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Applicability of Capillary Gas Chromatography to Substance Identification in Toxicology by Means of Retention Indices

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ABSTRACT: Three capillary columns, set up in a routine screening system, were tested in temperature-programmed runs. A narrow-bore fused silica capillary, Carbowax-deactivated and with a methylsilicone liquid phase, was found to be unstable at higher temperatures, giving irreproducible results and retention indices that varied considerably from those obtained on packed columns. The two other columns, a wide-bore glass capillary and a narrow-bore fused silica capillary, were polysiloxane-deactivated and had a dimethylpolysiloxane liquid phase. Although both showed good stability, reproducibility, and load capacity, retention indices for various drugs still showed discrepancies as compared to corresponding values on packed columns.

KEYWORDS: toxicology, chromatographic analysis, drug identification, systematic drug screening, capillary gas chromatography, retention indices

Gas-liquid chromatography (GLC) has proven to be an indispensable tool in screening for the presence of drugs in systematic toxicological analysis (STA). Owing to the work of Moffat and co-workers [*1,2*], it is now generally accepted that dimethylsilicone stationary phases like SE-30 and OV-1 provide optimum discriminating power. In addition, it has been shown that measurement of the retention indices [*3*] is the technique of choice for substance characterization as well as for the compilation of gas chromatographic data in a data bank and the exchange of those data between different laboratories [*2,4-11*]. The retention index (RI) of a substance on a given stationary phase can be considered a physical parameter of reasonable constancy, provided the method is adequately standardized. The interlaboratory standard deviation of measurement is usually between 15 and 20 RI units [*2,10,12*]. Although RI's are temperature-dependent [*10*], it has been shown that those obtained in a temperature-programmed run are usually in good agreement with those determined under isothermal conditions [*7,8*].

So far, almost all RI data for toxicological analysis have been obtained on conventional, packed, glass columns, with an inner diameter of 2 to 5 mm and with the stationary phase coated on relatively inert supports such as Chromosorb. Although open capillary columns were introduced in 1957, their use in toxicology has remained rather limited, probably

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because of their high cost, fragility, and limited load capacity. However, recent years have been a period of innovation with regard to column technology, resulting in a new generation of glass capillary columns and the so-called fused silica and fused quartz columns, which combine high separation power with good column stability, flexibility, load capacity, and so on [*13,14*]. These newer capillary columns are made from high purity materials that are relatively inert to susceptible solute molecules and exhibit a smooth surface. Although the content of total metal oxides is less than 1 ppm, to obtain optimum results deactivation of the wall surface is still necessary, the most common procedures being treatment with polyethylene glycol (Carbowax) or silylation. A second advantage of deactivation procedures is that they improve the wettability of the wall surface for the stationary phase. Although various deactivation procedures have been described [*14,15*], it should be realized that their performance requires a considerable amount of experience. For that reason, most toxicological laboratories would have to buy their capillary columns from commercial sources.

The present study was undertaken to evaluate the applicability of some of the new capillary columns for toxicological screening purposes. We chose to work with temperature-programmed runs to diminish analysis time, and special attention was paid to column stability, day-to-day reproducibility, load capacity, and the degree of agreement between RI's determined on capillary and packed columns with similar stationary phases.

Materials and Methods

The drugs used in this investigation were obtained from commercial suppliers and were used as received. All were dissolved in methanol to give solutions of approximately 0.5 mg/mL, of which 1-μL aliquots were injected. Straight chain alkanes (C_{11} to C_{32}) were used as references for the calculation of retention indices [*3*], with each dissolved in hexane methanol (99:1) to give a solution of about 0.5 mg/mL. Aliquots of 1 μL were injected into an HP 5880 gas chromatograph (Hewlett-Packard) with a splitless capillary injection system. Injections were performed with a HP 7671 A automatic injector. The columns and their operating conditions were as follows:

1. A Carbowax® 20 M-deactivated fused silica narrow-bore capillary column coated with methylsilicone fluid [*16*] was obtained from Hewlett-Packard. The column was 12 m in length and had an internal diameter of 0.20 mm, a film thickness of 0.12 μm, a coating efficiency of 72%, 4200 theoretical plates per meter (C_{15}), and a capacity ratio of 6.1. The maximum operating temperature was given as 280°C. During the first 20 days of this study, the temperature program of the oven was 2 min at 120°C, 8°C/min to 260°C, and then 8 min at 260°C; the injector and detector temperatures were 275°C. During the second part, the program was 2 min at 100°C, 8°C/min to 250°C, and then 15 min at 250°C, with the injector and detector at a temperature of 250°C.

