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An Evaluation of Fused Silica Capillary Columns for the Screening of Basic Drugs in Postmortem Blood: Qualitative and Quantitative Analysis

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ABSTRACT: Fused silica capillary columns (Durabond®) have been evaluated for the screening of more than 100 basic drugs in postmortem blood samples. The combination of these columns, nitrogen-phosphorus detectors, and SKF-525A (internal standard) allows for the simultaneous screening and quantitation of several basic drugs such as amphetamines, amitriptyline, and codeine. Approximately 2000 blood samples have been analyzed by this procedure. The use of capillary columns results in excellent baseline stability and this, together with an autosampler and data system, enables unattended overnight operation. "Double peaking" associated with splitless injection can be a problem as can sensitivity for some of the polar drugs; however, with the extraction procedure described and the equipment used, the screening of blood for basic drugs is improved when compared with packed column technology.

KEYWORDS: toxicology, blood, screening procedures, capillary column

High resolution capillary columns have been used in forensic toxicology for several years. Capillary gas chromatography (CGC) with a nitrogen-phosphorus detector (NPD) has also been used by several workers to determine the concentration of specific drugs or drug classes in biological fluids [*1-5*]. Anderson and Stafford [*6*] recently described a screening procedure for routine toxicological analysis using the split injection technique. The application of CGC in the splitless mode of injection to drug analysis has generally been unpopular although a few papers have appeared [*7-9*]. However, there have been no reports of a general CGC-NPD procedure suitable for qualitative and simultaneous quantitative analysis.

In developing the procedure described here, it was necessary to select an extraction method suitable to most of the common drugs and to automatic injection. It had to be applicable to multi-tube vortex and rotorack equipment so that large numbers of samples could be processed simultaneously for batch analysis by automated GC. NP detection and splitless mode injection were used to obtain adequate sensitivity. Modifications were necessary to existing GC equipment for capillary work and the characteristics of various columns had to be evaluated.

This paper describes the practical application of CGC-NPD to screening for the presence of basic drugs in postmortem blood samples. Also presented are qualitative and quantitative

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data generated during 18 months of use in case work. Chromatographic precision, quality control, and the overall precision and accuracy of the method were examined. The use of a composite standard curve, thus eliminating frequent extraction of calibration standards, was also studied and the limitation of the method for the detection of the more polar compounds considered.

Experimental Procedure

Automated Capillary Chromatography System

Two Hewlett-Packard (Model 5710A) gas chromatographs were modified for capillary work. The Universal injection system was supplied by Scientific Glass Engineering (SGE) and is used in the Grob-type splitless mode. Both instruments were equipped with an NP detector and with an autosampler (HP 7671A) and connected to Spectra Physics integrators (SP 4000 and SP 4100). The chemically bonded fused silica capillary columns (J & W Scientific, Inc.) used were: (1) DB-5 (film 0.25 μm), (2) DB-1 (film 0.10 μm), and (3) DB-1701 (film 0.15 μm). All three columns were narrow bore (inside diameter 0.25 mm) with a column length of 15 m.

The operating parameters were:

- injector temperature: 250°C;
- detector temperature: 300°C;
- program: 95°C (2 min) to 250°C (240°C for DB-5) at 8°C;
- final temperature hold: DB-1—20 min, DB-5—8 min, and DB-1701—16 min;
- detector gases: air—40 mL/min, 8% H_2 in He—30 mL/min, and He (makeup)—15 mL/min;
- carrier: He—1.5 mL/min;
- split vent activation time: 30 s; and
- vent flow (He): 60 mL/min.

Chemical and Reagents

All drug standards were obtained in pure form from pharmaceutical manufacturing companies. Stock solutions of each drug were prepared in methanol at a concentration of 1.0-mg/mL free base. Working standard solutions containing 13 selected drugs at concentrations of 10, 20, and 40 μg/mL were prepared by diluting appropriate volumes of the stock solutions with distilled water. A 2.0-μg/mL solution of Proadifen SKF-525A was prepared in distilled water for use as an internal standard. Stock solutions were stored in the dark at −15°C for up to four months; the diluted standard solutions were prepared every four weeks and stored at 5°C. Extracts of basic drugs used to establish relative retention times (RRTs) were prepared as follows: after the addition of internal standard to the aqueous standard solution, the solution was made alkaline with 0.5*N* sodium hydroxide and extracted with the appropriate volume of toluene to give a concentration equivalent to 2.0 μg/mL. Human blood (outdated) was obtained from a blood bank and artificially aged at room temperature for approximately three to four weeks; after the addition of 1.0-g sodium fluoride/100 mL the blood was stored at 5°C for several months. This blood was used as a control.

All solvents used were of "distilled in glass" quality and the inorganic reagents of analytical reagent grade.

Extraction Method

A 2.0-mL blood sample to which 7.0 mL of toluene and 0.1 mL of concentrated ammonium hydroxide has been added is rotated for 20 min. After centrifugation, the organic

phase is back-extracted for 1 min with 2.0 mL of 2*N* sulfuric acid using the multi-vortex. Following centrifugation, the upper toluene layer is removed and discarded. The remaining sulfuric acid is kept at −15°C for 10 min. After the addition of two drops bromothymol blue indicator and 0.1 mL of the internal standard solution the acid fraction is made alkaline with 5*N* sodium hydroxide and extracted on a vortex mixer for 1 min with 1.5 mL of toluene. After centrifugation for 5 min the toluene layer is transferred to a small glass culture tube containing 0.2 mL of distilled water. This mixture is vortexed for 20 s then centrifuged. The upper toluene layer is transferred to an autosampler vial. Aliquots of 2.0 μL are injected into the gas chromatograph. For GC/MS confirmation, the remaining toluene extract is evaporated under a stream of N_2 at 55°C. The residue is taken up in 100 μL of ethyl alcohol and 3 μL are injected.

Analysis

The identification of unknown peaks is made by comparing RRT data obtained from dissimilar columns. Further qualitative confirmation is obtained by mass spectrometry (Finnigan 1020 mass spectrometer with a DB-5 capillary column and a split/splitless Grob-type injector).

Quantitation is based on comparison with appropriate standards prepared in drug-free blood. The quantitative method employs the SP-4100 Computing Integrator Internal Standard procedure for multiple peaks corresponding to individual basic drugs. The calibration data points are fitted to a linear least square function. The calculated regression equations are kept in the system memory and are used for quantitation during the chromatography run of the blood extracts.

Results and Discussion

The RRT data for 102 drugs are listed in Table 1. Some neutral drugs are included as they are frequently detected using the described procedure.

