APPLICATION OF FUSED-SILICA CAPILLARY GAS CHROMATOGRAPHY TO THE ANALYSIS OF UNDERIVATIZED DRUGS

L. L. PLOTCZYK

Hewlett-Packard Company, Route 41, Avondale, PA 19311 (U.S.A.)

(First received October 20th, 1981; revised manuscript received February 1st, 1982)

SUMMARY

The feasibility of using fused-silica capillary chromatography for the routine analysis of several common drugs is illustrated. Considerations for optimizing on-column and splitless injection are discussed as part of a study of system discrimination and reproducibility. The peak shape of polar solutes is improved through the use of a binary solvent and non-extractable stationary phase columns. Cold on-column injection with cross-linked polysiloxane deactivated columns produced linear quantitation from 1 to 100 ng with precisions of 0.1–2 % for selected anticonvulsant drugs.

INTRODUCTION

Capillary chromatography was originally used as a qualitative technique for the high-resolution analysis of petroleum-based substances. Advances in instrument design and column technology have led to the quantitative analysis of not only relatively inert hydrocarbons, but also more polar solutes including phenols, amines, and mercaptans[1,2]. The development of the cold on-column injection technique and non-extractable stationary phase columns has enabled capillary chromatography to expand into more diverse application areas. One area is the analysis of underivatized drugs. Growth into biological or pharmaceutical drug related areas will be spurred by the high sensitivity, selectivity and speed offered by fused-silica column gas chromatography.

Methods using glass column chromatography for the biological analysis of several important drugs, steroids, and prostaglandins were recently reviewed[3,4]. Only marginal success has been experienced with these techniques due to problems with column surface activity. These problems can be minimized using sample derivatization procedures and column leaching techniques. The inertness and high-temperature stability of commercially available fused-silica columns eliminates the need for derivatization, for many samples, while providing enhanced sensitivity. Nanogram quantities of several drugs and their metabolites may be resolved on a single stationary phase.

Quantitative use in such areas as therapeutic drug monitoring, dosage phar-

macokinetics and forensic drug screening requires an investigation into the linearity and reproducibility of the method. In this study, several underivatized anticonvulsant drugs were selected to test factors affecting column selection and optimization of the chromatographic system. The fused-silica capillary gas chromatography of common alkaloids, barbiturates, analgesics and tricyclic antidepressants is illustrated.

EXPERIMENTAL

The analysis was performed on a Hewlett-Packard (Avondale, PA, U.S.A.) 5880A gas chromatograph equipped with splitless and dedicated on-column injection ports and a 7672A automatic liquid sampler. The injection volume was 1 μl. A flame ionization detector was used with nitrogen make-up gas at 45 ml/min, air at 400 ml.min and hydrogen at 35 ml/min. On-column syringes were prepared by fitting Hamilton 701-RN gas-tight syringes with fused-silica needle stock (100 × 0.14 mm I.D.). Siloxane deactivated cross-linked and gum phase SE-54* fused-silica columns were obtained from Hewlett-Packard. Distilled-in-glass methanol and toluene were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.).

RESULTS AND DISCUSSION

Two fused-silica capillary systems were defined for the purpose of this study. They include splitless injection on gum phase columns and cold on-column injection on non-extractable cross-linked columns. The first system is composed of instrumentation and column technology which is routinely available and has the advantage of automation. By comparison, technology for both the on-column injection and non-extractable columns only recently became available. Their combination, however, results in a state-of-the-art system specifically designed for accurate and reproducible quantitation. The degree of activity present in each of these systems was investigated along with factors affecting their optimization.

Splitless injection on gum phase columns

Splitless injection of polar solutes requires a careful choice of both solvent and inlet temperature. Considerations for solvent selection should include the ability to dissolve the sample, to provide a good "solvent effect" and to be compatible with the column stationary phase. In general, the "solvent effect" occurs when the solute encounters a steadily increasing stationary phase film (solvent plus stationary phase) at the head of the column. Molecules at the front of the solute band are slowed to a greater extent than those at the rear, creating an effective reconcentration of the solute[5,6]. Conditions for an optimized "solvent effect" require that the solvent be soluble in the stationary phase. Since primarily non-polar stationary phases are currently available with fused-silica columns, the usual capillary solvents are non-polar.

Several typical capillary solvents, including hexane, toluene and methylene chloride, were tested and found to lack sufficient solvent strength to dissolve the drug sample. More polar solvents, such as methanol and ethyl acetate, will dissolve most

---

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

common drugs but will also strip or extract the thin layer of column stationary phase. The result is a steadily decreasing stationary film thickness, at the front of the column and a subsequent increase in column activity. Secondly, the use of polar solvents will not provide an optimized "solvent effect". The low solubility of the solvent in the stationary phase leads to an ineffective reconcentration and poor polar solute peak shape.