2. The polysiloxane-deactivated glass, wide-bore capillary column, which was coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was obtained from Chrompack (Middelburg, The Netherlands). The column was 25 m in length and had an inner diameter of 0.49 mm, a film thickness of 1.14 μm, a coating efficiency of 90%, 2060 theoretical plates per meter (C_{14}), and a capacity ratio of 8.6. The upper temperature limit for isothermal use is given as 325°C and 350°C for temperature programming. With the injector and detector temperatures at 275°C, the temperature program for the oven was 2 min at 100°C, 8°C/min to 275°C, and then 15 min at 275°C.

3. The polysiloxane-deactivated fused silica narrow-bore capillary column coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was also obtained from Chrompack. The column was 12 m in length and had an inner diameter of 0.22 mm, a film thickness of 0.45 μm, a coating efficiency of 95%, 5160 theoretical plates per meter, and a capacity ratio of 7.0. The upper temperature limits were as under Condition 2. With the injector and detector temperatures at 300°C, the temperature program for the oven was 2 min

at 120°C, 8°C/min to 300°C, and then 12 min at 300°C. Helium was used throughout as carrier gas and the precolumn pressure was set such that the C_{32} alkane reference had a retention time of not more than 35 min. The temperature programs were chosen so that a nearly straight line was obtained when the carbon numbers of the reference alkanes were plotted versus their retention times. Flame ionization detectors were used.

The capillary data given in the figures represent the results of single determinations; those in the table represent the means of at least three determinations, with the individual observations differing by not more than ±5 RI-units. The P column data in the figures were obtained on packed SE-30 or OV-1 columns and were taken from Ref *12*.

Results

The first column tested was a fused silica wall-coated open tubular column with an internal diameter of 0.20 mm [*16*]. The stationary phase is described by the manufacturer as a methylsilicone fluid comparable to SE-30 and OV-1. Deactivation is achieved by thermally degrading Carbowax 20 M and feeding the pyrolysis products through the columns. The column is then extracted with solvents, but unextractable material remains on the column, resulting in substantial deactivation and increased wettability for the stationary phase [*18,19*]. Methylsilicone columns treated in this way are claimed to be thermally stable up to 280°C.

When first testing these columns during routine day-to-day operation for 24 h per day, seven days a week, it soon became apparent that the retention times of the reference alkanes were not constant (see Fig. 1A). The increase in retention time suggested that the column was becoming less polar with time of operation. This was presumed to be the result of the temperatures of the injection port and the detector being too high (both at 275°C), resulting in bleed-off of the Carbowax deactivation material at both ends of the columns. After 20 days of operation we then shortened each of the two ends 20 cm and lowered the injector and

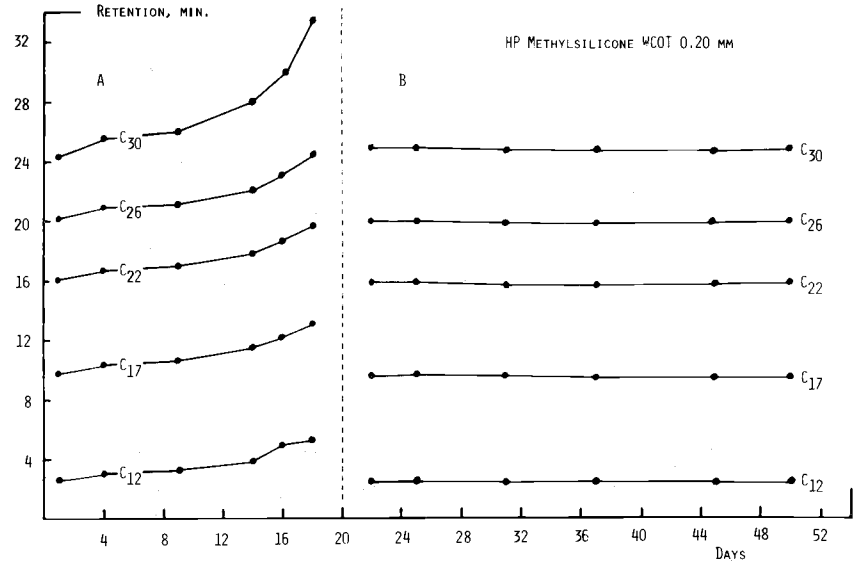

FIG. 1—*Retention behavior of reference alkanes on a Carbowax-deactivated methylsilicone fused silica column in temperature-programmed runs:* (A) *injector and detector temperatures 275°C, maximum oven temperature 260°C;* (B) *after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