Significant differences in elution order and in resolution were found between the three columns for such compounds as caffeine, diphenhydramine, lidocaine, pheniramine, and phencyclidine (Fig. 1). The close retention time of lidocaine and diphenhydramine precluded the simultaneous quantitation of these drugs on the DB-5 column. Insufficient separation of lidocaine and phencyclidine can also create some ambiguity in the evaluation of the chromatogram on this column. Pheniramine elutes close to caffeine, therefore, a positive pheniramine case could easily be interpreted as a negative case (positive caffeine). In general, better resolution was obtained with either DB-1 or DB-1701 columns and these were chosen for routine work.

Data for the single concentration long-term precision of relative retention times are shown in Table 2. The 35 drugs selected are representative of volatile amines as well as of the more polar compounds analyzed. Over a period of three months, single concentration (2.0-μg/mL) extracted aqueous standards were injected 20 times. On the DB-1 column, the mean value of the coefficient of variation (CV) was 0.235% ranging between 0.021 and 1.661%. However, 33 of the RRT values were in the 0.021 to 0.912 range. The mean values of the CV for the DB-1701 and DB-5 columns were slightly higher, at 0.402 and 0.314%, respectively. Out of the 35 compounds tested, 4 had a CV higher than 1% on both columns. Particularly interesting was oxycodone which eluted relatively close to the SKF standard yet had a CV as high as 2.93% on the DB-1701 column. The fact that oxycodone tails on this column most likely contributes to its higher CV; flurazepam having a much longer, RT, does not tail (CV = 0.392%). This demonstrates that the tailing of oxycodone and of other polar compounds such as amines results in a poor CV and the use of multiple reference standards would not solve the problem.

TABLE 1—*Relative retention time data for some basic drugs on Durabond capillary columns.*[a]

No.	Compound	DB-1	DB-1701	DB-5
1	Alphaprodine	0.701	0.679	...
2	Amantadine	0.282	0.198	0.174
3	Amitriptyline	0.935	0.926	0.914
4	Amoxapine	1.264	1.270	1.189
5	Amphetamine	0.204	0.151	0.115
6	Anileridine	1.625	1.589	...
7	Atropine	0.937	0.983	0.910
8	BD Vacutainer contaminant	1.022	1.083	...
9	Benztropine	1.005	1.003	0.995
10	Bromodiphenhydramine	0.911	0.911	0.889
11	Brompheniramine	0.884	0.880	0.853
12	Bupivacaine	0.982	1.038	...
13	Caffeine	0.715	0.782	0.650
14	Carbamazepine	0.797	0.847	...
15	Chlordiazepoxide	1.143 (1.281)[b]	1.224 (1.499)[b]	1.115 (1.404)[b]
16	Chlorpheniramine	0.826	0.813	0.786
17	Chlorphentermine	0.377	0.318	0.265
18	Chlorpromazine	1.127	1.124	1.096
19	Clomipramine	1.060	1.064	1.050
20	Cocaine	0.940	0.978	0.926
21	Codeine	1.047	1.095	1.028
22	Cyclobenzaprine	0.959	0.963	0.943
23	Cyproheptadine	1.028	1.028	...
24	Desipramine	0.963	0.979	0.949
25	Diazepam	1.085	1.167	1.068
26	Diethylpropion	0.487	0.488	0.384
27	Diphenhydramine	0.741	0.716	0.690
28	Diphenylpyraline	0.884	0.875	0.855
29	Disopyramide	1.134	1.173	...
30	Doxepin	0.953 (0.943)[b]	0.964 (0.949)[b]	0.937 (0.926)[b]
31	Doxylamine	0.773	0.748	0.726
32	Ecgonine methyl ester	0.472	0.444	0.365
33	Ephedrine	0.388	0.360	...
34	Ethyl aminobenzoate	0.534	0.595	0.443
35	Fentanyl	1.366	1.347	...
36	Fluphenazine	0.822	...	...
37	Flurazepam	1.442	1.448	1.340
38	Haloperidol	1.754	1.860	...
39	Hydrocodone	1.095	1.169	1.067
40	Imipramine	0.951	0.950	0.933
41	Isoephedrine	0.391	0.361	...
42	Ketamine	0.736	0.753	0.667
43	Ketazolam	1.085	1.167	...
44	Lidocaine	0.748	0.798	0.693
45	Lorazepam	1.066	1.138	1.052
46	Loxapine	1.211	1.197	1.159
47	Maprotiline	1.013	1.037	...
48	Mepazine	1.175	1.156	1.128
49	Meperidine	0.664	0.626	0.594
50	Mephentermine	0.297	0.218	0.190
51	Methadone	0.907	0.897	0.883
52	Methamphetamine	0.242	0.174	0.145
53	Methapyrilene	0.817	0.811	0.775
54	Methaqualone	0.917	0.968	0.887
55	Methaqualone metabolite	1.051	1.166	1.036
56	Methotrimeprazine	1.150	1.151	1.119
57	Methoxyamphetamine	0.393	0.347	0.285
58	Methylenedioxyamphetamine	0.467	0.428	0.362
59	Methylphenidate	0.653	0.627	0.582
60	Methyprylon	0.517	0.577	0.420

TABLE 1—(*Continued*)

No.	Compound	DB-1	DB-1701	DB-5
61	Metoclopramide	1.246	...	...
62	Metoprolol	0.839	0.875	...
63	*N*-desalkyl flurazepam	1.091	1.256	1.062
64	*N*-desmethyldiazepam	1.137	1.304	1.105
65	Nicotine	0.367	0.303	0.253
66	Norcocaine	0.923	0.954	...
67	Nordiphenhydramine	0.737	0.726	...
68	Norlidocaine	0.708	0.741	0.645
69	Normeperidine	0.684	0.672	0.615
70	Norpropoxypheneamide	1.179	1.244	1.136
71	Nortriptyline	0.945	0.953	0.927
72	Orphenadrine	0.785	0.762	0.743
73	Oxycodone	1.160	1.229	1.114
74	Pargyline	0.279	0.205	0.174
75	Pentazocine	0.977	1.037	0.967
76	Phencyclidine	0.760	0.687	0.696
77	Phendimetrazine	0.459	0.396	0.352
78	Phenindamine	0.915 (0.930)[b]	0.909 (0.927)[b]	0.855 (0.904)[b]
79	Pheniramine	0.707	0.674	0.645
80	Phenmetrazine	0.444	0.404	0.336
81	Phentermine	0.229	0.166	0.134
82	Phenyltoloxamine	0.789	0.771	0.744
83	Pipradrol	0.913	0.917	0.883
84	Pramocaine	0.973	0.996	0.968
85	Proadifen (SKF 525A)[c]	1.000	1.000	1.000
86	Procaine	0.836	0.911	...
87	Promazine	1.009	1.028	0.994
88	Promethazine	0.982	0.995	0.965
89	Propoxyphene	0.930	0.926	0.923
90	Propranolol	0.910	0.963 (0.978)[b]	0.864
91	Protriptyline	0.965	0.983	...
92	Pyrilamine	0.955	0.977	0.946
93	Quinidine	1.481	1.586	...
94	Quinine	1.487	1.569	1.548
95	Racemethorpan	0.904	0.882	0.870
96	Temazepam	1.316	1.345	...
97	Tranylcypromine	0.271	0.215	...
98	Trifluoperazine	1.299	1.244	1.226
99	Trimipramine	0.948	0.939	0.931
100	Tripelennamine	0.813	0.801	0.772
101	Triprolidine	0.969	0.976	...
102	Zolamine	0.953	0.983	0.943

