE-54 column and the four columns incorporating the different monomers are shown in Fig. 1. It is apparent that the

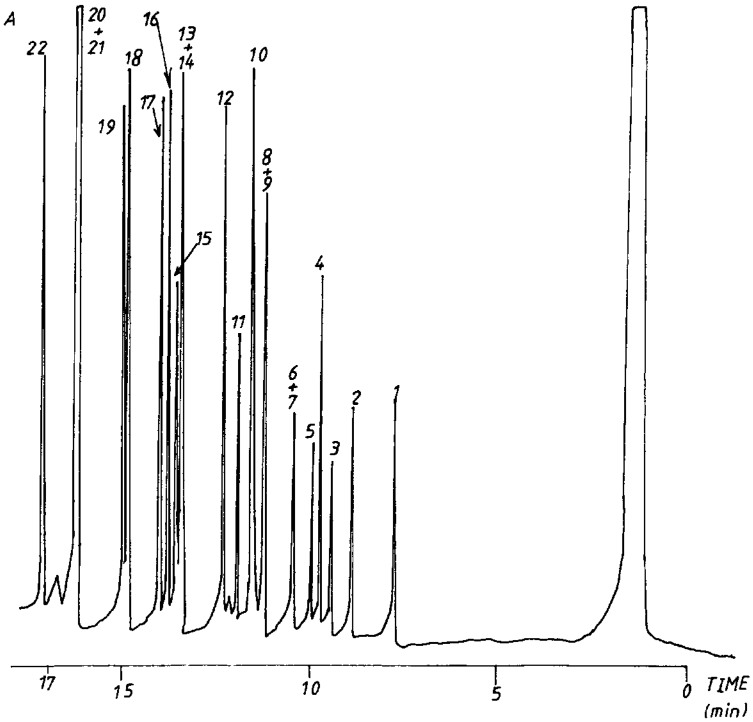

Fig. 1.

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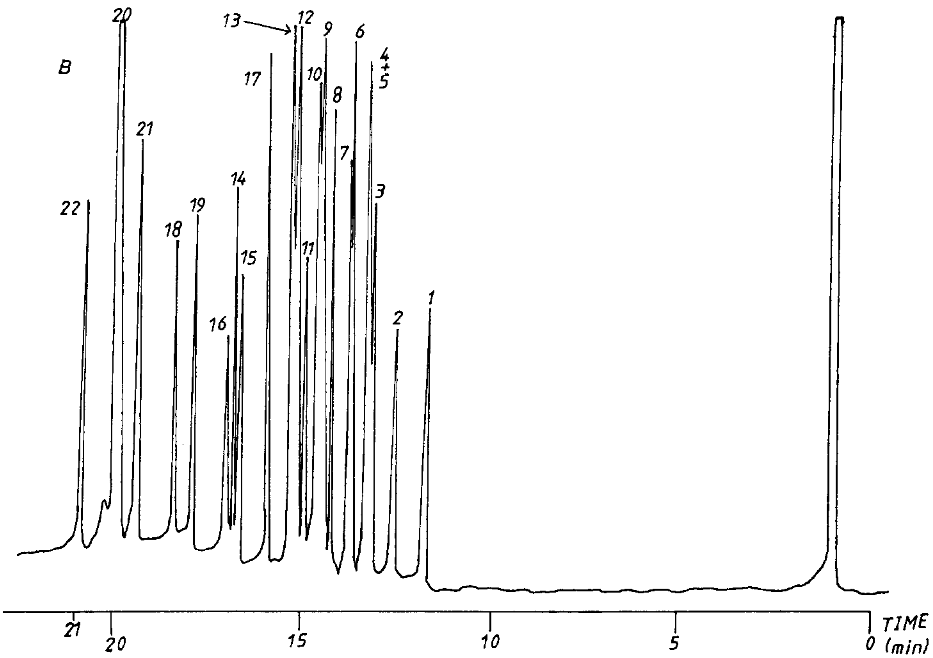
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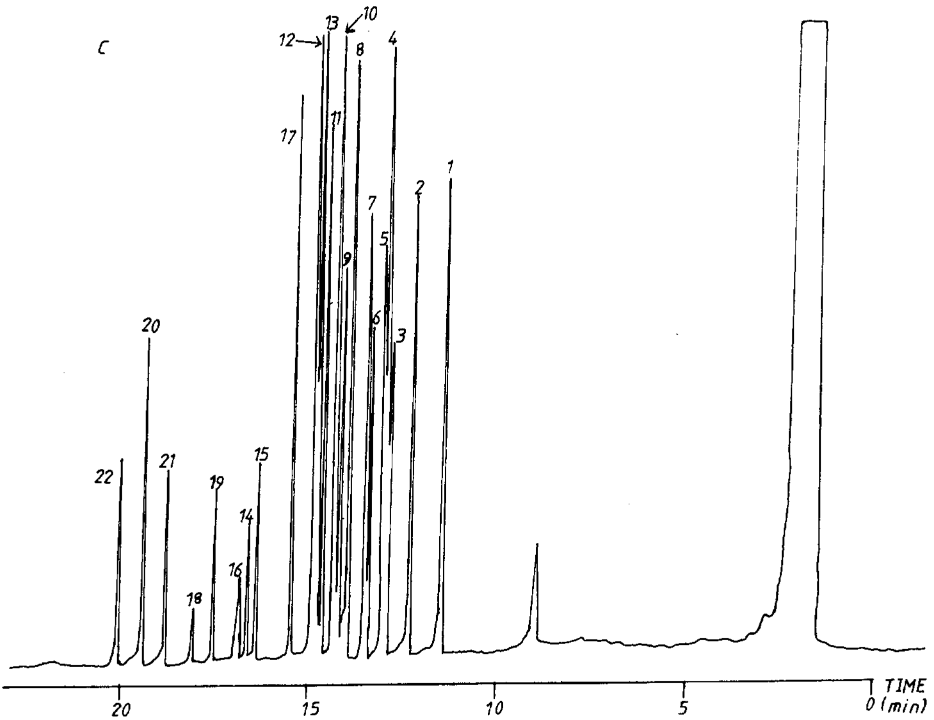
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Fig. 1.