detector temperatures to 250°C. The maximum temperature of the oven was also reduced to 250°C. As shown in Fig. 1B, after these changes had been made the alkane retention times remained constant for the rest of the testing procedure.

Figures 2 and 3 show the time versus retention behavior of a selection of drugs, expressed in terms of RI. Different tendencies can be observed. During the first 20 days the 5,5-disubstituted barbiturates showed declining RI's, which seemed to be less pronounced with the *N*-methylated derivatives hexobarbital and metharbital. Other substances, such as caffeine, benzocaine, bromisoval, bemegride, and acetylsalicylic acid, yielded fairly constant RI's, whereas some benzodiazepines with higher RI's showed some variation. Even after we lowered the injector and detector temperatures, RI's decreased for most substances, with some of the barbiturates and clonazepam giving somewhat more pronounced decreases.

Comparison of RI's measured on capillary columns with those obtained on normal packed columns (P in Figs. 2 and 3) clearly indicate marked differences. The 5,5-disubstituted barbiturates all have lower RI's on packed columns; the 1,5,5-trisubstituted barbiturates have quite comparable values, as do caffeine, benzocaine, and bemegride; the diazepines show some variations, with bromisoval and acetylsalicylic acid having higher RI's on packed methylsilicone columns than on Carbowax-deactivated ones.

The CP-Sil 5 wide-bore glass column was treated with polysiloxane according to the procedure of Houtermans and Boodt [*17*]. The upper temperature limit for the deactivation material is claimed to be 350°C. The stationary phase is dimethylpolysiloxane prepared specially from normal SE-30 and is stable to at least 325°C [*17*]. Its retention characteristics are similar to those of SE-30 or OV-1.

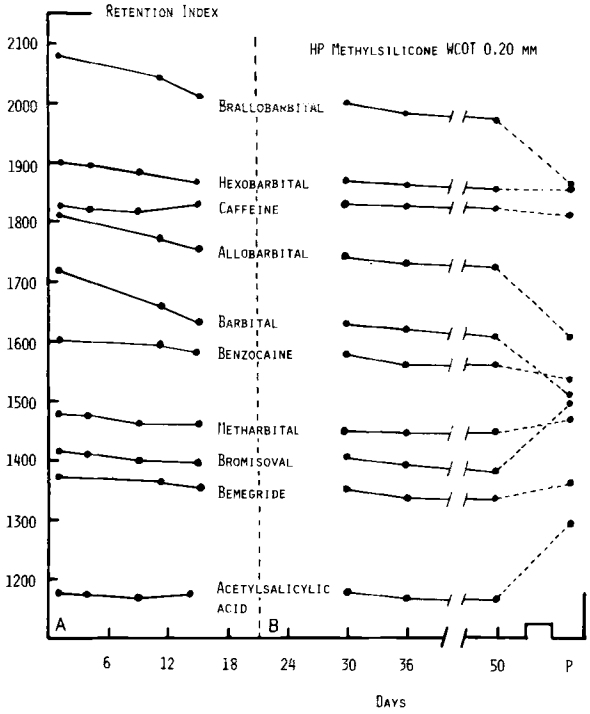

FIG. 2—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P):* (A) *injector and detector temperatures 275°C, maximum oven temperature 260°C;* (B) *after removing 20 cm at both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