[a] Retention time relative to SKF 525A.

[b] Secondary GC peak.

[c] Reference internal standard (SKF 525A) elutes at 21.78 min on DB-1, 19.00 min on DB-1701, and 18.10 min on DB-5.

Although the long-term variability of RRT for certain polar compounds was high the short-term (daily) reproducibility was excellent, less than 0.1% for all the 102 drugs studied.

The extraction procedure is outlined in Fig. 2. Toluene was found to be the most useful solvent for extraction as well as to ensure a "solvent effect" in the splitless mode of injection. Previous experiments showed that the addition of SKF to blood before analysis could lead to quantitative error [*10*]. Therefore, internal standard was added to the cooled acid phase; although this cannot be called a true internal standard, it does fulfill a function as reference compound and corrects for any change that might occur after its addition. The initial extraction losses are partially compensated by the use of co-extracted blood standards.

FIG. 1—*Illustration showing the comparative resolution and elution order of:* (1) *pheniramine,* (2) *caffeine,* (3) *diphenhydramine,* (4) *lidocaine, and* (5) *phencyclidine on Durabond capillary columns.*

TABLE 2—*Single concentration long-term RRT precision of selected basic drugs on Durabond capillary columns,*[a] N = *20.*

		DB-1		DB-1701		DB-5	
No.	Compound	Avg. RRT	CV, %	Avg. RRT	CV, %	Avg. RRT	CV, %
1	Amitriptyline	0.935	0.080	0.925	0.089	0.915	0.060
2	Amoxapine	1.260	0.168	1.277	0.647	...	...
3	Amphetamine	0.203	0.912	0.147	1.365	0.115	1.740
4	Bromodiphenhydramine	0.911	0.058	0.912	0.311	0.890	0.060
5	Brompheniramine	0.883	0.058	0.884	0.501	0.853	0.070
6	Caffeine	0.716	0.174	0.782	0.104	0.649	0.140
7	Chlorpheniramine	0.826	0.081	0.813	0.116	0.786	0.050
8	Chlorphentermine	0.375	1.077	0.317	0.755	0.265	0.760
9	Chlorpromazine	1.126	0.088	1.124	0.043	1.096	0.040
10	Codeine	1.047	0.060	1.094	0.047	1.028	0.040
11	Diazepam	1.084	0.092	1.166	0.089	1.068	0.040
12	Diphenhydramine	0.746	0.070	0.716	0.167	0.690	0.090
13	Diphenylpyraline	0.884	0.055	0.874	0.060	0.855	0.070
14	Flurazepam	1.443	0.128	1.445	0.392	1.340	0.060
15	Hydrocodone	1.094	0.123	1.169	0.044	1.067	0.060
16	Imipramine	0.952	0.067	0.950	0.126	0.933	0.040
17	Lidocaine	0.748	0.084	0.798	0.065	0.693	0.080
18	Meperidine	0.664	0.124	0.625	0.244	0.594	0.130
19	Mephentermine	0.296	0.434	0.216	0.763	0.190	2.104
20	Methadone	0.907	0.057	0.897	0.058	0.883	0.034
21	Methamphetamine	0.242	0.469	0.171	1.185	0.145	1.390
22	Methaqualone	0.916	0.131	0.968	0.053	0.887	0.060
23	Methotrimeprazine	1.150	0.159	1.150	0.080	1.119	0.040
24	*N*-desmethyldiazepam	1.137	0.159	1.302	0.302	1.105	0.060
25	Nicotine	0.366	0.409	0.305	0.634	0.253	0.390
26	Oxycodone	1.159	0.168	1.257	2.930	1.114	0.050
27	Pentazocine	0.977	0.053	1.037	0.021	0.967	0.050
28	Phencyclidine	0.759	0.089	0.687	0.182	0.696	0.080
29	Pheniramine	0.706	0.134	0.674	0.077	0.645	0.090
30	Phenmetrazine	0.444	0.242	0.404	0.577	0.336	0.300
31	Phentermine	0.228	0.453	0.165	1.575	0.134	2.270
32	Promazine	1.009	0.021	1.027	0.133	0.994	0.050
33	Racemethorphan	0.903	0.058	0.882	0.055	0.870	0.070
34	Trifluoperazine	1.291	1.661	1.243	0.246	1.226	0.050
35	Trimipramine	0.948	0.056	0.939	0.049	0.931	0.050

[a]Data are collected over a three-month period. RRT: retention time relative to SKF 525A.

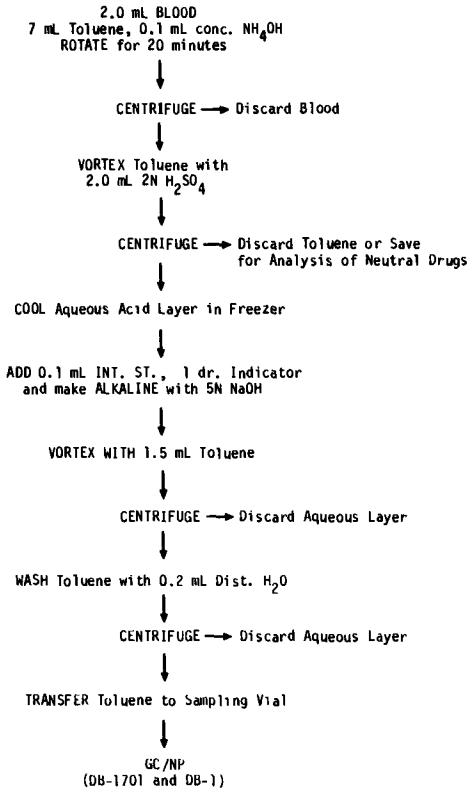

FIG. 2—*Flow diagram of method of basic drug extraction from whole blood.*

Figure 3 demonstrates the separation and the elution order of 30 compounds on 2 columns. Outdated Red Cross blood, previously found negative by GC screening was spiked at a concentration of 1.0 μg/mL and extracted as described. Both columns permitted good separation without excessive tailing. Better sensitivity was obtained on the DB-1 column for compounds such as normeperidine, nortriptyline, and maprotiline. However, on this column propoxyphene at a concentration below 2.0 μg/mL is almost undetectable.