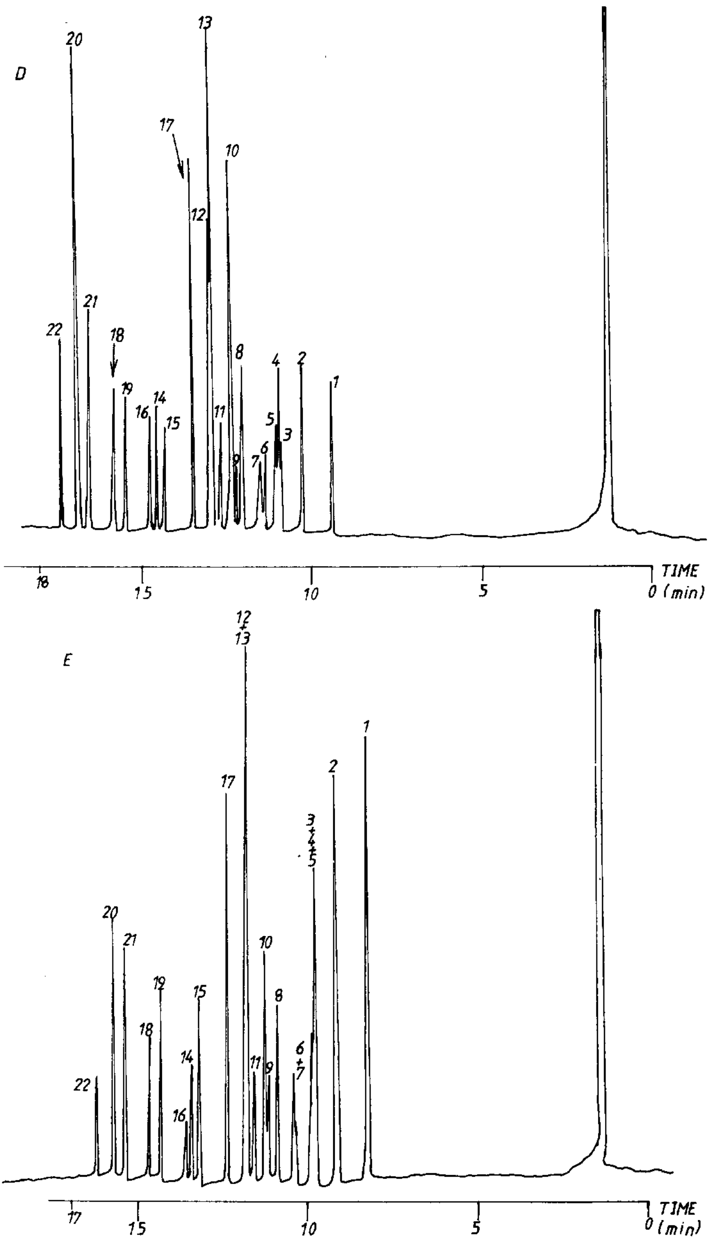


Fig. 1. Separation of a mixture containing 22 barbiturates commonly encountered in toxicological cases on five different columns. Chromatographed at 120°C for 2 min, programmed at 8°C/min to 240°C and held at the upper temperature for 5 min. Chart speed: 600 mm/h. (A) SE-54 column; (B) SE-54 column with 30% IV incorporated; (C) SE-54 column with 30% I incorporated; (D) SE-54 column with 30% II incorporated; (E) SE-54 column with 30% III incorporated. Peaks: 1 = barbital; 2 = probarbital; 3 = allobarbital; 4 = aprobarbital; 5 = ethylcrotylbarbital; 6 = butobarbital; 7 = butalbital; 8 = amylobarbital; 9 = nealbarbital; 10 = pentobarbital; 11 = vinbarbital; 12 = quinalbarbital; 13 = hexobarbital; 14 = brallobarbital; 15 = cyclopentobarbital; 16 = ibomal; 17 = methylphenylbarbital; 18 = phenobarbital; 19 = cyclobarbital; 20 = 5-ethyl-5-tolylbarbital; 21 = heptabarbital; 22 = reposal.

different phases show differences in selectivities towards the barbiturates. The SE-54 column is capable of resolving only 18 out of the 22 barbiturates and the column incorporating monomer III was also unable to resolve all the barbiturates. The column incorporating monomer IV resolves 21 out of 22 but the best resolution was provided by the one incorporating monomer I in which some resolution of all 22 barbiturates was demonstrated over 20 min.