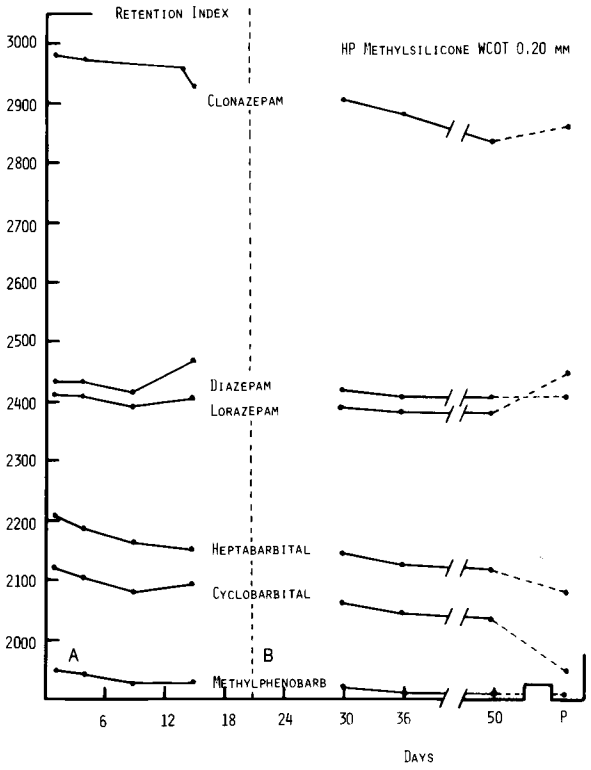

FIG. 3—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P):* (A) *injector and detector temperatures 275°C, maximum oven temperature 260°C;* (B) *after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

As can be seen in Fig. 4, retention times of the reference alkanes were constant over the entire test period of 60 days of continuous operation. The time versus RI of a selection of drugs is depicted in Figs. 5 and 6. It can be observed that RI's are relatively constant, the variations staying within acceptable limits of ± 5 RI units (open circles) for most compounds, and never exceeding ± 10 units. The majority of substances tested showed higher RI's on packed SE-30 or OV-1 columns, with the exception of two benzodiazepines, nitrazepam and diazepam. This trend was also found with some 120 other drugs: the majority gave higher values on packed columns, sometimes amounting to more than 40 RI's, but other substances showed the reverse (see Table 1).

The third column, which could only be tested during the latter part of our investigations, was a fused silica narrow-bore CP-Sil 5 capillary, deactivated with polysiloxane as described above. The stationary phase had a relatively large layer thickness of 0.45 μm to ensure adequate load capacity for biological samples. Over a period of four weeks of continuous operation, the RI's of the reference alkanes showed excellent reproducibility, comparable to that on the wide-bore CP-Sil 5 column. The RI's of the drugs investigated also showed good agreement with those measured on the wide-bore column, as is demonstrated in Figs. 5 and 6 and in Table 1. However, RI's determined on this fused silica capillary showed some discrepancies with those on packed columns similar to those mentioned for the wide-bore glass CP-Sil 5 capillary. Figure 7 depicts an actual chromatogram taken from case work on the narrow-bore fused silica CP-Sil 5 capillary, illustrating the excellent separation efficiency

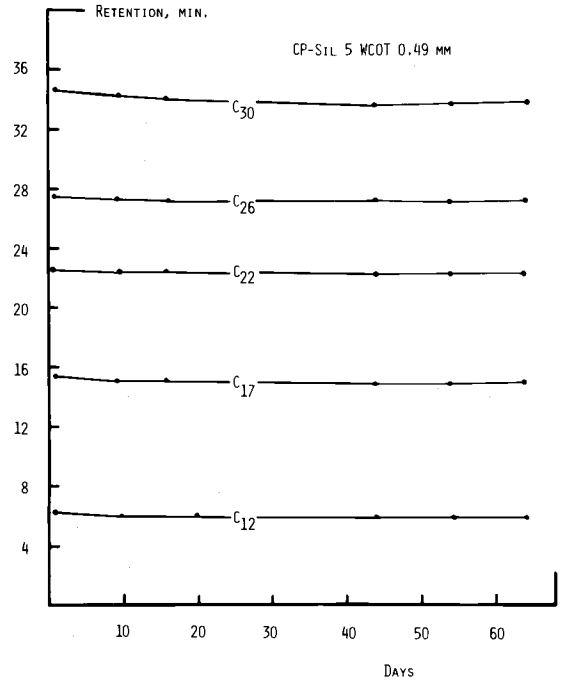

FIG. 4—*Retention behavior of reference alkanes on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) glass capillary column in temperature-programmed runs.*

(note that acetylcodeine and 6-monoacetylmorphine show almost baseline separation in this programmed run), the narrow peak shape, and the nearly flat baseline.