To evaluate the overall precision for quantitation, within-run and between-run CVs were calculated for selected drugs. Data were used from two concentrations (0.5 and 5.0 μg/mL) for each drug in blood. To estimate the within-run CV, the response factors of the basic drugs were calculated from seven assays of each concentration. The response factor was determined from the ratio of the response for the substance of interest divided by the response for the internal standard.

To calculate the day-to-day CV, five sample tubes of each concentration were kept in the refrigerator for subsequent analysis over a period of one month. Concentrations were determined using the daily standard curve obtained in routine analysis.

Data for within-run precision are presented in Table 3. At the lower concentration, the mean value of the CV was 5.64% ranging between 1.72 and 12.65%. Of the 21 drugs tested, only 5 were found to exhibit a wide variation. The CVs for amphetamine, flurazepam, *N*-desmethyldiazepam, methotrimeprazine, and trifluoperazine were around 10%. Better precision was obtained at the higher concentration. The mean value of the CV was 4.09% ranging between 1.38 and 13.25%. Although the upper limit is similar to that of the lower concentration, only flurazepam and *N*-desmethyldiazepam had a CV over 10%.

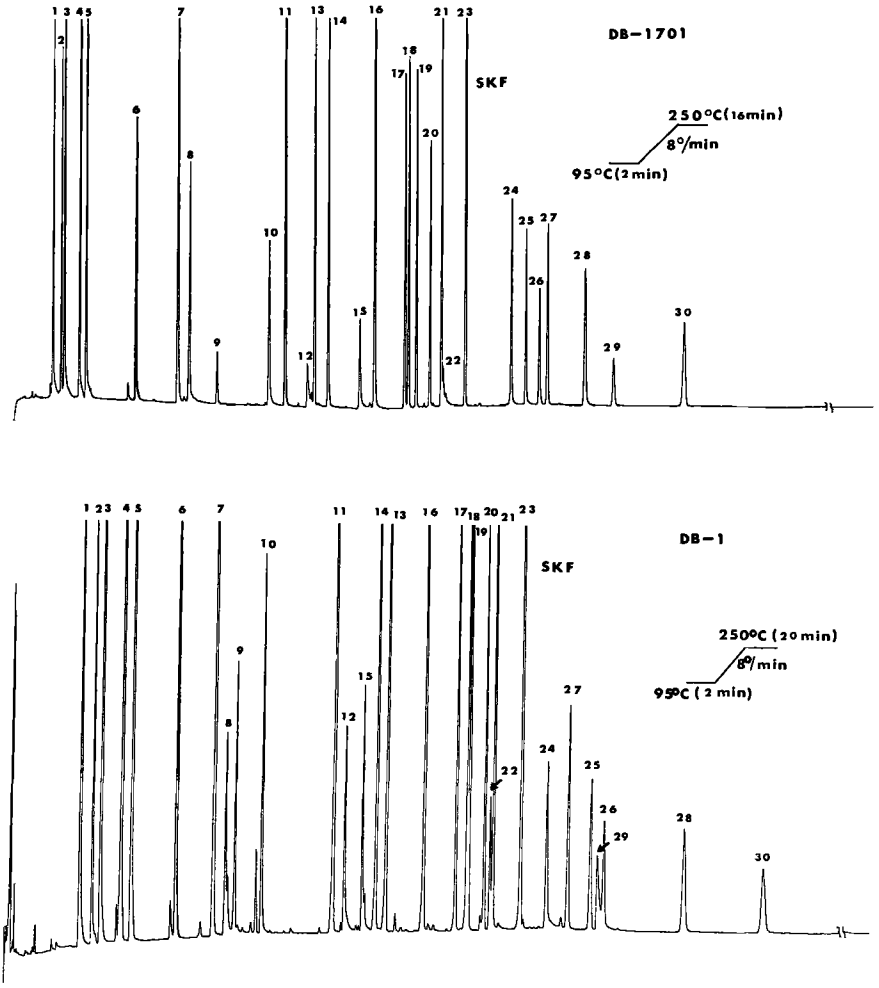

FIG. 3—*Chromatograms of a spiked blood extract on Durabond capillary columns. Peaks:* (1) *amphetamine,* (2) *phentermine,* (3) *methamphetamine,* (4) *pargyline,* (5) *mephentermine,* (6) *chlorphentermine,* (7) *phenmetrazine,* (8) *MDA,* (9) *diethylpropion,* (10) *ethyl aminobenzoate,* (11) *meperidine,* (12) *normeperidine,* (13) *phencyclidine,* (14) *diphenhydramine,* (15) *caffeine,* (16) *chlorpheniramine,* (17) *diphenylpyraline,* (18) *racemethorphane,* (19) *methadone,* (20) *amitriptyline,* (21) *imipramine,* (22) *nortriptyline,* (23) *SKF,* (24) *codeine,* (25) *chlorpromazine,* (26) *methotrimeprazine,* (27) *diazepam,* (28) *trifluoperazine,* (29) N-*desmethyldiazepam, and* (30) *flurazepam.*

Twelve drugs were selected for the daily calibration standard curves. Data on the day-to-day precision are shown in Table 4. The results are similar to those in Table 3. As expected, the CV for amphetamine and methamphetamine were greater than 10% at the lower concentration and the mean value of the CV for all drugs in the table was 5.40% ranging between 1.96 and 13.50%. At the higher concentration, the CV ranged between 1.40 and 4.69% with a mean of 3.04%.

