Journal of Chromatography, 257 (1983) 211–226
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,479

ADVANCES IN FUSED-SILICA COLUMN TECHNOLOGY FOR THE ANALYSIS OF UNDERIVATIZED DRUGS

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(First received June 28th, 1982; revised manuscript received October 28th, 1982)

SUMMARY

The chemical durability of a cross-linked 5% phenyl methylsilicone stationary phase is investigated for the analysis of underivatized drugs. Column performance is tested with respect to quantitative reproducibility, solvent extractability, selectivity for various drug families, and capacity for the polar solute. Data are presented showing the effects of a polar solvent and a serum extract on column lifetime with splitless and cool on-column injection. Examples of a human drug screen and the forensic analysis of street heroin are provided.

INTRODUCTION

High-resolution gas chromatography offers several distinct advantages in the area of drug analysis. The overall inertness of a fused-silica chromatographic system enables detection of most common drugs by flame ionization at the low-nanogram level. Resolution of several important drug families and their metabolites is possible on a single stationary phase[1]. Linear and reproducible quantitation over the range 1–100 ng of drug can be achieved using cool on-column injection.

The use of capillary gas chromatography for drug analysis requires that the system has the ability to chromatograph low to moderately polar substances in an underivatized form. With previous systems this proved to be difficult owing to discrimination occurring both in the inlet as well as on the column. Recent advances with the cool on-column injection technique have minimized sample discrimination associated with the split/splitless inlet[2]. Column contributions to sample discrimination then become significant. Previous forms of wall-coated open tubular (WCOT) column technology exhibited high levels of polar solute adsorption due to problems with column activity and stationary-phase extractability with solvents such as methanol, chloroform and ethyl acetate. Advances in column technology, such as the introduction of fused silica[3], polysiloxane deactivation[4], and non-extractable (cross-linked) stationary phases[5,6] have overcome problems with column inertness, thermal stability and solvent compatibility.

In this study, the limitations of each of these column parameters were in-

0021-9673/83/0000–0000/$03.00

vestigated for several cross-linked siloxane deactivated SE-54* WCOT capillary columns. Column effects on the reliability of the retention measurement were determined by monitoring the relative response factors and the linear retention indices of a number of underivatized barbiturates and alkaloids. Using these two parameters the chemical durability of the cross-linked column was investigated. Chemical durability was measured by monitoring column performance with respect to reproducibility, extractability, capacity and selectivity.

EXPERIMENTAL

For the purpose of this study, a drug test mixture was prepared consisting of selected underivatized barbiturates including barbital, amobarbital, pentobarbital, secobarbital and phenobarbital as well as selected alkaloids including procaine, methadone, cocaine, codeine, heroin and quinine. Each drug was present at 10 ng/μl in a binary solvent of methanol–toluene (4:96) (Burdick & Jackson, Muskegon, MI, U.S.A.). An inert hydrocarbon, n-C_{16}, is added as an internal standard so that response factors can be generated. By periodically injecting this sample and monitoring changes in drug retention time and response factor, the usable lifetime of the column can be followed.

A column test sample was prepared containing 800 ng/μl each of an organic acid (4-chlorophenol) and an organic base (1-decylamine). The preparation of this sample is described elsewhere[7]. The base-to-acid ratio of the column (B/A) is determined by the relative peak height of the base to that of the acid obtained with an isothermal 125°C oven and a 70:1 split ratio. When cool on-column injection was employed the B/A ratio was determined under the same oven conditions as the drug test sample. Any undeactivated exposed surface silanol groups impart an acidic nature to the column surface ($pK_a \approx 6.5$). By monitoring the relative amount of acid to base eluted by the column, changes in the quality of the deactivation and efficiency of the stationary phase coating can be indirectly measured.

Instrumentation included a Hewlett-Packard (Avondale, PA, U.S.A.) 5880A gas chromatograph equipped with dedicated cool on-column and split/splitless injectors, an HP 7672A automatic liquid sampler, and flame ionization detectors. Manual cool on-column injections were made with a Hamilton 701 gas-tight syringe and fused-silica needle stock (100 × 0.14 mm I.D.). Fused-silica polysiloxane deactivated cross-linked SE-54 columns (25 m × 0.32 mm I.D.) were obtained from Hewlett-Packard with a film thickness of 0.52 μm.

A system for retention indexing was established by using a linear oven-temperature ramp. Retention index systems have been described elsewhere for petroleum-[8], environmental-[9], and pharmaceutical-[10] related substances. Splitless injections were made at 50°C (1.5 min). The oven was then increased to 280°C at 10°/min. On-column injections were made at 75°C (0.5 min) and the oven temperature increased to 280°C at 10°/min. Hydrogen carrier gas was used at a flow-rate of 165 cm/sec. The high linear velocity decreases analysis time while still providing baseline resolution of all drugs in the test mixture. It also ensures that all drugs elute at a time within the linear temperature ramp of the oven.

* SE-54 is a registered trademark of General Electric.

A standard blend (10 ng per component) of the even-numbered hydrocarbons from n-C_{10} to n-C_{32} is first injected as an external calibration. The standard alkaloid–barbiturate mix is then injected and the retention index of the drug calculated by linear interpolation between the retention indices (I) of the hydrocarbons which bracket the drug according to the equation

$$I(\text{drug}) = 100(N) + 200 \cdot \frac{[t_R(d) - t_R(n)]}{[t_R(n+2) - t_R(n)]}$$

where I(drug) is the retention index of the drug, (N) is the hydrocarbon number of the hydrocarbon eluting just prior to the drug, $t_R(d)$ is the retention time of the drug, $t_R(n)$ is the retention time of the previous hydrocarbon and $t_R(n + 2)$ is the retention time of the hydrocarbon eluting just after the drug.