Jenkins[7] recently proposed a technique to improve polar solute peak shape by co-injection of a low-polarity secondary solvent. A plug of an immiscible non-polar solvent is drawn into the syringe barrel followed by the sample plug. A second plug of the secondary non-polar solvent is drawn into the syringe after the sample. The three liquid plugs are separated by air spaces. During the "solvent effect", the polar solvent is essentially encapsulated at the front and rear by a non-polar solvent–stationary phase film. A more efficient reconcentration results in an improvement in solute peak shape. Co-injections of methanol–hexane, methanol–cyclohexane, and acetonitrile–benzene were investigated.
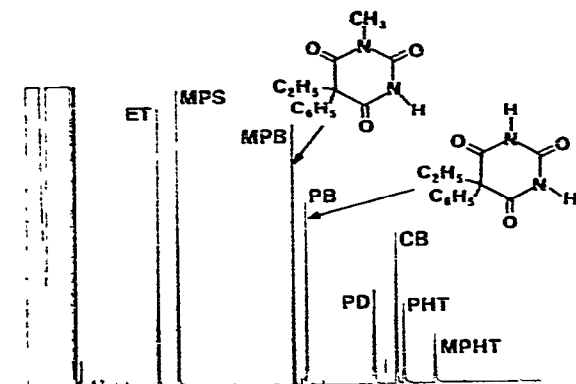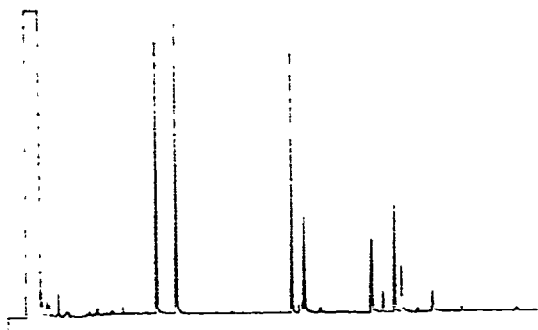

Fig. 1. Solvent selection in the splitless sampling of anticonvulsant drugs at 20 ng per drug. Column: SE-54 gum (25 m × 0.32 mm I.D.), $\beta = 450$; oven profile: 45°C for 1 min, 15°/min to 240°C. ET = ethosuximide; MPS = methyl-propylsuccinimide; MPB = mephobarbital; PB = phenobarbital; PD = primidone; CB = carbamazepine; PHT = phenytoin; MPHT = methyl phenytoin.

A second technique to improve polar solute peak shape is the use of a low percentage polar solvent in a miscible, higher boiling and inert non-polar solvent. A binary mixture of 1 to 10% methanol in toluene eliminates the need for co-injection while providing a means for dissolving the drug sample, optimizing the solvent effect and minimizing column deterioration. Fig. 1 shows a splitless injection of the anticonvulsants at 20 ng per drug using methanol (b.p. 64°C) as the solvent. A suitable solvent effect is obtained at an initial oven temperature of 10–30°C below the boiling point. However, the low relative response of the reactive secondary amine, phenobarbital, *versus* the methylated tertiary amine, mephobarbital, suggests that solvent extraction of the SE-54 gum phase has occurred. Phenobarbital adsorption to newly exposed surface silanol groups may account for the loss. This is supported by the observation that repeated injections produces steadily declining phenobarbital recoveries.