To investigate the feasibility of the use of composite standard curve thus eliminating the necessity of frequent standard extraction, statistical analysis was done on 20 standard curves obtained over a period of more than 3 months. The results for the twelve drugs used in the day-to-day operation are summarized in Table 5. The correlation coefficients computed

TABLE 3—*Within-run precision*[a] *of selected basic drugs,* N = *7.*

No.	Compound	0.5 μg/mL		5.0 μg/mL	
		Avg. RF	CV, %	Avg. RF	CV, %
1	Amitriptyline	1.06	2.31	1.25	2.02
2	Amphetamine	1.58	11.40	2.05	5.00
3	Chlorpheniramine	2.46	2.40	2.76	1.97
4	Chlorphentermine	1.19	2.98	1.15	2.57
5	Chlorpromazine	0.67	7.25	1.08	3.04
6	Codeine	0.68	4.46	0.84	4.78
7	Diazepam	0.86	2.97	0.93	4.71
8	Diphenhydramine	1.84	3.43	1.86	1.95
9	Diphenylpyraline	1.33	4.86	1.33	2.80
10	Flurazepam	0.65	12.65	1.04	13.25
11	Imipramine	2.20	2.36	2.79	1.38
12	Meperidine	2.15	1.72	1.99	2.19
13	Mephentermine	2.27	5.08	2.28	3.09
14	Methadone	1.33	3.29	1.50	4.50
15	Methamphetamine	2.76	6.75	2.93	2.94
16	Methotrimeprazine	0.42	9.87	0.99	5.25
17	*N*-desmethyldiazepam	0.56	11.57	0.72	10.60
18	Phencyclidine	1.74	2.33	1.89	2.46
19	Phenmetrazine	2.55	1.89	2.41	2.38
20	Phentermine	0.99	6.69	1.02	3.43
21	Trifluoperazine	0.72	12.20	1.34	5.62

[a]Precision data based on response factor RF of basic drugs on capillary column and calculated as:

$$RF = \frac{Qs}{Qx} \frac{Ax}{As}$$

where

Qs = injected quantity of internal standard,
Qx = injected quantity of basic drugs,
As = peak area of internal standard, and
Ax = peak area of basic drugs.

TABLE 4—*Day-to-day precision for determination of selected basic drugs,* N = *5.*

No.	Compound	0.5 μg/mL		5.0 μg/mL	
		Avg.[a]	CV, %	Avg.[a]	CV, %
1	Amitriptyline	0.50	3.74	4.74	2.29
2	Amphetamine	0.51	13.00	4.72	4.77
3	Chlorpheniramine	0.51	3.80	5.47	1.40
4	Chlorphentermine	0.50	5.35	4.88	2.77
5	Codeine	0.51	1.96	4.69	3.73
6	Diazepam	0.41	4.60	5.00	4.69
7	Diphenhydramine	0.48	5.57	4.87	2.47
8	Imipramine	0.51	3.59	4.74	2.08
9	Meperidine	0.50	2.60	5.15	2.33
10	Methadone	0.51	3.79	4.80	3.50
11	Methamphetamine	0.53	13.50	4.73	2.95
12	Phenmetrazine	0.49	3.39	4.99	3.50

[a]Avg.: average of five determinations over one-month period.

TABLE 5—*Day-to-day precision for standard linear regression curves,*[a] N = *20.*

No.	Compound	Linear Regression[b] $Y = a + bX$	r	Avg. Slope[c] b	Avg. Y intercept[c] a
1	Amitriptyline	$Y = -0.0639 + 5.60X$	0.9903	5.60 ± 0.16	−0.0654 ± 0.0140
2	Amphetamine	$Y = -0.1618 + 5.78X$	0.9646	5.68 ± 0.43	−0.1563 ± 0.0215
3	Chlorpheniramine	$Y = -0.1743 + 9.95X$	0.9852	9.93 ± 0.37	−0.1760 ± 0.0350
4	Chlorphentermine	$Y = -0.0364 + 4.25X$	0.9707	4.19 ± 0.29	−0.0387 ± 0.0158
5	Codeine	$Y = -0.0609 + 3.98X$	0.9712	3.96 ± 0.23	−0.0628 ± 0.0132
6	Diazepam	$Y = -0.0235 + 2.83X$	0.9536	2.78 ± 0.22	−0.0231 ± 0.0138
7	Diphenhydramine	$Y = -0.0546 + 6.94X$	0.9858	6.90 ± 0.29	−0.0542 ± 0.0235
8	Imipramine	$Y = -0.1444 + 9.19X$	0.9848	9.06 ± 0.27	−0.1380 ± 0.0261
9	Meperidine	$Y = -0.0344 + 7.05X$	0.9818	7.04 ± 0.30	−0.0367 ± 0.0279
10	Methamphetamine	$Y = -0.1375 + 9.25X$	0.9817	9.15 ± 0.43	−0.1270 ± 0.0493
11	Phenmetrazine	$Y = -0.0588 + 9.23X$	0.9818	9.23 ± 0.37	−0.0606 ± 0.0367
12	Racemethorphan	$Y = -0.0659 + 5.94X$	0.9857	5.93 ± 0.20	−0.0675 ± 0.0179

[a] Y: peak area ratio; a: Y intercept; b: slope; X: drug concentration; and r: correlation coefficient. Composite standard curves were analyzed for linearity of regression [*14*]. The tested curves did not deviate significantly from linearity ($p > 0.2$).

[b] Linear regression equation derived from composite standard curves.

[c] Average of 20 individual standard linear regression curves (±95% confidence limits).

from all data points of the 20 standard curves are within the acceptable range (>95). A nearly perfect correlation was observed for amitriptyline. Data obtained from the new fitted lines are within the 95% confidence limit of the calculated average slope b and intercept a of the 20 individual regression equations and thus confirm the validity of the new regression equation. Despite the fact that the correlation coefficient of diazepam is close to the acceptable limit the data in Table 5 are supportive of the contention that the use of a composite standard curve is possible and practical.

Typical standard linear regression curves from composite standards are illustrated in Fig. 4. The confidence band was constructed using the critical value of the variance ratio with 2 and $N - 2$ degrees of freedom at a level of significance equal to 0.95. As expected because of the range of the standard curve, the confidence interval was greater at the very low and high values of the regression line than in the middle. However, no significant difference was found between the regression lines during the three-month period. The overall coefficient of variation for the slope of the line was less than ±10%.

The main criteria for assessing the value of capillary chromatography of blood extracts are sensitivity, resolution, and reproducibility of the qualitative and quantitative results. Figure 5 illustrates the chromatograms of an extract of a blood control containing 13 drugs, caffeine, and SKF. Selection of these drugs as calibration standards was based on either their good precision or their frequent finding in case samples. The excellent reproducibility of results and the adequate sensitivity achieved can be attributed mainly to several key aspects of the procedures: (a) the method used for the extraction of blood samples resulted in an extract sufficiently free from endogenous blood impurities, (b) the inherent sensitivity of the NP detector and the employment of the splitless mode of injection made possible the application of an efficient and accurate automatic injection system, (c) the internal standard added was able to compensate to some extent for possible changes in detector response, and (d) the use of Durabond® capillary columns provided excellent resolution and baseline stability during temperature programming.