RESULTS AND DISCUSSION

Reproducibility

The precision of both the retention index (I) and the response factor (Rf) must be established if they are used as column performance indicators. Each column tested was first subjected to a baseline study consisting of ten injections of the drug test mixture. The reproducibility of I and Rf are presented in Table I. For this particular column the variation in I is 0.27 units and of the Rf is 1%.

The column-to-column variation in I and Rf for three randomly selected cross-linked columns is shown in Table II. On-column injection was employed since it is a non-discriminating injection technique; consequently, the data reflect phenomena occurring on the column. Repeatability of the Rf averages 4.6%. Some of the drugs

TABLE I

INJECTION-TO-INJECTION VARIATIONS

n = 10, cool on-column injection. Retention index ±0.27 units; $1500 \leqslant I \leqslant 2900$. Response factor ±1.02%. R.S.D. = Relative standard deviation; σ = standard deviation.

Drug	*Retention index*		*Response factor* $\left(\frac{Drug}{n\text{-}C_{16}}\right)$	
	$x \pm \sigma$	*R.S.D. (%)*	$x \pm \sigma$	*R.S.D. (%)*
Barbital	1510.42 ± 0.15	0.010	0.251 ± 0.004	1.76
Amobarbital	1736.79 ± 0.21	0.012	0.373 ± 0.002	0.49
Pentobarbital	1758.72 ± 0.21	0.012	0.379 ± 0.003	0.75
Secobarbital	1808.79 ± 0.22	0.012	0.336 ± 0.002	0.54
Phenobarbital	1986.85 ± 0.26	0.013	0.430 ± 0.007	1.62
Procaine	2053.98 ± 0.23	0.011	0.444 ± 0.003	0.71
Methadone	2178.09 ± 0.26	0.012	0.615 ± 0.003	0.54
Cocaine	2242.00 ± 0.26	0.012	0.440 ± 0.002	0.43
Codeine	2419.60 ± 0.40	0.017	0.392 ± 0.014	3.50
Heroin	2695.75 ± 0.34	0.013	0.426 ± 0.001	0.27
Quinine	2862.98 ± 0.46	0.016	0.593 ± 0.003	0.58

TABLE II

COLUMN-TO-COLUMN VARIATIONS

n = 10, cool on-column injection. Repeatability of ±0.74 index units, $1500 \leqslant I \leqslant 2900$; ±4.65% response factor (drug/C_{16}).

Drug	*Retention index*			*Response factor*		
	Col. I	*Col. II*	*Col. III*	*Col. I*	*Col. II*	*Col. III*
Barbital	1510.42	1511.09	1510.63	0.251	0.288	0.279
Amobarbital	1736.79	1737.07	1736.75	0.373	0.374	0.376
Pentobarbital	1758.72	1759.43	1758.73	0.379	0.392	0.386
Secobarbital	1808.79	1809.41	1808.73	0.336	0.317	0.312
Phenobarbital	1986.85	1987.81	1986.83	0.430	0.406	0.387
Procaine	2053.98	2054.94	2054.21	0.444	0.411	0.388
Methadone	2178.09	2179.25	2178.64	0.615	0.565	0.547
Cocaine	2242.00	2243.44	2242.62	0.444	0.426	0.425
Codeine	2419.60	2423.02	2420.99	0.392	0.374	0.364
Heroin	2695.75	2698.68	2696.88	0.426	0.397	0.390
Quinine	2862.98	2865.30	2863.04	0.593	0.307	0.460

show little change in the *Rf* such as amobarbital with a percent relative standard deviation of 0.4, while other drugs, in particular quinine, show poor reproducibility. Differences in the coating efficiency of the stationary phase and quality of the deactivation layer may account for these differences. Depending upon the particular drug structure, its solubility in the apolar stationary phase and its binding properties to exposed column active sites, some drugs may be quite sensitive to small differences between columns. If the high and low values are discarded, the overall column-to-column repeatability is 5%. This is far superior to the repeatability obtained using packed columns and is readily achievable without employing such techniques as "priming" or conditioning of the column.

The column-to-column variation in I ranges from 0.18 to 1.72 units. The average is ±0.7 units which is approximately twice the injection-to-injection value. Even so, an overall retention index reproducibility of 1 unit from injection-to-injection and column-to-column assures a consistent drug identification.

Once baseline values for reproducibility are established, column performance can easily be determined. Periodic injections of the drug sample and the column test sample were made to monitor any changes which occurred as a result of column stress.

Solvent and serum stress

The analysis of any polar solute places an additional level of stress on the capillary system, both in terms of the types of solvents which are necessary as well as the type of complex matrix often encountered. The effect of repeated injections of a polar solvent on column performance is shown in Fig. 1. Over three hundred injections of methanol were made using an automatic liquid sampler. Column performance was monitored by measuring the base-to-acid ratio (*i.e.* the relative peak height of the 1-decylamine to the 4-chlorophenol), the response factor of phenobarbital and the retention index of phenobarbital. Phenobarbital was chosen to test the degree of

