of the three components which have widely spaced boiling points.

REFERENCES

(1) Brochmann-Hanssen, E., and Svendsen, A. B., *J. Pharm. Sci.*, **51**, 938(1962).
(2) Fales, H. M., and Pisano, J. J., *Anal. Biochem.*, **3**, 337(1962).
(3) Parker, K. D., Fontan, C. R., and Kirk, P. L., *Anal. Chem.*, **34**, 1345(1962).
(4) *Ibid.*, **35**, 356(1963).
(5) Massingill, J. L., Jr., and Hodgkins, J. E., *ibid.*, **37**, 952(1965).
(6) Mule, S. J., *ibid.*, **36**, 1907(1964).
(7) Fontan, C. R., Smith, W. C., and Kirk, P. L., *ibid.*, **35**, 591(1963).
(8) MacDonald, A., Jr., and Pflaum, R. T., *J. Pharm. Sci.*, **53**, 887(1964).
(9) Celeste, A., and Turczan, J., *J. Assoc. Offic. Agr. Chemists*, **46**, 1055(1963).
(10) Schmerzler, E., Yu, W., Hewitt, M. I., and Greenblatt, I. J., *J. Pharm. Sci.*, **55**, 155(1966).
(11) Horning, E. C., Moscatelli, E. A., and Sweeley, C. C., *Chem. Ind. (London)*, **1959**, 751.
(12) Ray, N. H., *J. Appl. Chem.*, **4**, 21(1954).
(13) Advisory Board, *Anal. Chem.*, **38**, 2010(1966).

Keyphrases

Methapyrilene fumarate, ephedrine HCl, codeine PO_4 syrup—analysis
Simultaneous determination
GLC—analysis
Amobarbital—internal standard

Quantitative Determination of Some Single and Multiple Component Drugs by Gas-Liquid Chromatography

By BOBBY R. RADER and EMMA S. ARANDA

A gas-liquid chromatographic procedure has been developed to separate and quantitate various drug mixtures. The samples are extracted by various techniques and determined by the use of a polar (4 percent cyclohexanedimethanol succinate) or a polar–nonpolar (1 percent cyclohexanedimethanol succinate plus 10 percent SE 52 silicone gum rubber) gas-liquid column. Retention data relative to pentobarbital are presented for 50 drug materials. Quantitative data are presented for 25 different drugs found in 19 commercial preparations, and for 7 synthetically prepared drug combinations. Recoveries ranged from 96 to 106 percent.

GAS-LIQUID CHROMATOGRAPHY (GLC) has been used to successfully separate antihistamines (1–5), barbiturates (6–11), and alkaloids (12–14) where several of the same class occur together. Many multiple component drugs, however, are not confined to a single class of ingredient (*e.g.*, barbiturates only) but have a variety of active ingredients. The purpose of this study was to find appropriate column materials for GLC which could be used to separate various classes of drugs in a single dosage form.

Previous reports on drug separation by GLC (2, 5, 11, 12) indicate that columns containing either polar or polar–nonpolar liquid phases give more symmetrical peaks for a larger number of drugs than nonpolar liquid phases. Peak symmetry is desirable for accurate quantitation of drugs. Two such columns were investigated in this study.

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Nineteen commercial drug preparations were analyzed. These drugs were in several dosage forms including tablets, capsules, liquids, and lotions. They contained from one to ten active ingredients each, but a maximum of six ingredients was analyzed in any one sample.

Seven synthetic drug mixtures were also analyzed. These mixtures were prepared, in most cases, with the concentration of active ingredients and excipients (starch, lactose, and magnesium stearate) equivalent to commercial drug preparations.

EXPERIMENTAL

Column Preparation

A mixed column of 1% HI-EFF-8BP (cyclohexanedimethanol succinate) + 10% SE 52 (a methylphenyl silicone gum rubber) on Gas Chrom Q (Column A) and a polar column of 4% HI-EFF-8BP on Gas Chrom Q (Column B) were prepared and conditioned in the following manner.