A binary solvent of 4% methanol in toluene restores the relative response of the phenobarbital peak. Toluene (b.p. 110°C) is a suitable choice for the secondary solvent since it is both miscible with methanol and shows less tendency to extract the
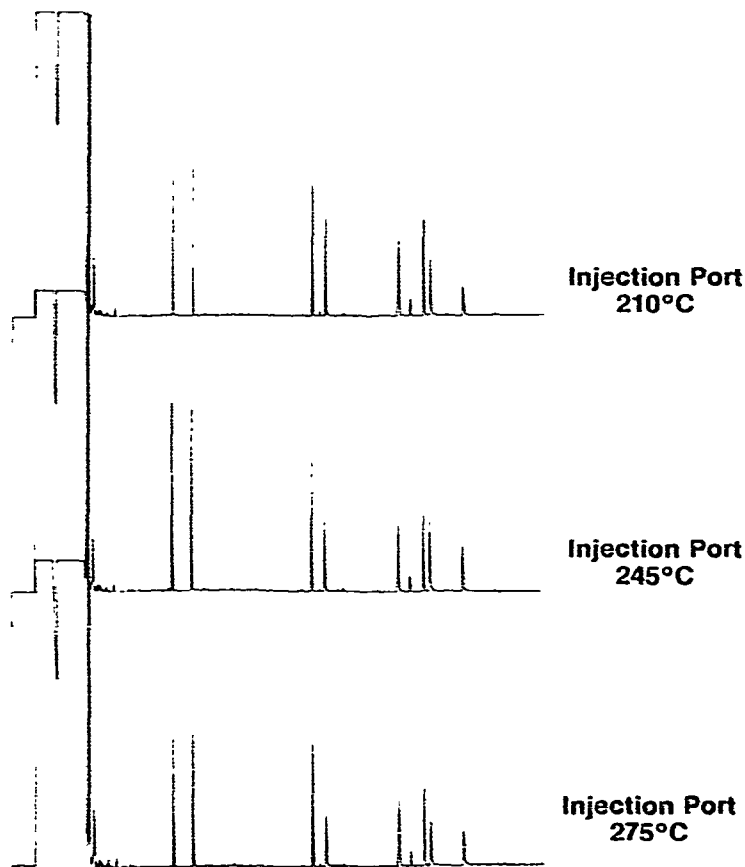

Fig. 2. Effect of injection port temperature on splitless sampling of anticonvulsant drugs at 4.8 ng per drug.

non-polar gum phase. Peak splitting or other forms of peak irregularities may occur with the use of binary solvents or co-injected solvents if liquid–liquid partitioning and/or zones of differing phase ratios are formed during the solvent effect. Co-injections will be particularly prone to these effects since the use of relatively immiscible solvents creates a multi-phase system. These problems are avoided with the binary solvent if the initial oven temperature is far enough below the boiling points of the miscible solvent pair or some form of secondary cooling is used. A value of 20°C below the boiling point of methanol was adequate for a methanol–toluene mix of 1–10% with splitless injection. There was no evidence of peak splitting over a range of initial oven temperatures near the boiling point of methanol. The formation of a single phase "solvent effect" may account for these observations.

Thermal lability of the underivatized drug is minimized in splitless sampling by operating at the lowest inlet temperature possible. Increasing thermal degradation with unnecessarily high inlet temperatures is evidenced by the declining phenobarbital response (Fig. 2). Typically, an inlet temperature of 200–250°C will be sufficient. Lower inlet temperatures than are used with either split or packed column injection are possible due to an increased residence time in the inlet liner and a longer volatization time.

TABLE I

SPLITLESS REPRODUCIBILITY OF ANTICONVULSANTS (4.8 ng PER DRUG)

HP 7672A automatic liquid sampler; 1-μl injections; purge activation time, 0.5 min.

	Area counts							
	ET	MPS	MPB	PB	CB	PD	PHT	MPHT
	27.54	27.82	28.22	18.56	24.08	26.55	23.71	16.37
	26.20	26.74	27.19	18.18	23.49	26.16	23.75	15.59
	24.18	24.72	24.70	16.76	21.07	23.36	19.63	13.82
	24.78	25.13	25.32	16.90	21.25	23.57	19.42	13.20
	25.00	25.36	25.92	17.80	22.38	24.92	18.87	14.85
	27.19	27.87	29.16	20.70	25.94	28.53	20.63	17.63
	24.61	24.97	25.07	15.67	21.49	24.09	21.26	12.68
Mean	25.64	26.09	26.51	17.79	22.81	25.31	21.04	14.87
S.D.	1.33	1.36	1.71	1.61	1.79	1.87	2.00	1.78
Relative S.D. (%)	5.20	5.22	6.45	9.05	7.85	7.41	9.51	11.99

Reproducibility of splitless sampling on a gum phase SE-54 column was tested with the aid of an HP 7672A automatic liquid sampler. Results appear in Table I. Deviations in peak area are caused by both reversible and irreversible adsorption phenomena to active sites present in the fused silica inlet liner and column. In addition, sample may be lost at the moment of injection by back flow against the inlet septum or syringe needle as a result of the flash vaporization technique.

Relative standard deviations in absolute area of 5–12% were typical for the anticonvulsant drugs at 4.8 ng per drug. Quantitation in the splitless mode will benefit from the use of internal standards but will be limited to those analyses which tolerate precisions in the order of 5%. Into this category fall many of the clinical methods

which routinely use internal standards and often report therapeutic concentrations to within 10%.