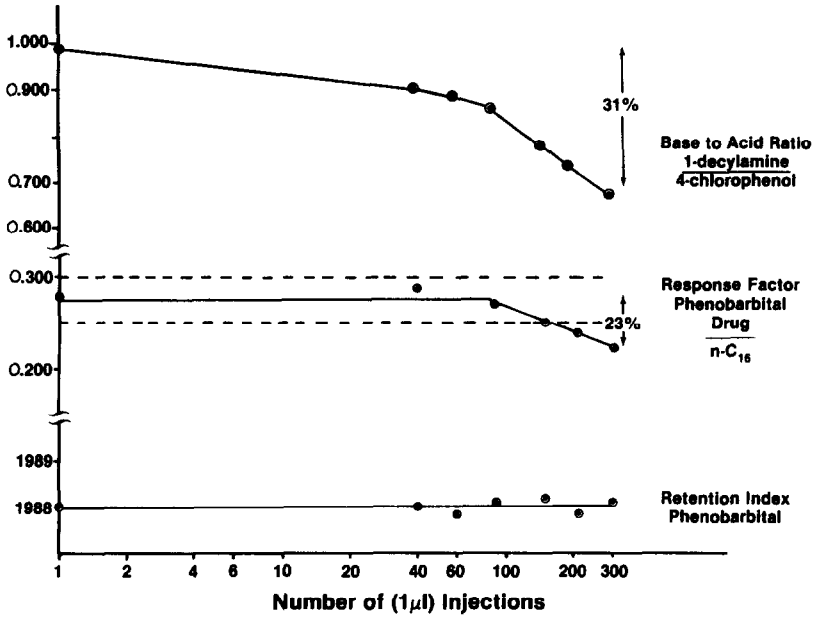

Fig. 1. Effects of repeated splitless injection of methanol on column B/A ratio, *Rf* of phenobarbital and *I* of phenobarbital. Changes in the column-inertness level, which are monitored by the declining B/A ratio, have resulted in a quantitative performance change but not a qualitative performance change.

stationary phase extractability as it is highly sensitive to subtle column changes. For over 300 splitless injections of methanol, the B/A ratio decreases by 31% and the *Rf* of phenobarbital decreases by 23%. The *I* of phenobarbital, however, remains constant. Changes in inertness level which are monitored by the declining base-to-acid ratio have resulted in a quantitative, but not a qualitative, performance change. The area of the column affected by the methanol is undoubtedly small when compared to the total length of the column.

Another way of showing the effect of the methanol stress is to observe the chromatograms (Fig. 2). For most of the drugs the relative peak heights are unchanged. Phenobarbital and quinine show the greatest change. Overall, the change in *Rf* is less than 10%. Note that the retention time of cocaine is the same in injection No. 1 as in injection No. 310. The average change in *I* was 0.3 units, or simply random scatter.

The same observations can be made if the injections are made using on-column injection (Fig. 3). After 100 manual on-column injections the *I* of phenobarbital is unchanged. Both the *Rf* and the B/A ratio begin to show deviations after approximately ten injections. The B/A ratio rises by approximately 14% and the *Rf* falls by 8%. Note that the absolute value of the *Rf* using on-column injection ($Rf = 0.387$) is greater than that obtained using splitless injection ($Rf = 0.282$). This is consistent with our earlier findings[1] and is a result of discrimination occurring during the flash vaporization associated with a splitless injection. The change in the *Rf* after ten on-column injections when compared to 100 splitless injections attests to the severity of

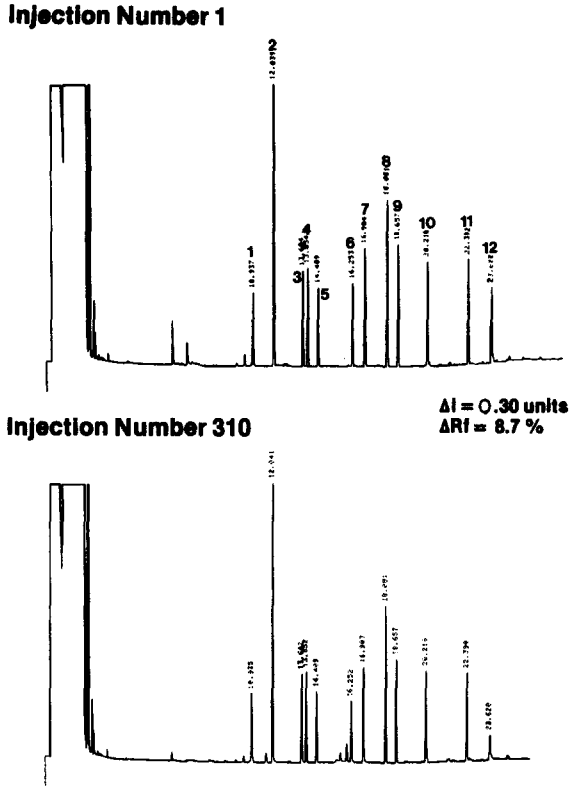

Fig. 2. Drug test-mixture chromatograms following 310 splitless injections of methanol. Peaks: 1 = barbital; 2 = n-C_{16}; 3 = amobarbital; 4 = pentobarbital; 5 = secobarbital; 6 = phenobarbital; 7 = procaine; 8 = methadone; 9 = cocaine; 10 = codeine; 11 = heroin; 12 = quinine.

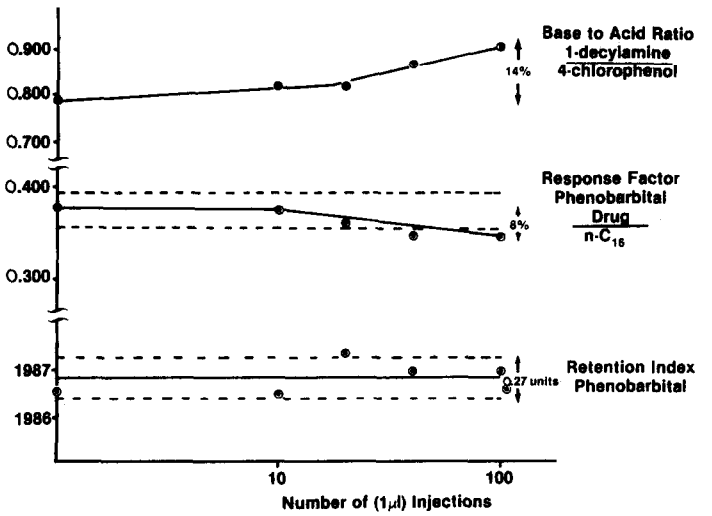

Fig. 3. Effects of repeated cool on-column injection of methanol. Changes in the drug response factor following only ten injections of the polar solvent attests to the severity of the on-column technique. Chemical durability of the cross-linked stationary phase is shown by the reproducible retention index.