The versatility of the new phases is further demonstrated by the analysis of some controlled drugs which are frequently abused. The best separation of six benzodiazepines is illustrated in Fig. 2 for the new phase incorporating monomer III and is compared with the result obtained using a standard SE-54 column. Different selectivities were shown for the separation of the benzodiazepines using columns incorporating the different monomers as illustrated by the different order of elution.

The separation of some common central nervous system stimulant drugs on the column incorporating monomer I is shown in Fig. 3, while several important drugs of abuse extracted from a human urine, as would be the case for a drug screen, is illustrated in Fig. 4. All components were resolved using either the columns incorporating monomers I or II although the separations of methaqualone and cocaine (peaks 6 and 7, respectively) and of codeine and ethyl morphine (peaks 8 and 9, respectively) were slightly better in the latter column.

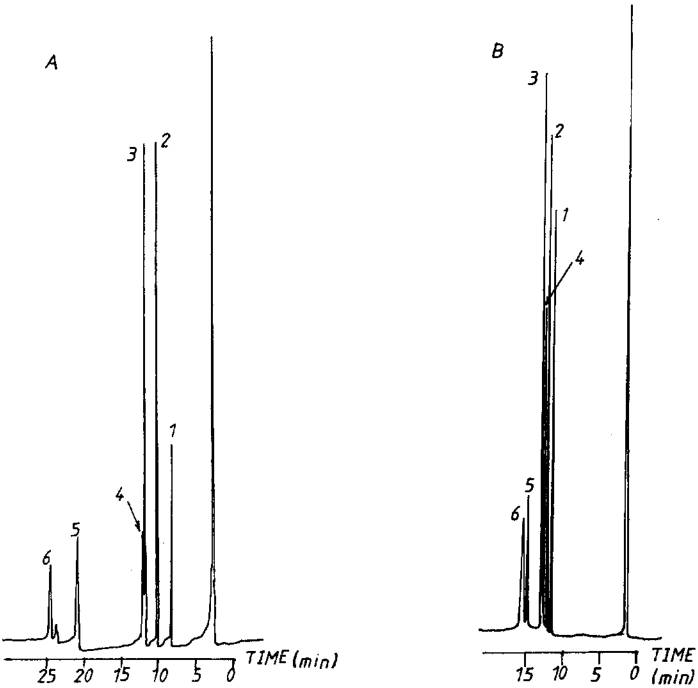
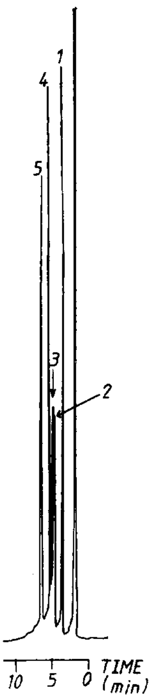