Column A—Into a 600-ml. beaker, 200 mg. of HI-EFF-8BP (Applied Science Laboratories) and

2.0 Gm. of SE 52 (Analabs, Inc.) were weighed, then dissolved in 350 ml. of benzene–toluene (1 + 2) by heating on the steam bath with mixing. To this solution, 20 Gm. of 80–100 mesh Gas Chrom Q (Applied Science Laboratories) was added.

Column B—Into a 600-ml. beaker, 800 mg. of HI-EFF-8BP was weighed, then dissolved in 350 ml. of benzene by heating on the steam bath with mixing. To the solution, 20 Gm. of 80–100 mesh Gas Chrom Q was added.

The solvent in each case was removed by evaporation on a steam bath with frequent stirring. The packing material was slowly added to a 1.8 m. × 63 mm. (6 ft. × 1/4 in. i.d.) glass coiled column, using vacuum and tapping. The columns were conditioned overnight at 250° with a flow rate of nitrogen of about 50 ml./min.

Apparatus

A Packard model 7621S gas chromatograph equipped with a hydrogen flame ionization detector and a 1-mv. recorder was used with the following conditions: Column temperature, 200–250°; inlet temperature, 280°; detector temperature, 250°; voltage, 250; carrier gas, nitrogen at 100 ml./min.; sensitivity, 1×10^{-7} to 1×10^{-10} amp. full scale.

Because of the concentration range of the components in some of the drugs, the concentrations in the sample solutions to be analyzed ranged from 0.2 to 3 mg./ml. Column temperatures and instrument sensitivity were varied to obtain adequate separation and response for quantitation.

Preparation of Standard Solution

A weighed amount of the drug to be determined was diluted with chloroform or methanol to approximate the same concentration as that in the sample solution (0.2–3 mg./ml.).

Barbiturates as sodium salts used as standards were extracted in the same manner as the sample.

Sample Preparations

For tablets, 20 tablets were weighed and powdered; then a portion was weighed for assay. For liquids, an aliquot was pipeted. For lotions, the density was determined and a sample was weighed for analysis.

Extraction Procedures

In the first stage of sample analysis, the active ingredients were extracted from the inert materials by one of three techniques: direct solution in an appropriate solvent, liquid-liquid extraction using a separator, or liquid-liquid partition column chromatography. The purpose of these procedures was not necessarily to separate one component from another but to isolate the active ingredients for subsequent chromatographic separation.

The following extraction procedures were used. (The ether and chloroform used in Procedures 3 and 4 were water-saturated just before use.)

Procedure 1—A sample aliquot was diluted to a suitable volume with chloroform or methanol and mixed.

Procedure 2—The solvent was added by pipet to an accurately weighed portion of the powdered sample. The sample was placed in an ultrasonic bath for 10 min. to aid solution, then mixed, and filtered.

Procedure 3—The sample was mixed with 3–4 ml. of 1 *N* NaOH and 4–5 Gm. of acid-washed diatomaceous earth[1] and transferred to a chromatographic column. The beaker and funnel were rinsed with 1 Gm. of acid-washed diatomaceous earth, which was also added to the column. The column was topped with a piece of glass wool, then eluted with one or two of the following: (*a*) 200–300 ml. of ether; (*b*) 200–300 ml. of chloroform; (*c*) 10 ml. of 10% acetic acid in chloroform, followed by 100 ml. of 1% acetic acid in chloroform; (*d*) 100 ml. of 1% triethylamine in chloroform; (*e*) 5 ml. of 20% triethylamine in chloroform, followed by 100 ml. of 1% triethylamine in chloroform.

Procedure 4—The sample was treated as in Procedure 3, except that water was used instead of 1 *N* NaOH.

Procedure 5—The sample was transferred to a separator, diluted to approximately 20 ml. with distilled water, and made either (*a*) basic with 1 *N* NaOH, or (*b*) acid with HCl and extracted 6–8 times with 25-ml. portions of chloroform.

All extracted sample solutions in Procedures 3, 4, and 5 were evaporated nearly to dryness, then dissolved in an appropriate solvent and transferred to a volumetric flask.

When multiple components in a drug were determined, the drug with the lowest concentration was determined first; then appropriate dilutions were made for assay of the other drugs present in greater concentrations.