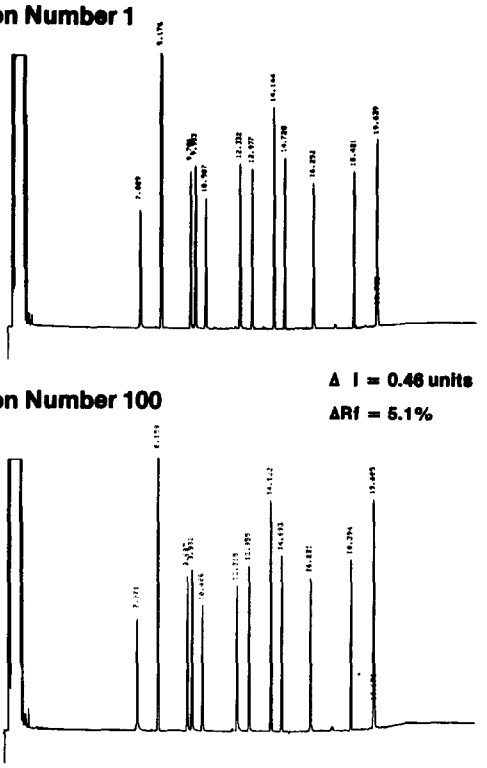

Fig. 4. Drug test-mixture chromatograms following 100 cool on-column injections of methanol. Elution order same as for Fig. 2.

the on-column technique. Again, the chromatograms (Fig. 4) indicate that the drug profiles are essentially unchanged, with phenobarbital and quinine showing the greatest effect. A highly reproducible retention index was possible for most of the drugs in the test mixture even with repeated on-column injections of the polar solvent.

The second major type of column stress is the accumulation of material of low-volatility at the head of the column. When splitless injection is used, this problem is not usually serious because the sample is first vaporized and then transferred to the column. The low-volatility material remains in the injection port liner. By careful selection of extraction pH and solvent, the amount of co-extracted material can be minimized. Good laboratory practice requires periodic replacement or cleaning of this liner. When on-column injection is used, the low volatility material deposited directly on the column. As this material accumulates, it may adversely affect either column efficiency or inertness. In fact, very often both characteristics are affected. The ability of a column to withstand either the high temperature necessary to elute these materials or solvent rinsing is a measure of its chemical durability.

In order to measure the durability of the cross-linked columns and to determine their suitability for clinical analyses, 100 on-column injections were made of a serum extract. The results of this study are shown in Fig. 5. A 10-min post-run hold at 325°C was necessary to elute serum material of low-volatility from the column. The *I*

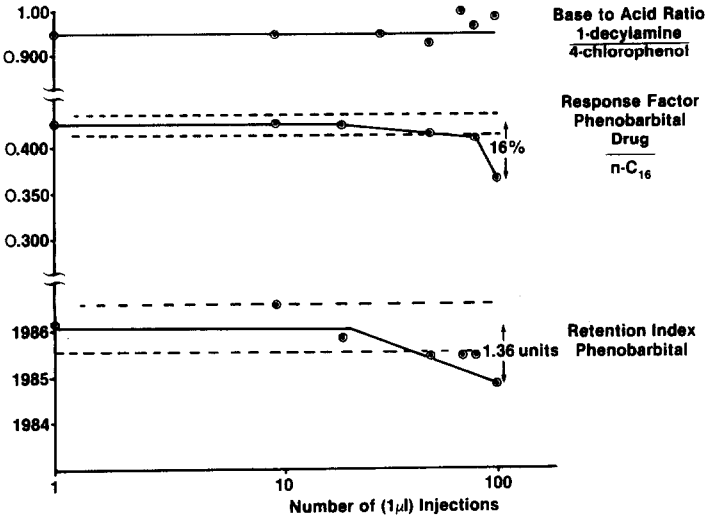

Fig. 5. Effects of repeated cool on-column injection of a human serum extract. Extractions were performed with 1 ml of pH 6 buffered serum into 5 ml of hexane. The organic layer was dried and reconstituted with 100 μl of methanol–toluene (4:96).

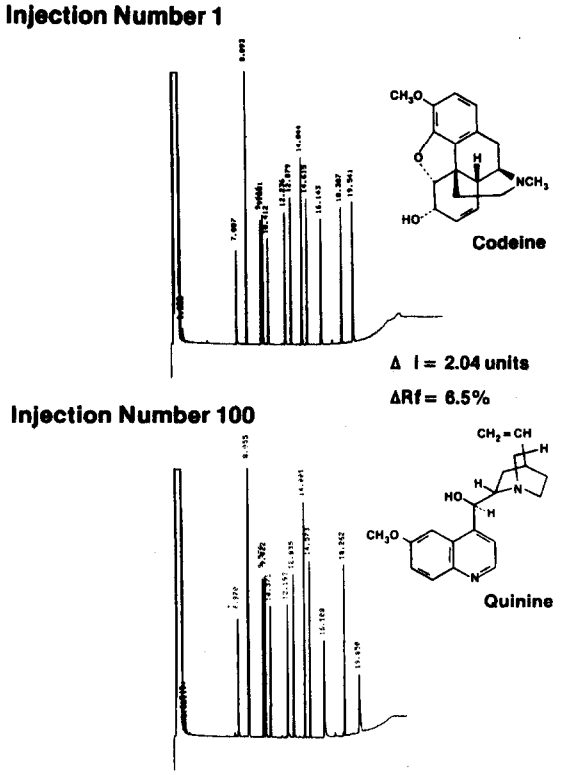

Fig. 6. Drug test chromatograms following 100 cool on-column injections of the serum extract described in Fig. 5. Elution order is the same as for Fig. 2. Most drugs are unaffected but codeine and quinine show a loss in peak height and increased tailing. These are the only two drugs in the test mixture which contain hydroxyl groups.