Discussion

This investigation has clearly demonstrated that the maximum operation temperature of the Carbowax-deactivated column was a rather critical factor to separation efficiency. Although the temperatures were kept within the limitations recommended by the manufacturer, high temperatures at the injector and detector ports apparently caused significant bleedoff of the deactivation material in the adjoining column ends. By lowering the maximum oven temperature and that of the injector and detector to 250°C, the bleedoff could be virtually eliminated. It should be noted, however, that this temperature is too low to obtain full, effective use for STA because of the prolonged analysis time required. Moreover, the RI's obtained on this type of capillary column were found to be quite different from those obtained on comparable packed columns. These discrepancies were more pronounced in certain drug classes yet did not show a clear and predictable pattern. This might have been due to the fact that the Carbowax deactivation material and the methylsilicone liquid phase acted as a mixed stationary phase, interacting differently with certain components than did methylsilicone alone. In view of their thermal instability, which substantially affects both retention behavior and RI, plus the observed discrepancies with RI's measured by packed columns, Carbowax-deactivated methylsilicone capillaries cannot be recommended for general use in STA.

Both polysiloxane-deactivated capillary columns showed excellent stability as well as reproducibility at temperatures up to 300°C and thus were quite effective in STA, permitting rapid screening (within 45 min) for components with RI's up to 3400. The CP-Sil 5 col-

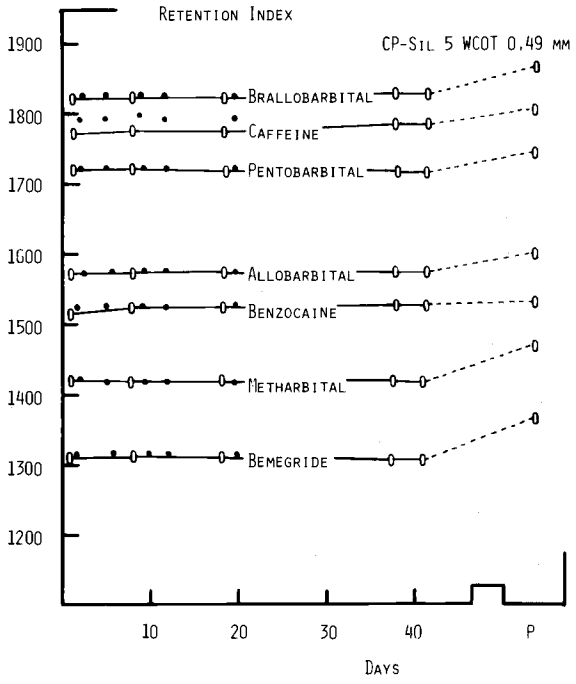

FIG. 5—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

umn produced excellent chromatograms both in this study and in intermittent case work and appeared to have adequate load capacity. Even when overloading did occur, which happened occasionally during case work, there was no residual detrimental effect on column performance. The fused silica column was more flexible and easier to handle than the glass column. Purchased at a cost of about $200, the former has now been in continuous operation for three months and provides excellent value for the money.

Although at first sight there seems to be a fairly good agreement between RI's measured on the CP-Sil 5 capillaries and those reported on the comparable SE-30 or OV-1 packed columns, deviations do occur, possibly because the deactivation material and the dimethylpolysiloxane coating may act as a mixed stationary phase. Of the 120-odd substances studied, 16 gave differences of more than 40 RI units. This has an important impact on the use of RI data compilations. The presently available RI compilations have all been obtained on packed columns, and the above results indicate that it may be unwise to carry out STA on capillary columns and then use a packed column data base for identification.

Yet, the highly increased separation efficiency, reproducibility, stability, and flexibility of the fused silica CP-Sil 5 columns argue that it may be worthwhile to set up a separate capillary column data base. It will be clear, however, that, before starting such an endeavor, additional investigations must be performed on a much larger selection of drugs and over a longer period of time and spread over different institutions to evaluate interlaboratory variations. On the other hand, it should be realized that the manufacture of capillary columns, especially of the newer fused silica types, is undergoing a process of rapid development, so that even better deactivation and coating techniques may become available in the near future. Thus, presently available capillary columns and the capillary materials being developed need to be evaluated further.