The actual extraction method used for a given sample can be determined from Table I. For example, the extraction for a drug mixture (No. 7) of orphenadrine citrate, caffeine, and phenacetin (with aspirin) is listed in the table as *3 bd*. This sample was extracted on the NaOH–diatomaceous earth column, and eluted with solution *b* (chloroform), followed by solution *d* (1% triethylamine in chloroform).

Injection Technique

From 1–3 μl. of solvent was drawn into a 10-μl. syringe, followed by 1–3 μl. of air and then by 5 μl. of the sample solution. The plunger was drawn back, and the volume of sample solution was read and injected.

This technique allows the entire sample in the syringe to be injected, and eliminates the need for an internal standard (15).

Quantitation of Peaks

A number of methods can be used to quantitate chromatographic peaks (16). In this study, calculations were based on peak heights for drugs with retention times of less than 5 min., because it was difficult to accurately measure the width of sharp, narrow peaks. For peaks with retention times greater than 5 min., quantitation was based on peak area obtained by triangulation.

To obtain accurate results, an amount of standard was injected to produce a peak area within ±20% of sample peak area. Peak heights were between 20 and 90% of full-scale deflection.

DISCUSSION

The experimental conditions used were selected to allow the analysis of a variety of multiple component

[1] Celite, Johns-Manville Corp., New York, N. Y.

TABLE I—ANALYSIS OF COMMERCIAL AND SYNTHETIC DRUG PREPARATIONS

No.	Drugs Analyzed	Method of Extraction	Column Used	Amount Declared	% Found of Declared	Recovery of Synthetic Mix Equivalent to Sample	% Found of Declared by UV Method
1	Orphenadrine citrate	1	A	30 mg./ml.	96	...	100
2	Orphenadrine citrate	2	A	100 mg./tab.	98	...	99
3	Orphenadrine HCl	2	A	50 mg./tab.	103	...	...
4	Mephenesin	2	A	0.5 Gm./tab.	100	...	98
5	Pyrilamine maleate	2	A	50 mg./tab.	102	...	...
6	Mephenesin	3*ac*	B	300 mg./tab.	105	...	...
	Salicylamide			300 mg./tab.	93	...	...
7	Orphenadrine citrate	3*bd*	B	25 mg./tab.	98	101	...
	Caffeine			30 mg./tab.	95	98	...
	Phenacetin			160 mg./tab.	100	101	...
	(with aspirin)			225 mg./tab.	...	...	...
8	Phenindamine tartrate	2	A	25 mg./tab.	80	...	80
9	Caffeine	3*b*	B	30 mg./tab.	107	...	...
	Phenacetin			160 mg./tab.	102	...	...
	Chlorpheniramine maleate (with aspirin)			2 mg./tab.	114	...	...
10	Benzocaine	5*a*	B	0.5% soln	98	98	...
	Methapyrilene HCl			0.5% soln.	102	99	...
	Pyrilamine maleate			0.5% soln.	99	103	...
11	Salicylamide	3*ae*	A	3.5 gr./cap.	97	106	...
	Acetaminophen			2 gr./cap.	106	98	...
	Caffeine			0.5 gr./cap.	104	98	...
	Pheniramine maleate			12.2 mg./cap.	98	102	...
	Methapyrilene HCl			10.2 mg./cap.	98	102	...
	Pyrilamine maleate			12.2 mg./cap	91	102	...
12	Salicylamide	3*ae*	A	3.5 gr./cap.	106	...	...
	Acetaminophen			2 gr./cap.	112	...	...
	Caffeine			0.5 gr./cap	106	...	...
	Pheniramine maleate			6.25 mg./cap.	89	...	...
	Methapyrilene HCl			8.33 mg./cap.	97	...	...
	Pyrilamine maleate			8.33 mg./cap.	90	...	...
13[a]	Pheniramine maleate	3*be*	A	10 mg/tab.		103	...
	Methapyrilene HCl			15 mg./tab.		104	...
	Salicylamide			300 mg./tab.		101	...
14	Salicylamide	3*ae*	A	250 mg./tab.	104	...	...
	Acetaminophen			200 mg./tab.	89	...	...
	Caffeine			15 mg./tab.	93	...	...
	Chlorpheniramine maleate			2 mg./tab.	112	...	...
	Pyrilamine maleate			10 mg./tab.	98	...	...
15	Phenylpropanolamine HCl	1	A	12.5 mg./ml.	95	...	...
	Chlorpheniramine maleate			5 mg./ml.	104	...	...
16[a]	Phenobarbital	2	A	4.09 mg.	...	96	...
	Theophylline			20.44 mg.	...	100	...
	Glyceryl guaiacolate			20.42 mg.	...	103	...
17[a]	Caffeine	2	A	29.8 mg.	...	99	...
	Hexobarbital			100.6 mg.	...	102	...
	Phenacetin			200.2 mg.	...	98	...
	Dihydrocodeinone bitartrate			5.0 mg.	...	101	...
18	Sodium butabarbital	5*b*	B	15 mg./5 ml.	99	104	...
	Sodium pentobarbital			15 mg./5 ml.	103	103	...
	Sodium secobarbital			15 mg./5 ml.	105	103	...
	Sodium phenobarbital			15 mg./5 ml.	104	98	...
19	Dextromethorphan (and terpin hydrate elixir)	1	B	200 mg./100 ml.	103	...	...
20	Dextromethorphan (and terpin hydrate elixir)	1	B	11 mg./ml.	99	...	...
21	Diphenhydramine HCl	1	A	0.05% soln.	102	...	...
	Ephedrine sulfate (with sodium sulfacetamide and *dl*-deoxyephedrine)			0.5% soln.	97	...	...
22	Trifluoperazine	3*a*	A	2 mg./tab.	113	...	115