of phenobarbital remains constant for up to 30 injections and then begins to decrease. The *Rf* also decreases but there is little change in the B/A ratio. When the first and final chromatograms are inspected (Fig. 6), two of the drugs, quinine and codeine, show a significant change in *Rf*. This may be explained on the basis of the chemical structures of these two alkaloids. Both drugs contain hydroxyl groups. For quinine, the position of the hydroxyl group is aliphatic in nature while for codeine it is cyclic.

A possible explanation can be found by looking at the results from the column test sample (Fig. 7). It is apparent that the 4-chlorophenol and the 1-decylamine have not been affected by the repeated injection of the serum extract. However, another component of the test mixture, the dodecanol peak (an aliphatic hydroxyl group) begins to tail. One explanation is that the accumulation of material of low volatility on the column results in a change in the adsorptive properties (*i.e.* activity) of the column surface. Column discrimination for free hydroxyl groups appears to be the result. The original performance of the column can be restored if approximately 1 m of the front of the column is removed. When splitless injection is used the inlet liner is simply replaced or cleaned.

The additional level of durability provided by cross-linking of the stationary phase minimizes many of the effects caused by the use of polar solvents or the ac-

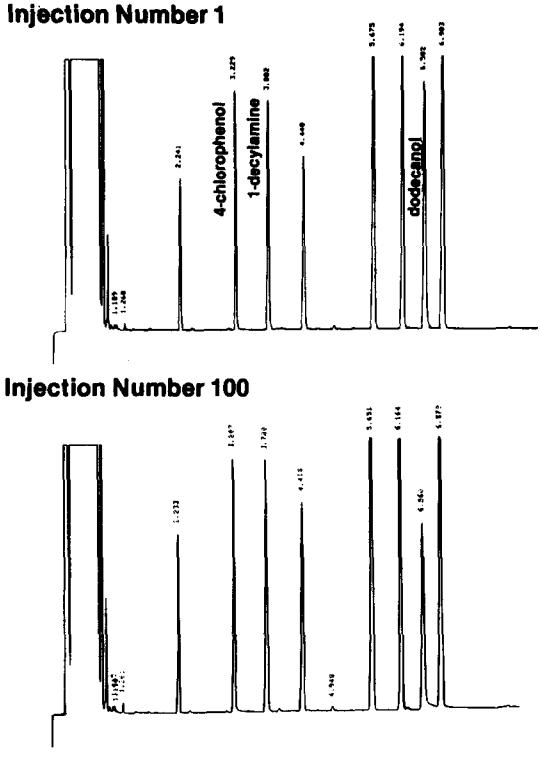

Fig. 7. Results of column test mixture following 100 cool on-column injections of the serum extract described in Fig. 5. Column B/A ratio is unaffected but dodecanol shows signs of increased reversible adsorption. Column discrimination for hydroxyl groups appears to be the result of the accumulation of co-extracted serum material.

cumulation of material of low volatility on the column. It is this property of non-extractable fused-silica columns which will accelerate their use in biological and pharmaceutical areas.

Column capacity

The third column parameter of importance to the analysis of underivatized drugs is capacity, or the degree to which the retention time or retention index may vary with the amount of sample injected. As most drugs in their underivatized form are polar in nature, the limited capacity of the essentially apolar stationary phase can decidedly affect the dynamic range of the technique. The degree to which I may vary as a result of column overload is represented in Fig. 8.

Heroin was selected as a representative member of the alkaloid family and barbital as a representative member of the barbiturates. Columns of length 50 m (0.52 μm film) were selected to maximize column capacity. As the sample amount is varied from 1 to 100 ng, the retention index of barbital increases by 7 units, while that of heroin by only 2. Differences between these drugs may be understood in terms of their structure. Although the structure of heroin is complex, the polar functionalities are considered to be shielded. These include the tertiary cyclic amine and the acetyl oxygen groups. By comparison, barbital contains two secondary amines