On-column injection with cross-linked columns

Sample losses associated with the splitless inlet are eliminated with the cold on-column injection technique. Sample is introduced onto the capillary column as a liquid aerosol with the aid of a specifically designed inlet and fused-silica syringe[8,9]. Reconcentration of the solutes occurs via the "solvent effect" when the initial oven temperature is equal to or near the boiling point of the solvent.

An on-column injection of the anticonvulsants with an SE-54 gum phase column is shown in Fig. 3. Improvement in the relative response of phenobarbital, carbamazepine, and phenytoin is evident when compared to splitless injection (see Fig. 1). Improved accuracy with on-column *versus* splitless injection is apparent for polar and thermally labile solutes. Optimization of the "solvent effect" for the methanol–toluene mix occurred at an initial oven temperature between the boiling points of the solvent pair. A value of 75°C was chosen because it is slightly above, but near, the boiling point of methanol yet below the boiling point of toluene.
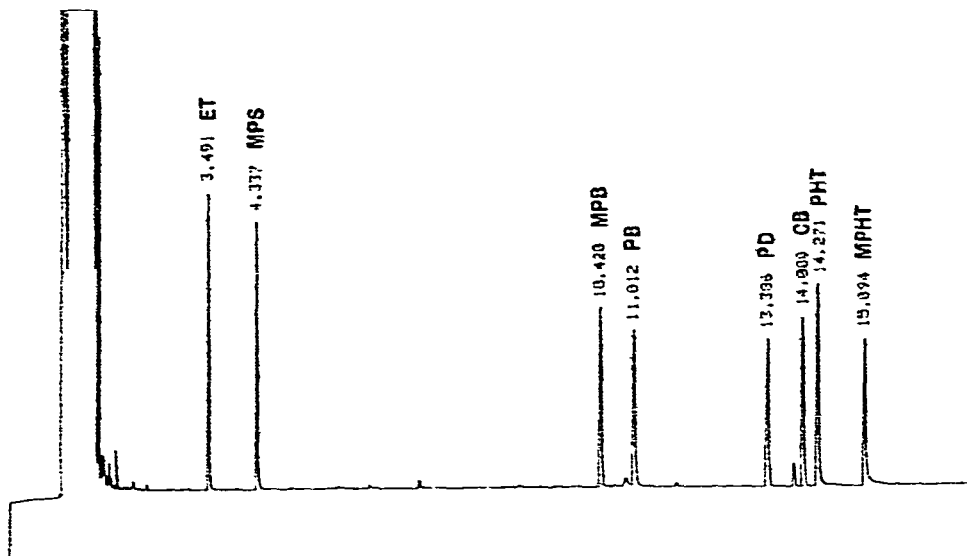

Fig. 3. On-column injection of anticonvulsants at 4.8 ng per drug. Column: SE-54 gum (25 m × 0.32 mm I.D.). $\beta = 450$; solvent: methanol–toluene (4:96); oven profile: 75°C for 0.5 min, 10°C/min to 260°C.

Effects of stationary phase solvent extraction will be even more severe than with splitless injection since the liquid is placed directly on the column. The use of a binary solvent will reduce these effects but will not eliminate them. Columns compatible with the on-column injection of polar solvents may be prepared by a process known as cross-linking. The technique was first described by Noll[10] in 1960 and later by Madani and Chambaz[11], Blomberg et al.[12], and Grob et al.[13]. The process involves the addition of peroxides to the gum phase to initiate *in situ* cross-linking. Methylene linkages are formed in the case of the methylsilicone OV-1*, and a combination of methylene and vinyl linkages in the methyl-phenylsilicone phases. When

coated onto a siloxane deactivated fused silica surface, columns are produced which exhibit inertness, high thermal stability and low solvent extractability.