Fig. 2. Separation of six commonly abused benzodiazepines (chart speed: 120 mm/h). (A) SE-54 column, chromatographed at 250°C for 16 min, programmed at 1°C/min to 260°C. (B) SE-54 column incorporating monomer III, chromatographed at 130°C for 2 min, programmed at 16°C/min to 300°C and held at the upper temperature for 5 min. Peaks: 1 = oxazepam; 2 = diazepam; 3 = desmethyl diazepam; 4 = librium; 5 = nitrazepam; 6 = clonazepam.



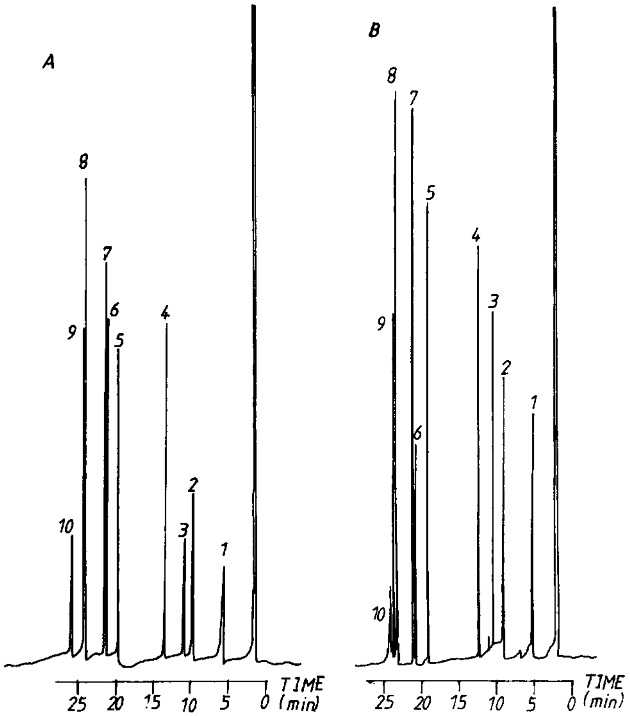


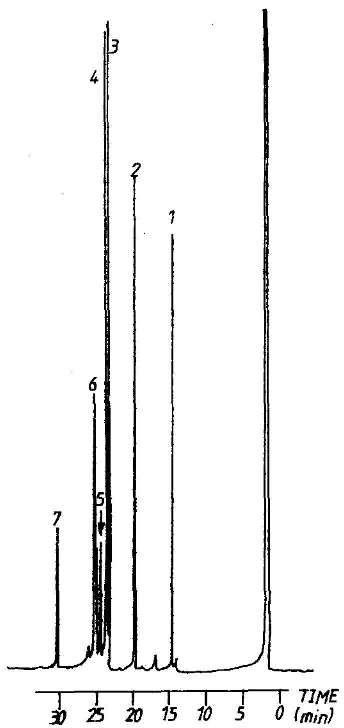
Fig. 3. Separation of some common central nervous system stimulants on the column incorporating monomer I. Oven temperature: isothermal at 120°C. Chart speed: 120 mm/h. Peaks: 1 = cyclopentamine; 2 = norfenfluramine; 3 = phentermine; 4 = methylamphetamine; 5 = ethylamphetamine.

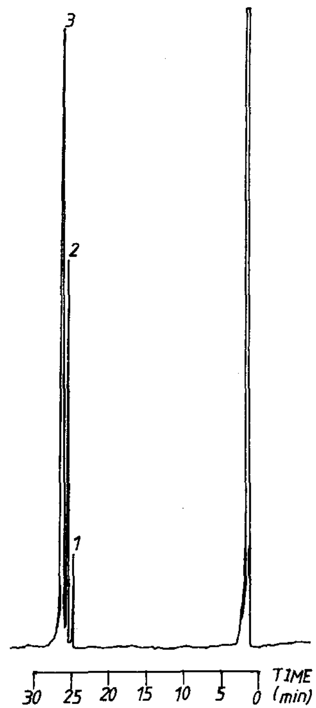
Fig. 4. Separation of several important drugs of abuse in a human urine drug screen. (A) Column incorporating monomer I; (B) column incorporating monomer II. Chromatographed at 100°C for 2 min, programmed at 8°C/min to 280°C and held at the upper temperature for 5 min. Chart speed: 120 mm/h. Peaks: 1 = amphetamine; 2 = ephedrine; 3 = phendimetrazine; 4 = benzocaine; 5 = procaine; 6 = methaqualone; 7 = cocaine; 8 = codeine; 9 = ethylmorphine; 10 = morphine.