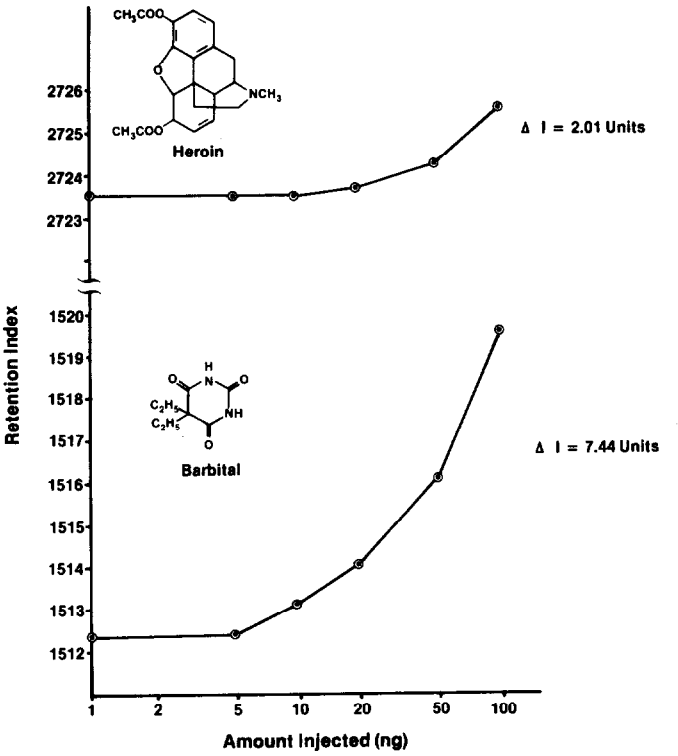

Fig. 8. Capacity effects on the value of the drug retention index using splitless injection. A 50 m × 0.32 mm cross-linked SE-54 column of 0.52 μm film thickness was selected to maximize sample capacity.

which, because of the electron-withdrawing effects of the α carbonyl groups, are polarized to the extent of being considered acidic. It can be concluded on the basis of structure that barbital is more polar than heroin and therefore shows less solubility in the apolar stationary phase.

Table III summarizes the change in retention index for all members of the drug test sample. If a criterion of no more than a two-unit change in I is set, then the capacity of the thick-film SE-54 column for barbiturates is between 20 to 50 ng while for alkaloids it may be as high as 100 ng. For other classes of drug, tests must be carried out to determine the level of drug which can be injected on to the column, and the resultant change in retention index determined. Usually a visual distortion in peak shape (*i.e.* leading front) will be apparent when the sample size is sufficient to cause index shifts of two units or greater. The sample should then be diluted and re-injected. Care should be taken when using the retention index for drug identification that the amount injected does not exceed the capacity of the column or an inaccurate drug identification may result. The future availability of more polar stationary phases which exhibit the same inertness and durability as the cross-linked phenyl methylsilicone phases will improve this situation.

TABLE III

RETENTION INDEX *vs.* AMOUNT INJECTED

Splitless injection. Column same as Fig. 8.

Drug	*1 ng*	*5 ng*	*10 ng*	*20 ng*	*50 ng*	*100 ng*	*ΔRT*
Barbiturates							
Barbital	1512.36	1512.54	1513.15	1514.18	1516.24	1519.80	7.44
Amobarbital	1738.41	1738.62	1739.09	1739.92	1741.84	1744.95	6.53
Pentobarbital	1762.42	1762.57	1762.99	1764.07	1766.04	1769.41	6.99
Secobarbital	1813.12	1813.06	1813.63	1814.48	1816.19	1819.50	6.38
Phenobarbital	1996.40	1996.23	1996.57	1997.54	1999.54	2003.08	6.68
							$\bar{x} = 6.80$
Alkaloids							
Procaine	2065.81	2065.63	2066.06	2066.38	2066.99	2068.37	2.56
Methadone	2193.51	2193.52	2193.39	2193.64	2193.95	2195.08	1.57
Cocaine	2259.11	2258.98	2259.32	2259.25	2259.79	2260.94	0.83
Codeine	2452.15	2451.94	2452.15	2452.37	2452.74	2454.27	2.12
Heroin	2723.66	2723.58	2723.58	2723.89	2724.44	2725.67	2.01
Quinine	2898.53	2898.19	2898.11	2898.19	2898.53	2900.46	1.93
							$\bar{x} = 1.84$

Selectivity

Several important drugs of abuse were separated in a human serum (Fig. 9) and urine (Fig. 10) drug screen. The serum and urine standards were prepared by a simple one-step extraction from the buffered sample into methylene chloride, the organic layer dried, and reconstituted with ethyl acetate. A portion of the reconstituted material is injected onto the capillary column using a splitless injection technique. Underivatized barbiturates, as well as cocaine, methaqualone, amitriptyline,

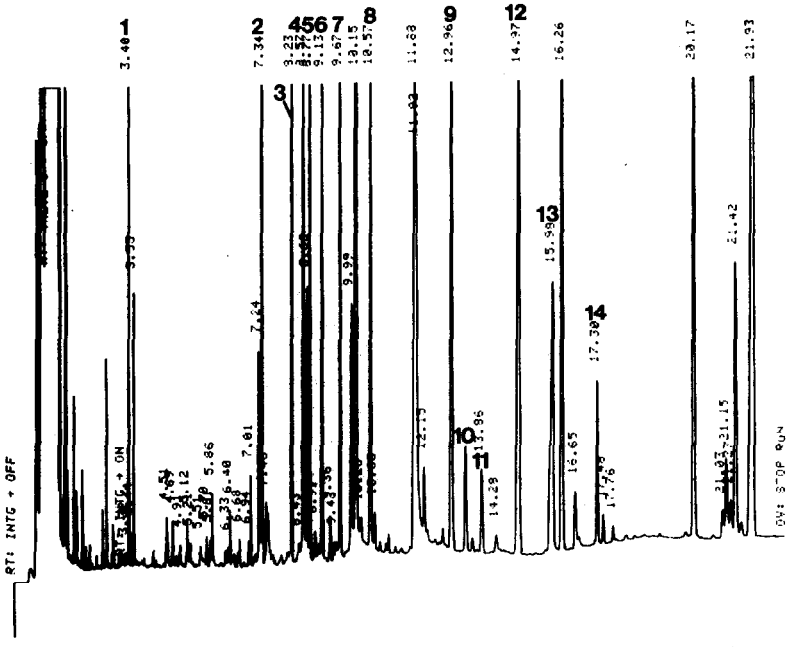

Fig. 9. Human serum drug screen*. Selected drugs were added to 1 ml of pH 1 buffered serum. The mixture was extracted into methylene chloride, dried and reconstituted with 100 μl of ethyl acetate, and 1 μl was injected. Drug levels in the serum standard varied from 0.5 to 5 μg/ml. The amount injected onto the column varied from 5 to 50 ng. A 25 m × 0.32 mm cross-linked SE-54 column with well over 100 injections of the biological extracts was used. Peaks: 1 = ethclorvynol; 2 = methyrprylon; 3 = butalbital; 4 = amobarbital; 5 = pentobarbital; 6 = secobarbital; 7 = glutethimide; 8 = phenobarbital; 9 = methaqualone; 10 = amitriptyline; 11 = imipramine; 12 = cyheptamide (internal standard); 13 = phenytoin; 14 = diazepam.