Column selection for the analysis of underivatized drugs is further aided by the use of column test mixtures. The fused silica surface is inherently acidic in nature due to the presence of surface silanol groups ($pK_a = 6.5$). Drugs, such as the anticonvulsants, which are normally considered "acidic" may appear "basic" or even "neutral" relative to the silanol groups. Substantial adsorption of the drug would occur with an undeactivated surface. The analysis of both acidic and moderately basic drugs will be improved through deactivation of the fused silica. Column test mixtures are a useful means of defining the level of surface activity remaining after deactivation and coating of the stationary phase.
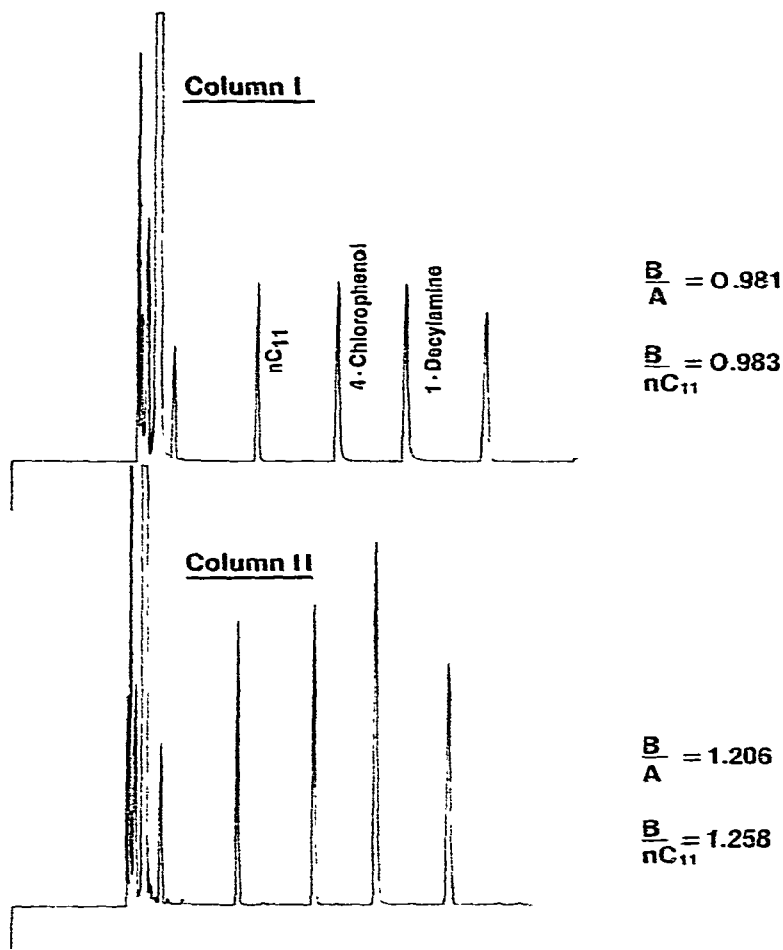

Fig. 4. Test mixture comparison of two cross-linked SE-54 siloxane deactivated columns (25 m × 0.32 mm I.D.), β = 150. The higher base to acid ratio of column II indicates a better level of deactivation resulting in decreased reversible and irreversible adsorption. Column II is preferred for the analysis of moderately polar drugs.

---

* OV-1 is a registered trademark of Ohio Valley.

TABLE II

RESPONSE FACTOR REPRODUCIBILITY OF ANTICONVULSANTS (10 ng PER DRUG; $n = 6$)

Area ratio	Column I	Column II
	Mean ± S.D. (Rel. S.D.)	Mean ± S.D. (Rel. S.D.)
ET/n C$_{16}$	0.5965 ± 0.0169 (2.83)	0.6307 ± 0.0009 (0.14)
MPB/n C$_{16}$	0.4721 ± 0.0135 (2.86)	0.4972 ± 0.0019 (0.37)
PB/n C$_{16}$.	0.4215 ± 0.0092 (2.18)	0.4679 ± 0.0046 (0.99)
PHT/n C$_{16}$	0.5132 ± 0.0193 (3.77)	0.5520 ± 0.0095 (1.72)

Results (Fig. 4) of a column test mixture containing equal amounts of an inert hydrocarbon (undecane), an organic acid (4-chlorophenol), and an organic base (1-decylamine) are compared for two cross-linked polysiloxane deactivated columns. The increased amount of tailing evident in column I indicates a lower level of siloxane deactivation. Adsorption phenomena are minimized on column II which yields a higher base to acid (B/A) and base to hydrocarbon (B/n C$_{11}$) ratio. Column II will be more suitable for the analysis of moderately polar drugs.