Narcotic analgesics, without derivatisation, can be resolved on a column incorporating monomer I (Fig. 5) and underivatised tricyclic antidepressants can also be resolved on this same column.

The usefulness of these columns is indicated by Fig. 6 which shows the forensic analysis of a "street" heroin sample. Separation of heroin from the impurities, without derivatisation, was achieved using the column incorporating monomer I. Information derived from such impurities can be used to help trace the heroin source[7].

The new phases are immobilised which means that they are also suitable for samples presented in aqueous solution since the immobilised phase cannot be displaced from the capillary surface by water. Any non-volatile material deposited in the column inlet system can also be removed by solvent rinsing, provided that any contamination is soluble. It must be borne in mind that injection of acids and bases together with water can lead to hydrolysis of the stationary phase, a problem also known to occur with HPLC silanized silica. The chromatogram of an aqueous solution of LSD 25 (0.1 mg LSD 25 tartrate in 1 ml water) is demonstrated in Fig. 7





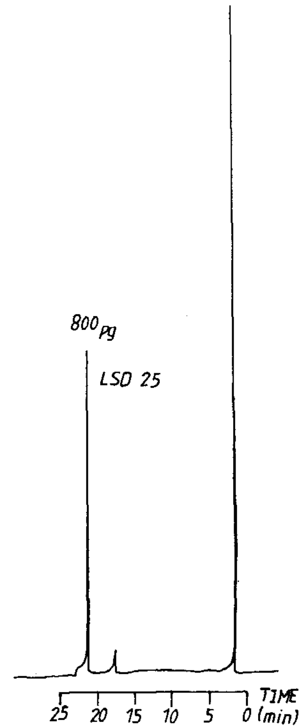


Fig. 5. Total separation of some common narcotic analgesics on the column incorporating monomer I. Chromatographed as in Fig. 4. Chart speed: 120 mm/h. Peaks: 1 = pethidine; 2 = methadone; 3 = dipipanone; 4 = codeine; 5 = morphine; 6 = heroin; 7 = dextromoramide.

Fig. 6. Forensic analysis of a "street" heroin sample in methanol on the column incorporating monomer I. Chromatographed as in Fig. 4. Chart speed: 120 mm/h. Peaks: 1, 2 = impurities; 3 = heroin.

Fig. 7. Chromatogram of an aqueous solution of LSD 25 (0.1 mg LSD 25 tartrate in 1 ml water) on the column incorporating monomer I. Chromatographed as in Fig. 4. Chart speed 120 mm/h. Attenuation: 1 × 256. Injection volume: 0.4 μl.

TABLE II

DRUG LIBRARY FOR THE COLUMN INCORPORATING MONOMER I

GC conditions: chromatographed at 100°C for 2 min, programmed at 8°C/min to 280°C and held at the upper temperature for 5 min. Chart speed: 120 mm/h.

No.	*Drug*	t_R*	*No.*	*Drug*	t_R*
1	Amphetamine	11.0	11	LSD 25	41.8
2	Ephedrine	19.0	12	Methaqualone	42.0
3	Phendimetrazine	21.5	13	Cocaine	42.8
4	Benzocaine	26.5	14	Desipramine	43.0
5	Pethidine	28.5	15	Dipipanone	46.0
6	Methadone	39.0	16	Codeine	47.5
7	Procaine	39.5	17	Ethylmorphine	48.5
8	Imipramine	41.0	18	Morphine	49.5
9	Trimipramine	41.0	19	Heroin	50.5
10	Nortriptyline	41.5	20	Dextromoramide	60.0

* Absolute retention time (mm).

on the column incorporating monomer I. The injection volume was 0.4 μl which enables *ca.* 800 pg of the hallucinogen to enter the column. The drug shows good peak shape and indicates the potential of such a column for forensic analysis.

Finally, it should be mentioned that the same temperature programme rate was employed for the separation of the human urine drug screen, the tricyclic antidepressants, the narcotic analgesics, the "street" heroin sample and the aqueous sample of LSD 25 on the column incorporating monomer I, hence a drug library, if desired, can easily be generated for identification purposes (Table II).

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