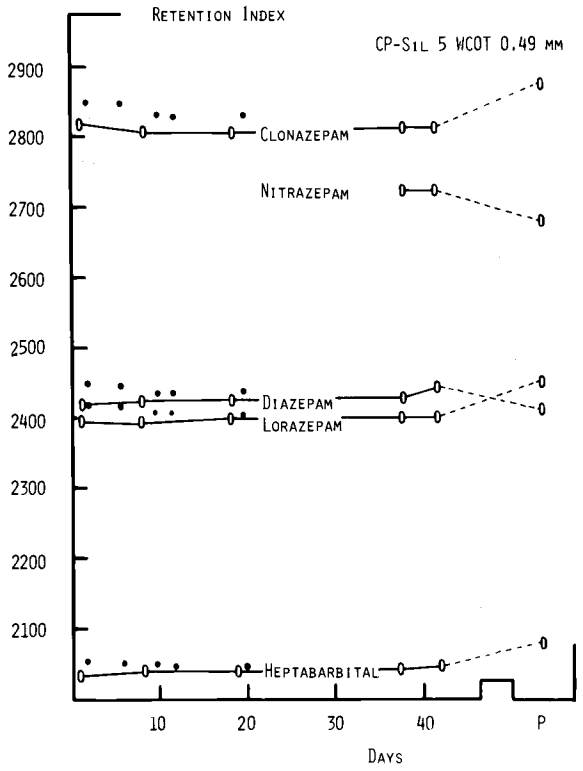

FIG. 6—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

TABLE 1—*Comparison of gas-liquid chromatographic retention indices on methylsilicone-packed columns and capillary columns of compounds of toxicological interest.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a] SE-30 or OV-1
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	
Allobarbital	1577	1577	1605
Amethocaine	2218	...	2230
Amidopyrine	1900	1903	1895
Amitriptyline	2195	...	2205
Amphetamine	1105	...	1105
Amobarbital	1696	1698	1720
Antazoline[b]	2295	...	2350
Aprobarbital	1592	1598	1620
Atropine	2184	...	2190
Barbital	1469	1467	1495
Bemegride[b]	1309	1314	1365
Benzocaine	1526	1528	1535
Brallobarbital	1828	1828	1860
Bromodiphenhydramine	2148	...	2155

TABLE 1—*Continued.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a] SE-30 or OV-1
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	
Buphenine[b]	2519	...	2315
Butacaine	2445	...	2460
Butabarbital	1637	...	1655
Butethamate	1742	...	1750
Butobarbital	1642	1646	1660
Caffeine	1780	1796	1810
Carbinoxamine	2067	...	2060
Chlorcyclizine	2232	...	2215
Chlorpromazine	2499	...	2465
Cinchonine	2585	...	2575
Clomipramine	2419	...	2415
Clonazepam[b]	2813	...	2860
Cocaine	2191	...	2195
Codeine	2376	...	2385
Cyclizine	2017	...	2020
Cyclobarbital	1950	1952	1960
Desipramine	2241	...	2250
Diamorphine	2630	...	2615
Diazepam	2426	2439	2410
Dimethoxanate[b]	1990	...	2030
Diphenhydramine	1857	...	1870
Diphenylpyraline	2101	...	2100
Dipipanone	2490	...	2470
Doxepin	2226	...	2210
Ephedrine	1337	...	1355
Ethinamate	1352	1348	1360
Ethoheptazine	1848	...	1860
Ethopropazine	2378	...	2355
Fluphenazine	3035	...	3045
Glutethimide	1820	1818	1830
Guanethidine	0000	...	0000
Heptabarbital	2041	2047	2080
Hexobarbital	1841	1843	1855
Hydroxyzine	2867	...	2850
Hyoscine	2310	...	2300
Imipramine	2222	...	2220
Iproniazid[b]	1531	...	1580
Isocarboxazid	1926	...	1950
Isothipendyl	2268	...	2260
Levallorphan	2348	...	2350
Lignocaine	1869	...	1870
Lorazepam[b]	2399	2411	2450
Malathion	1920	1922	1900
Meclozine	3034	...	3045
Mephenesin	1531	1533	1545
Meprobamate	1762	1752	1790
Mepyramine	2225	...	2220
Methadone	2150	...	2150
Methapyrilene	1974	...	1985
Methaqualone	2142	...	2115
Metharbital[b]	1421	1417	1470
Methoin	1786	...	1795
Methotrimeprazine	2532	...	2515
Methylamphetamine	1163	...	1155
Methylphenobarbital	1875	1880	1905