methadone, propoxyphene and phencyclidine (PCP), may be analysed on a single capillary column. Previous packed-column screening techniques required splitting the serum sample into acidic/neutral and basic extractions and subsequent separate analysis. Because of the sensitivity and selectivity of the capillary system, basic drugs, such as the antidepressants, are extracted from a single acid pH in sufficient quantities to be detected simultaneously with any acidic/neutral drugs which may be present. Both chromatograms were obtained from a column which had been in use for several months and had experienced well over 100 injections of the human extracts. The increased tailing exhibited by the codeine peak in the urine screen is a practical example of how the performance deteriorates following routine use with biological samples.

Table IV lists just a few of the important drugs which have been analysed by this technique. Included in this drug library are drugs found in samples obtained over-the-counter, from the street or therapeutic in nature. Several amphetamines are included in addition to the heroin and cocaine already shown. Dextromethorphan is an active ingredient in non-prescription cough syrup, meperidine is a common analgesic and pyrilamine is the active ingredient in several over-the-counter sleeping pills. Therapeutic drugs include anticonvulsants, antidepressants and benzodiazepines.

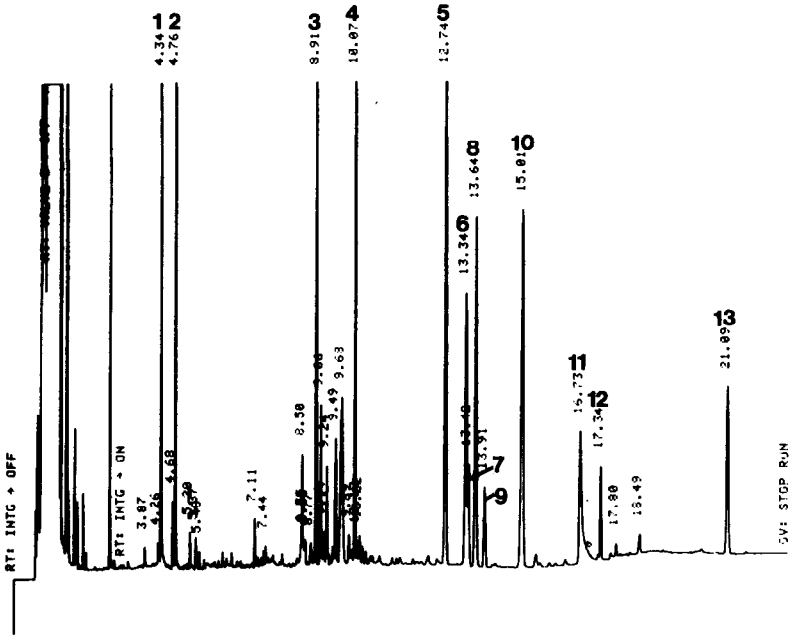

Fig. 10. Human urine drug screen*. Selected drugs were added to 2 ml of pH 9 buffered urine. The mixture was extracted into methylene chloride, dried and reconstituted with 150 μl of ethyl acetate, and 1 μl was injected. Drug levels in the urine standard varied from 0.5 to 2.5 μg/ml. The amount injected onto the column varied from 4 to 20 ng. The column was the same as in Fig. 9. Peaks: 1 = amphetamine; 2 = methamphetamine; 3 = meperidine; 4 = phencyclidine (P.C.P.); 5 = methadone; 6 = propoxyphene; 7 = amitriptyline; 8 = cocaine; 9 = imipramine; 10 = cyheptamide (internal standard); 11 = codeine; 12 = diazepam; 13 = flurazepam.

Although well over 30 drugs and their metabolites may be analysed on a single stationary phase, a region of fairly low resolution exists, generally bounded by hydrocarbon numbers C_{22} and C_{24}. In this region, ten to fifteen common drugs may elute with index differences of ten units or less. Considering that the magnitude of the retention index shifts due to column capacity alone may be on the order of two units, drug identification in this region will be obscured by capacity effects. Again, more polar stationary phases are necessary to improve sample capacity and to help separate these drugs on the basis of polarity instead of slight differences in boiling point.

Fig. 11 shows the analysis of a "street" heroin sample. All that is required is to dissolve the sample in methanol. Several additives do not dissolve and may be centrifuged out. An aliquot of the methanol is then injected onto the capillary column and heroin, as well as trace impurities of acetylcodeine and monoacetylmorphine, detected by their retention indices. Separation of these impurities is difficult either with packed-column gas chromatography or conventional liquid chromatography. Information derived from such impurities can be used to help trace the heroin source[11].

* The sample preparation schemes and chromatograms of Figs. 9 and 10 were contributed by D. Ehresman, St. Paul-Ramsey Hospital, St. Paul, MN 55101, U.S.A.

TABLE IV

DRUG LIBRARY

Cross-linked SE-54, 50 m × 0.32 mm, β = 150, $\bar{\mu}(H_2)$ = 80. Splitless injection at 45°C (1.5 min) then 6°C/min to 300°C.