The capillary system performance is calibrated and verified at the beginning of each working day. Thus, if changes in RRT values occur or the expected concentration exceeds ±10% of the target value, readjustment or recalibration is necessary. After a period of more than one year, the DB-1 column was found to be extremely stable with no measurable decrease in

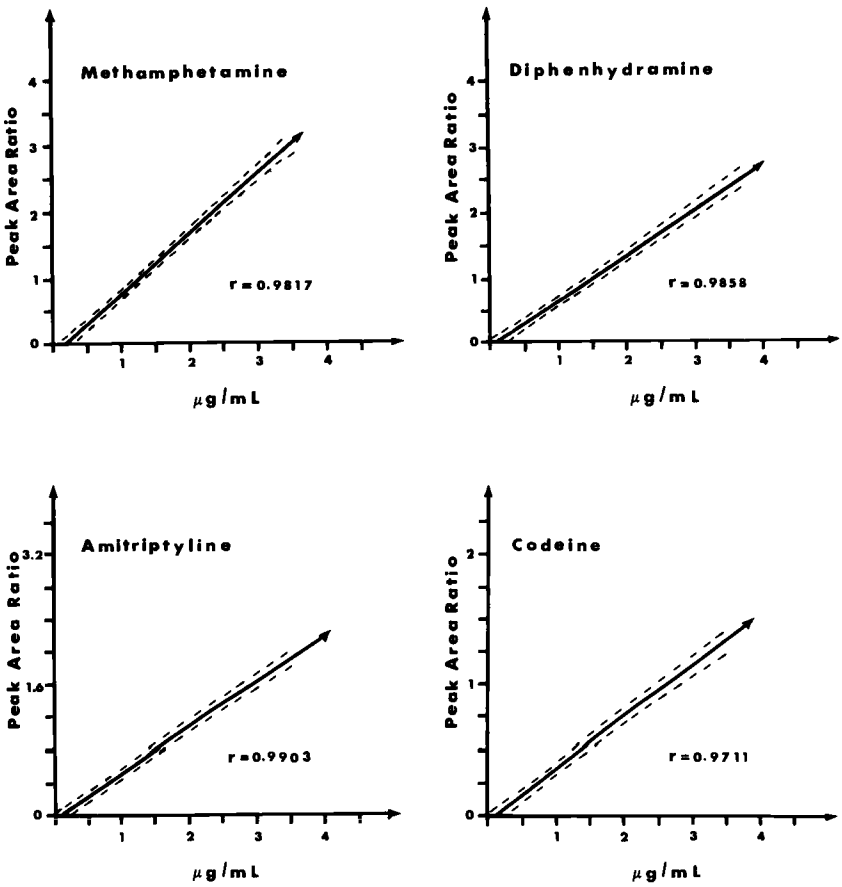

FIG. 4—*Standard linear regression curves, derived from composite standard curves* (N = 20). r = *correlation coefficient.*

efficiency; however, with the DB-1701 phase peak tailing, absolute retention time change and baseline noise were noticed after a period of four months. Because of the extensive use of the instrument, replacement of the NP detector bead is required more frequently. Consequently, recalibration of the system is inevitable. In view of the fact that the instrument is in use 24 h a day, the six to eight weeks of life time of the NP detector bead is acceptable.

Accuracy data for drugs used in routine calibration curves are given in Table 6. The concentration range covers the values most commonly found in blood samples.

The "true" relation between found and added should be a straight line passing through the origin with a slope equal to one. As is demonstrated in Table 6, the intercept a is slightly different from zero, and the slope b is very close to one for most of the drugs. However, chlorpheniramine and diazepam have an intercept equal to 0.122 and 0.125 μg/mL, respectively. The measure of precision obtained from quintuplicate analysis is demonstrated by showing the standard error of estimate using a 95% confidence limit. The overall mean value was 0.097 μg/mL. These statistical data are considered especially valid since separate blood samples were spiked and extracted to produce each data point.

The reliability of the calibration line over its range of applicability is illustrated in Fig. 6. It is based on a 95% joint confidence region for the slope and intercept of the data shown in Table 6. The correlation coefficients for all twelve drugs were greater than 0.99; however, the