TABLE 1—*Continued.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a] SE-30 or OV-1
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	
Morphine	2423	...	2435
Naphazoline[b]	1993	...	2065
Nialamide[b]	1673	...	1500
Nicotine	1328	...	1345
Nicotinyl alcohol[b]	1092	...	1150
Nikethamide	1515	1536	1510
Nitrazepam[b]	2724	...	2675
Nortriptyline	2211	...	2215
Noscapine[b]	3154	3170	3100
Orphenadrine	1932	...	1935
Papaverine	2815	...	2805
Parathion	1946	1947	1925
Pentobarbital	1721	1720	1745
Pethidine[b]	1739/2490	...	1765
Phenelzine	1266	...	1340
Phenindamine	2147	...	2160
Pheniramine	1799	...	1810
Phenobarbital	1938	1939	1960
Phensuximide	1618	...	1630
Phenylbutazone	2368	...	2375
Phenylpropanolamine	1291	...	1305
Phenyramidol	1957	...	2010
Phenytoin	2308	...	2330
Piperidolate	2347	...	2325
Piperocaine	1984	...	1975
Pramoxine	2275	...	2290
Primidone[b]	2202	...	2250
Procaine	2007	...	2010
Procyclidine	2177	...	2170
Promazine	2326	...	2305
Promethazine	2276	...	2270
Propiomazine	2736	...	2725
Propranolol	2141	...	2150
Propyphenazone	1917	...	1925
Prothipendyl	2343	...	2330
Protriptyline	2246	...	2230
Pyrrobutamine	2428	...	2430
Quinine	2796	...	2785
Secobarbital	1768	...	1790
Strychnine	3115	...	3115
Thenyldiamine	1992	...	2010
Theophylline[b]	1947	...	2105
Thiopentone	1846	...	1855
Thioridazine[b]	3116	...	3180
Tranylcypromine	1195	...	1210
Trimipramine	2228	...	2205
Tripelennamine	1974	...	1980
Triprolidine	2250	...	2250
Yohimbine[b]	3168	...	3290

[a]Data taken from Ref *12*.

[b]Substance showing a difference of more than 40 RI units between RI measured on capillary column and on packed column.

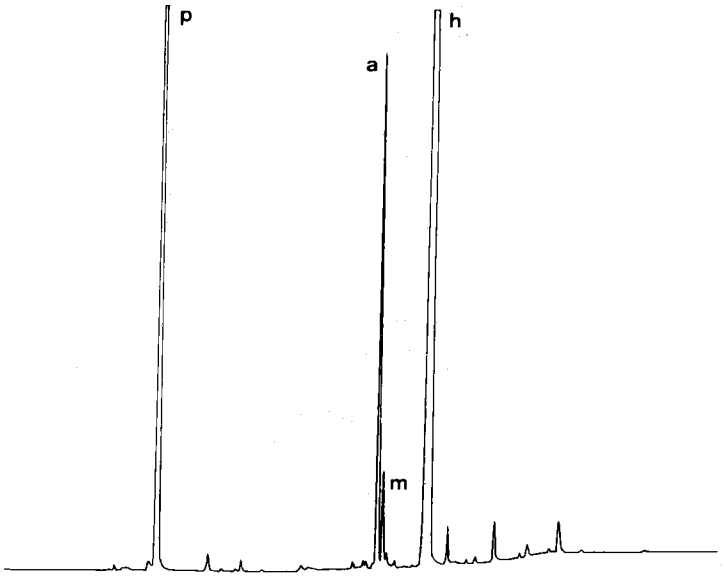

FIG. 7—*Chromatogram of an illicit heroin sample on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) fused silica capillary column in a temperature-programmed run. Only that portion of the chromatographic trace between 12 and 32 min is presented. Retention time, in minutes:* p = *procaine, 14.98;* a = *acetylcodeine, 20.66;* m = *6-monoacetylmorphine, 20.80;* h = *heroin, 22.01; other peaks not identified.*

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