Drug	*I*	*Drug*	*I*
D-Amphetamine	1128.25	Procaine	2066.27
Methamphetamine	1186.34	Dextromethorphan	2184.23
Ethosuximide	1240.62	Methadone	2193.84
Talbutal	1738.08	Methaqualone	2210.28
Methyl-propyl succinimide	1329.23	Amitriptyline	2247.68
Probarbital	1593.49	Cocaine	2259.38
Barbital	1512.78	Nortriptyline	2266.42
Amobarbital	1738.91	Pyrilamine	2289.21
Pentobarbital	1762.90	Primidone	2290.56
Meperidine	1776.37	Carbamazepine	2378.89
Secobarbital	1813.25	Phenytoin	2397.77
Meprobamate	1830.67	Methyl phenytoin	2508.98
Mephobarbital	1936.38	Diazepam	2515.26
Tropacocaine	1990.52	Heroin	2723.82
Phenobarbital	1997.32	Quinine	2898.07
Methapyrilene	2031.33	Librium	2939.33

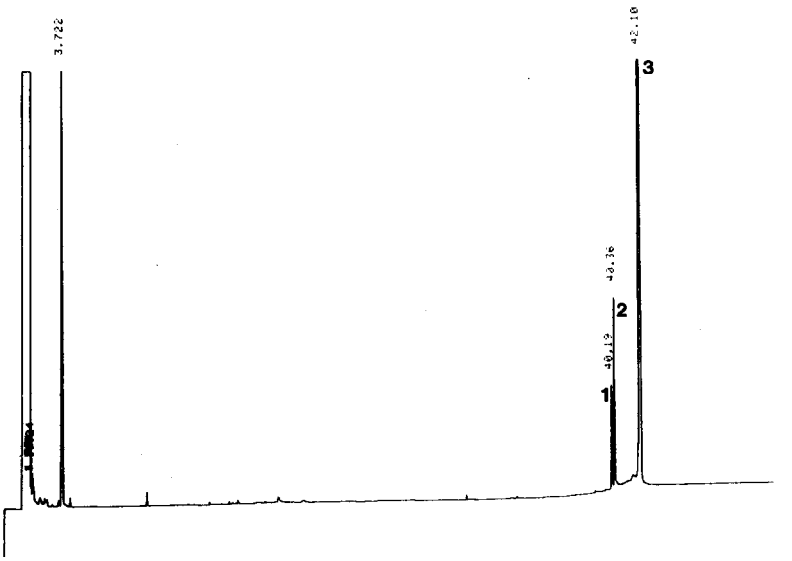

Fig. 11. Analysis of "street" heroin. Column and conditions were the same as in Table IV. This sample was obtained prior to cutting with quinine. Peaks: 1 = acetylcodeine, I = 2597.1; 2 = monoacetylmorphine, I = 2608.9; 3 = heroin, I = 2728.5.

Fig. 12 shows how the selectivity of the capillary technique can be used for the identification of drugs in unknown tablets. Quite often an overdose case will be found with several unidentified pills nearby. The pill can be pulverized in the presence of methanol. Several fillers in the pill do not dissolve and are centrifuged out. The methanol is injected onto the capillary column to obtain a drug profile. The retention index of the major peak eluting at 37 min correlates well with that of diazepam or Valium* in the drug library (Table IV). In fact, the difference in the retention indices is less than 0.5 units. Information obtained from the retention index can be combined with mass-spectral analysis to provide an accurate identification of the tablet. Reproducibility in the retention index of no more than ± 1 unit column-to-column enables a higher degree of confidence to be placed in the drug identification using retention data alone.

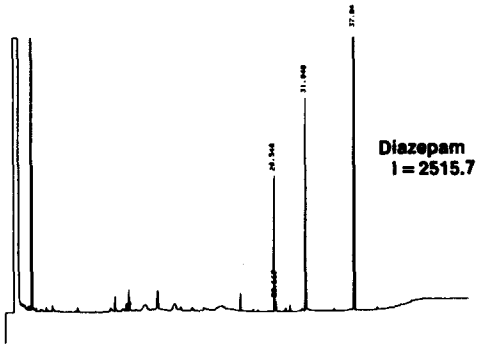

Fig. 12. Analysis of a Valium tablet. Diazepam is identified at a retention time of 37.04 min (I = 2515.7). Column and conditions were the same as in Table IV. Other peaks are unidentified.

CONCLUSION

The application of capillary gas chromatography as a routine technique for the analysis of underivatized drugs is possible due to recent advances in column technology. Cross-linking of the phenyl methylsilicone stationary phase has produced WCOT fused-silica columns capable of a very high degree of reproducibility and chemical durability, while providing reasonable selectivity and capacity. Limitations of the technique include restriction on polar solute loadability and selectivity, as well as eventual performance deteriorations for some drugs caused by the accumulation of co-extracted biological materials. The future availability of more polar stationary phases and more efficient means of automated sample clean-up should minimize or eliminate these effects. Even considering these limitations, there is currently no other commercial chromatographic technique which offers both the sensitivity and selectivity of fused-silica capillary chromatography. The combination of information provided by a reliable retention index with information provided by the mass spectrum can only improve the accurate identification of drugs in unknown samples.

* Valium is a registered trademark of Hoffmann–La Roche.

ACKNOWLEDGEMENTS

Appreciation is expressed to D. Ehresman (St. Paul-Ramsey Hospital) for providing the human serum and urine chromatograms and his helpful contributions to the understanding of the inlet phenomena associated with the capillary analysis of biological samples. We also thank R. R. Freeman for his patient assistance in the preparation of this manuscript.

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