This hypothesis is verified by the response factor reproducibility shown in Table II. In this case, hexadecane has been added as an internal standard to a solution containing 10 ng of each anticonvulsant drug. For ten manual injections, the more
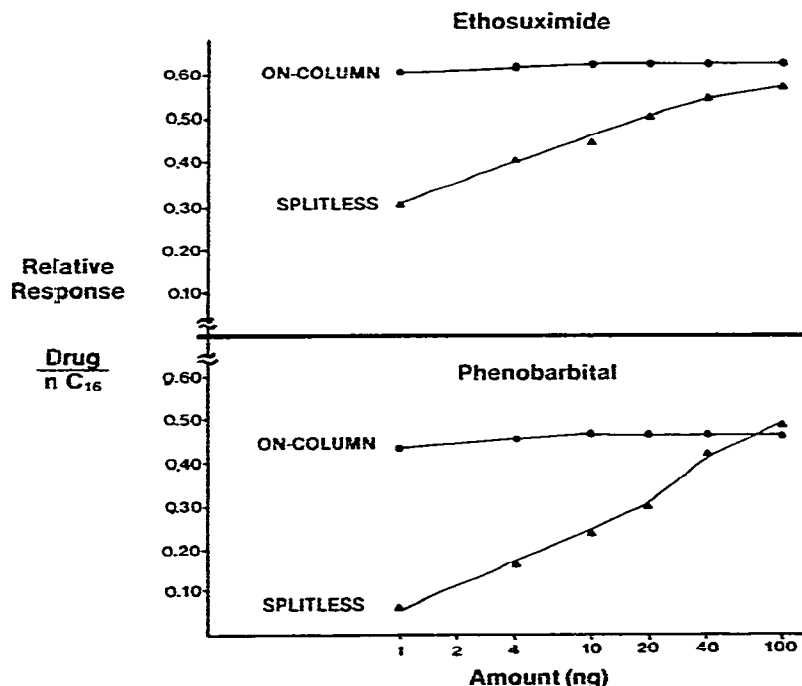

Fig. 5. Linearity of selected anticonvulsant drugs under splitless versus on-column injection. Inlet effects are the major contribution to discrimination occurring from 1 to 100 ng per drug. Column effects will predominate for the on-column injection at less than 1 ng. Column: cross-linked SE-54 (column II); same conditions as in Fig. 4.

inert column (column II) exhibits a slightly higher relative response and much lower relative standard deviation. Improvement in accuracy and precision is due to decreased irreversible and reversible adsorption effects.

Variations in relative response with the amount of drug injected were used as a measure of discrimination occurring with on-column *versus* splitless sampling (Fig. 5). To reduce the effects of discrimination which may be occurring on the column itself, the same cross-linked column was used for both injection techniques. The result is a comparison of the relative activities of the two sampling systems, including syringe effects and inlet adsorption phenomena. Non-linearities in the splitless data are due to the chemically active inlet system and the use of a vaporizing injection technique.

Cold on-column injection yields a linear response from 1 to 100 ng of drug with

Standard Alkaloid Mix 10ng/µl
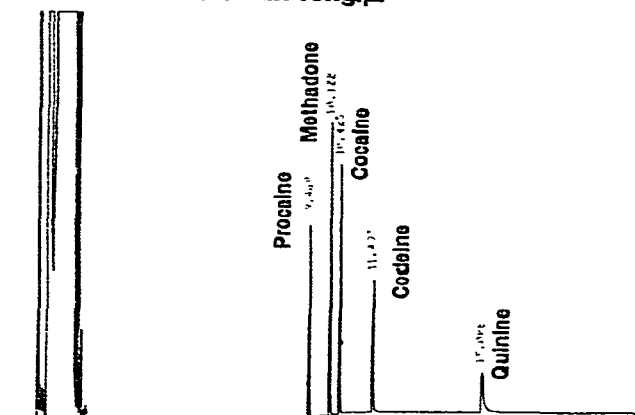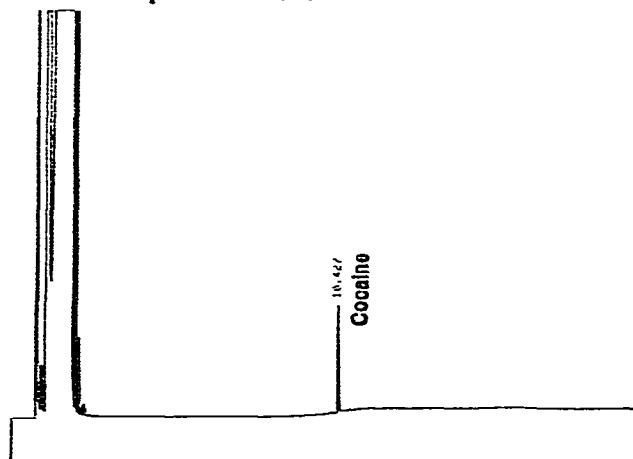

Street Sample 42% Pure

Fig. 6. Identification and quantitation of cocaine with on-column injection. Column: cross-linked SE-54 (25 m × 0.32 mm I.D.), $\beta = 150$; oven profile: 80°C for 0.5 min, 20°/min to 280°C; solvent; methanol–toluene (1:99).

reproducibilities of 0.1–2% at the 10-ng level. Precisions in this range indicate that the technique may be useful for the accurate quantitation of underivatized drugs, in relatively pure samples, without the need for internal standards or multilevel calibration. The on-column injection of serum, or other complex biological samples, will still benefit from the use of internal standards as a check on extraction efficiency in the sample clean-up.