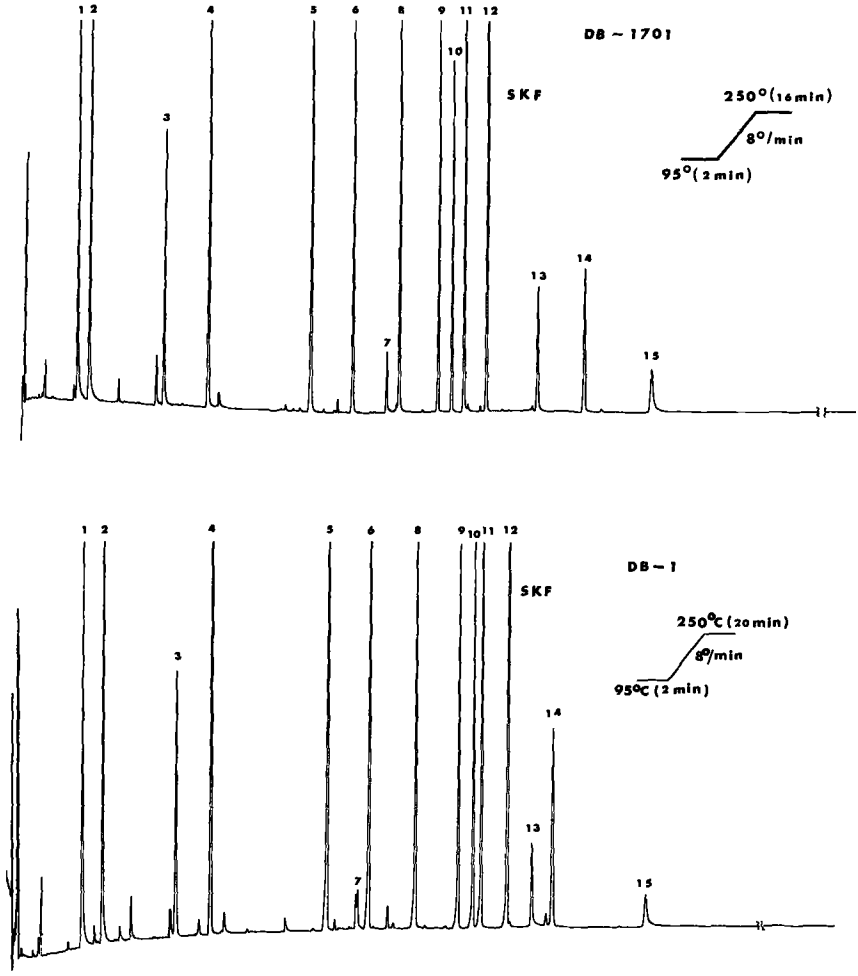

FIG. 5—*Chromatograms of a blood extract used as a control sample. Concentrations of each drug are: 0.5 μg/mL of blood. Peaks:* (1) *amphetamine,* (2) *methamphetamine,* (3) *chlorphentermine,* (4) *phenmetrazine,* (5) *meperidine,* (6) *diphenhydramine,* (7) *caffeine,* (8) *chlorpheniramine,* (9) *methadone,* (10) *amitriptyline,* (11) *imipramine,* (12) *SKF,* (13) *codeine,* (14) *diazepam, and* (15) *amoxapine.*

line is most accurate in the mid region of the range. Therefore, care must be taken when the concentration is outside this range. In this laboratory, higher concentrations are quantitated either by diluting the sulfuric acid phase before extraction into toluene, or by extracting standards in the range of the concentration encountered. Concentrations below 0.50 μg/mL must also be evaluated with caution because of possible drug loss through column or injection port adsorption.

When several compounds are present in a blood sample to be screened without any prior knowledge of their presence the efficiency of column separation becomes crucial. Figure 7 shows chromatograms of multiple drugs and metabolites found in an actual case. Both chromatograms are free of interference. Identification of the substances was indicated by the relative retention times on the DB-1701 column and confirmed by retention times on the DB-1 column. Diphenhydramine and codeine were quantified simultaneously. The remaining extract was used for final confirmation by GC/MS. Using the same extraction method,

TABLE 6—*Accuracy*[a] *of some basic drugs using linear regression data.*

No.	Compound	Linear Regression[b] $Y = a + bX \pm Sy \cdot x$	r
1	Amitriptyline	$Y = 0.0050 + 0.94X \pm 0.007$	0.9987
2	Amphetamine	$Y = -0.0039 + 0.95X \pm 0.011$	0.9979
3	Chlorpheniramine	$Y = -0.0122 + 1.11X \pm 0.010$	0.9987
4	Chlorphentermine	$Y = -0.0009 + 0.97X \pm 0.008$	0.9989
5	Codeine	$Y = 0.0027 + 0.93X \pm 0.010$	0.9981
6	Diazepam	$Y = -0.0125 + 1.02X \pm 0.014$	0.9971
7	Diphenhydramine	$Y = 0.0037 + 0.97X \pm 0.010$	0.9983
8	Imipramine	$Y = 0.0061 + 0.94X \pm 0.007$	0.9990
9	Meperidine	$Y = -0.0036 + 1.03X \pm 0.008$	0.9990
10	Methadone	$Y = 0.0016 + 0.98X \pm 0.010$	0.9982
11	Methamphetamine	$Y = 0.0045 + 0.94X \pm 0.013$	0.9971
12	Phenmetrazine	$Y = 0.0022 + 1.00X \pm 0.009$	0.9985

[a]Evaluated in the 0.5- to 5.0-μg/mL range, using quintuplicate analysis for each concentration (0.5, 1.0, 2.0, and 5.0 μg/mL).

[b]Drug concentration (added); Y: drug concentration (found); a: Y intercept; b: slope; r: correlation coefficient; and $Sy \cdot x$: standard error of estimate.

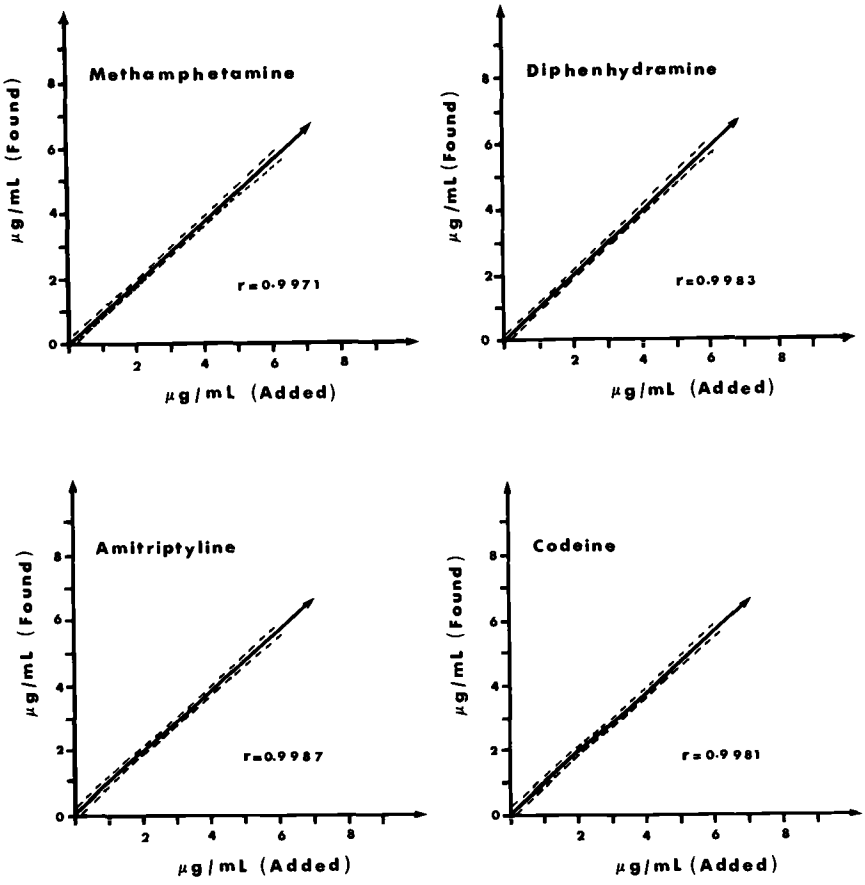

FIG. 6—*Correlation of the observed concentration and the theoretical drug blood concentration.* r = *correlation coefficient.*

another 2.0-mL aliquot of the blood was extracted and pheniramine, doxepin, pyrilamine, and flurazepam were simultaneously quantitated on the DB-1701 column. A specific extraction was required for quantitation of metoprolol. Figure 7 clearly demonstrates the advantage of two capillary columns different in polarity. Doxepin and pyrilamine eluted at the same retention time on the DB-1 column while on DB-1701 these two compounds were well resolved. Figure 7 also demonstrates "column overload;" the level of metoprolol exceeded 2.0 mg/100 mL. No problem was encountered with carry over to the next sample analyzed.