Applications

Forensic screening of "street" drug samples is one application of this chromatographic method. Fig. 6 shows the quantitative and qualitative analysis of cocaine. The "street" sample is dissolved in methanol–toluene (1:59). Sugars and other undissolved additives are centrifuged and separated. The remaining solution is injected on-column. An alkaloid mix containing 10 ng/μl of drug is injected as an external standard. With just two injections, the unknown drug is both identified via its retention time and determined to be 42% pure. Retention indexing or mass spectral identification could also be employed to support the results.

An on-column injection of a serum extract is shown in Fig. 7. A single extraction was performed on 100 μl of human serum from a patient on phenytoin therapy for the treatment of epilepsy. The serum was buffered to pH 6, extracted into chloroform, dried, reconstituted with 25 μl of the binary solvent and 1 μl was injected on-column. The amount of co-extracted substances could be reduced and column life lengthened through the use of a back extraction in the sample preparation sequence. With splitless injection, non-volatile material will remain in the replaceable inlet liner.
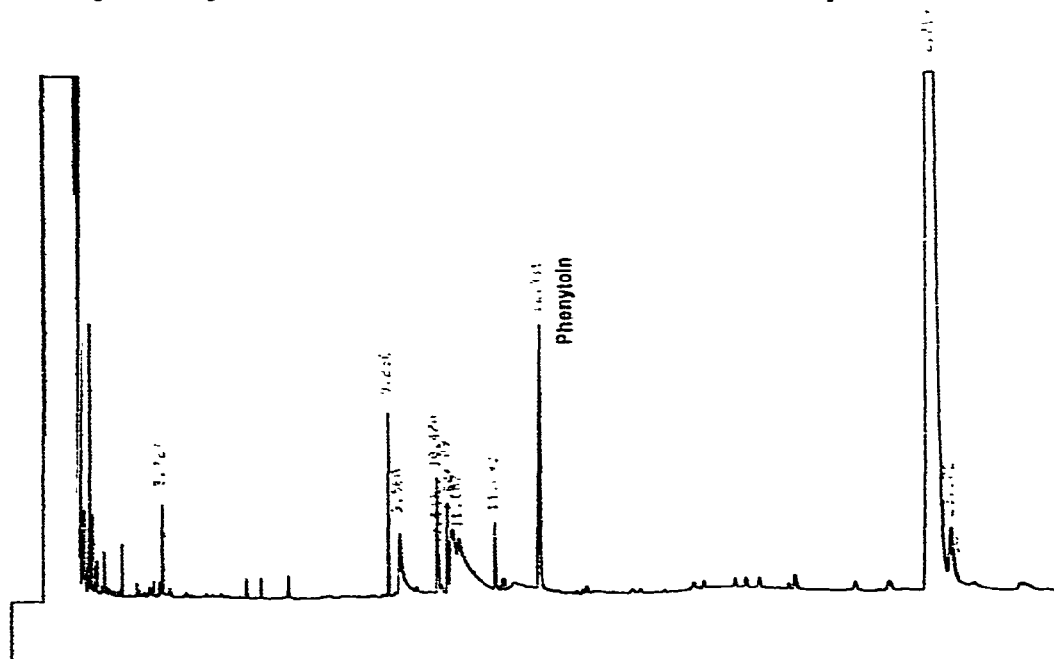

Fig. 7. On-column injection of a human serum extract. Sample: 100 μl of serum with a reported phenytoin concentration of 10.9 μg/ml. Serum was buffered to pH 6, extracted into chloroform, dried, reconstituted with 25 μl of the binary solvent and 1 μl was removed for injection. Column: cross-linked SE-54 (25 m × 0.32 mm I.D.), β = 150; solvent: methanol–toluene (4:96); oven profile: same as in Fig. 8.

Dirtier samples will be more easily tolerated and sample preparation time reduced with splitless injection. Quantitation with splitless injection, however, should only be done with the use of internal standards and multi-level calibration.

CONCLUSION

Fig. 8 summarizes several types of drugs which have been analyzed by fused-silica capillary chromatography. Underivatized anticonvulsants, alkaloids, barbiturates, and tricyclic antidepressants have been combined into a single drug screen. The high resolution offered by this technique makes a single stationary phase suitable for multi-drug identification. The use of the on-column injection technique with non-extractable siloxane deactivated stationary phases can lead to the linear and reproducible trace quantitation of selected members from each of these drug families.
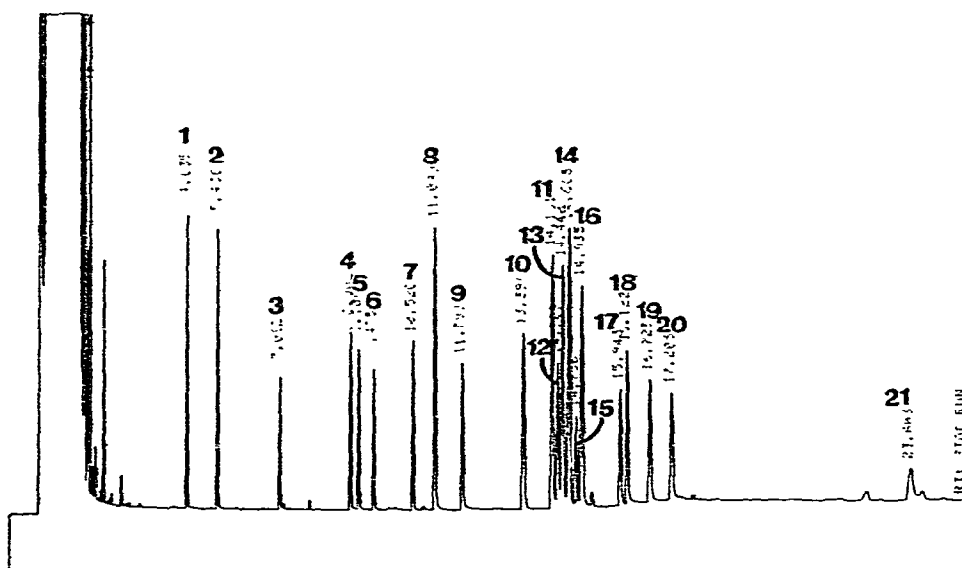

Fig. 8. Underivatized drug screen with on-column injection of 5 ng per drug; solvent: methanol–toluene (4:96); oven profile: 75°C for 0.5 min, 10°/min to 200°C, 5°/min to 210°C, 15°/min to 280°C; column: cross-linked SE-54 (25 m × 0.32 mm I.D.), β = 150. 1 = Ethosuximide; 2 = methyl-propylsuccinimide; 3 = barbital; 4 = amobarbital; 5 = pentobarbital; 6 = secobarbital; 7 = mephobarbital; 8 = phenobarbital; 9 = procaine; 10 = methadone; 11 = amitriptyline; 12 = cocaine; 13 = imipramine; 14 = nortriptyline; 15 = primidone; 16 = desipramine; 17 = carbamazepine; 18 = phenytoin; 19 = codeine; 20 = methylphenytoin; 21 = quinine.

ACKNOWLEDGEMENT

REFERENCES

1 G. Schomburg, H. Husmann and H. Behlau, *Chromatographia*, 13 (1980) 321.
2 R. Dandeneau and E. Zerenner, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 351.
3 W. J. A. VandenHeuvel and J. S. Zweig, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 381.
4 W. J. A. VandenHeuvel, J. R. Carlin and R. W. Walker, in R. E. Kaiser (Editor), *Proc. 4th Int. Symp. on Capillary Chromatography, Hindelang, 1981*, Institute of Chromatography, Bad Dürkheim, 1981, p. 211.
5 R. R. Freeman, *High Resolution Gas Chromatography*, Hewlett-Packard, Avondale, PA, 2nd ed., 1981.
6 W. Jennings, *Gas Chromatography with Glass Capillary Columns*, Academic Press, New York, London, 2nd. ed., 1980.
7 R. G. Jenkins, in R. E. Kaiser (Editor), *Proc. 4th Int. Symp. on Capillary Chromatography, Hindelang, 1981*, Institute of Chromatography, Bad Dürkheim, 1981, p. 803.
8 K. Knauss, J. Fullemann and M. P. Turner, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 641.
9 K. Knauss, J. Fullemann and M. P. Turner, *Hewlett-Packard Technical Paper No. 94*, Hewlett-Packard, Avondale, PA, 1982.
10 W. Noll, *Chemie und Technologie der Silicone*, Verlag Chemie, Weinheim, 1960.
11 C. Madani and E. M. Chambaz, *J. Amer. Oil Chem. Soc.*, 58 (1981) 63.
12 L. Blomberg, K. Markides and T. Wännman, *J. Chromatogr.*, 203 (1981) 217.
13 K. Grob, G. Grob and K. Grob, Jr., *J. Chromatogr.*, 211 (1981) 243.