Finally, the stability of some basic drugs in preserved blood was tested. Blood standards of 30 drugs at concentrations of 0.5, 1.0, and 2.0 μg/mL were stored under refrigeration and analyzed more than 5 times in duplicate over a period of 1 year. This limited experiment indicated that all 30 drugs could be readily detected at a concentration of 1.0 μg/mL after a year (Fig. 3). They showed little or no loss. The presence of all 30 drugs was confirmed by GC/MS.

Discrimination against the more polar drugs using the splitless injection system is probably inevitable [*11*] and is not helped by the fact that with the SGE capillary conversion kit the

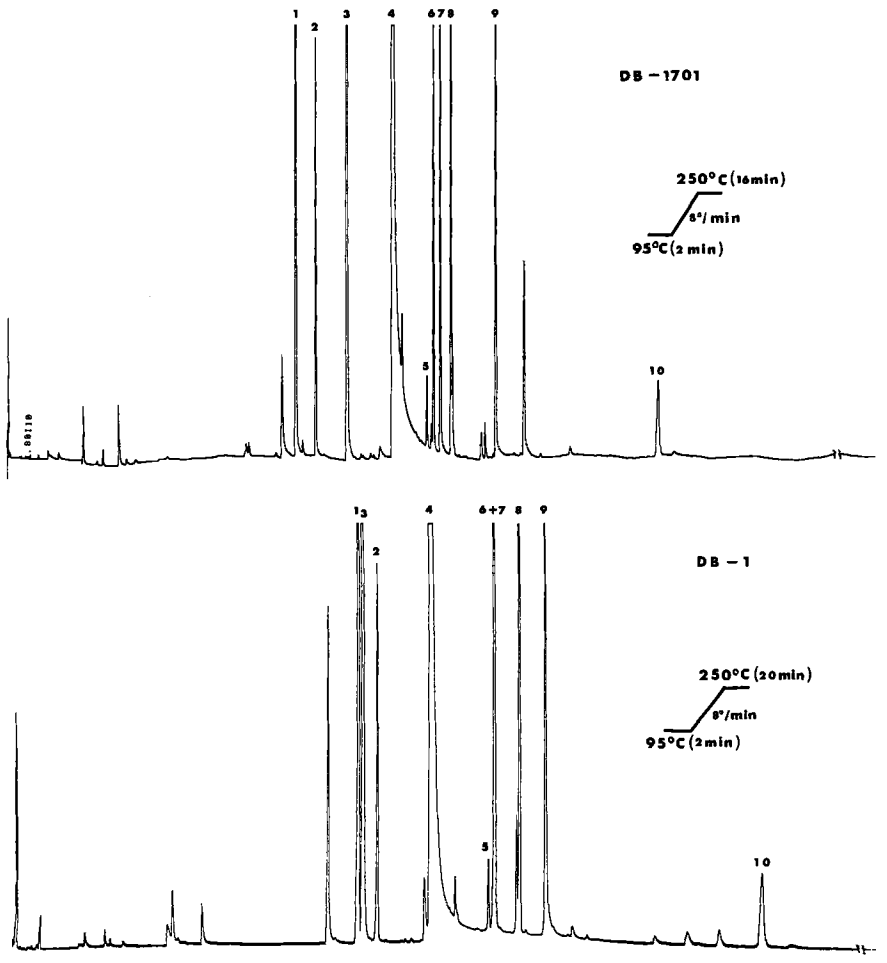

FIG. 7—*Chromatograms of a case blood extract.* (1) *pheniramine,* (2) *diphenhydramine,* (3) *caffeine,* (4) *metoprolol,* (5) cis-*doxepin,* (6) trans-*doxepin,* (7) *pyrilamine.* (8) *SKF-525A,* (9) *codeine, and* (10) *flurazepam.*

injector barrel protrudes into the GC oven. The injection volume is large (2 μL) and the rate of injection cannot be controlled using the present equipment; in fact it was somewhat surprising that the GC system worked as well as it did. Another critical point in splitless injection is peak splitting as a result of the trapping effect of the warm zone of the capillary connection [*12*]. In practice, peak splitting is minimized or eliminated by optimizing column head pressure, vent time, and column position at the injector inlet.

The CGC procedure described has been used successfully in this laboratory for over 18 months. Table 7 shows the positive findings. During the 18-month period, 1970 cases were routinely screened and 857 (43.5%) were found to be positive. The distribution of these 857 cases involving 1 or more than 1 drug were 562 and 295, respectively.

Conclusion

In this study, the use of capillary columns (Durabond) for the screening of basic drugs in blood has been evaluated. Precision and accuracy data and results for 1970 case blood samples have been presented. The extraction method described simplifies the handling of large numbers of samples and makes efficient use of the automatic injector. Using only 2.0 mL of blood, an effective screening method for qualitative identification, simultaneous quantitation, and further confirmation by GC/MS can be carried out. The lower limit for accurate quantitation is about 0.2 μg/mL. The use of composite standard curves for quantitation has been shown to be feasible. The automated CGC system operated in the splitless mode had

TABLE 7—*Drugs most frequently detected by capillary gas chromatography screen over 18 months.*

Drug	Number of Cases[a]		
	A	B	Total
Diazepam	118	125	243
Lidocaine	161	64	225
Codeine	27	81	108
Amitriptyline	37	56	93
Diphenhydramine	31	58	89
Chlordiazepoxide	23	35	58
Propoxyphene	22	27	49
Doxepin	11	24	35
N-desmethyldiazepam	19	10	29
Flurazepam	4	21	25
Meperidine	5	20	25
Imipramine	9	14	23
Methaqualone	7	14	21
Pheniramine	3	14	17
Chlorpromazine	7	9	16
Trimipramine	10	5	15
Ephedrine	5	10	15
Methyprylone	5	8	15
Pentazocine	4	9	13
Maprotiline	6	7	13
Carbamazepine	5	6	11
Chlorpheniramine	3	7	10
Quinine	2	8	10
Others[b]	38	61	99

[a]A: no other drug, except caffeine and B: in combination with other drugs.

[b]Thirty-five drugs, each encountered fewer than ten times.

proved to be reliable, accurate, and a significant improvement over packed column gas chromatography [*13*].

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