[a] Authentic samples only were analyzed.

drugs. Hence, the specified conditions may not be the optimum conditions for the analysis of a single drug material.

Different extraction procedures were necessary because of the variety of dosage forms and interferences from tablet excipients. A simple extraction with

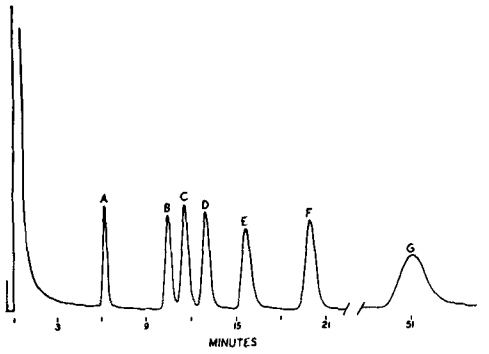

Fig. 1—Separation of barbiturates using a 1.8-m. (6-ft.) 1% HI-EFF-8BP + 10% SE 52 column on Gas Chrom Q 80–100 mesh at 220°. Key: A, *barbituric acid;* B, *butabarbital;* C, *amobarbital;* D, *pentobarbital;* E, *secobarbital;* F, *mephobarbital; and* G, *phenobarbital.*

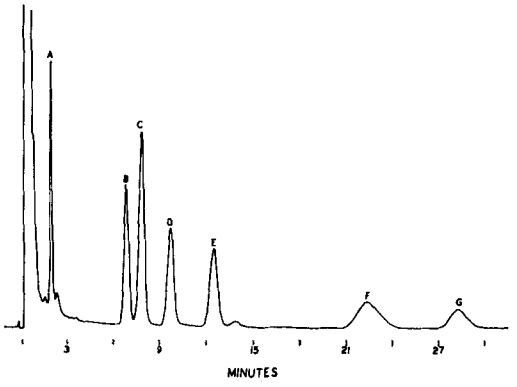

Fig. 2—Separation of antihistamines on 1.8-m. (6-ft.) 1% HI-EFF-8BP + 10% SE 52 column on Gas Chrom Q 80–100 mesh at 220°. Key: A, *phenylpropanolamine HCl;* B, *pheniramine maleate;* C, *diphenhydramine HCl;* D, *orphenadrine citrate;* E, *methapyrilene HCl;* F, *phenindamine tartrate; and* G, *pyrilamine maleate.*

chloroform or methanol is preferable when interferences are not encountered.

The 1% HI-EFF-8BP + 10% SE 52 column (Column A) is preferred for analysis since it gives sharper chromatographic peaks and can separate a greater variety of drugs. Figures 1, 2, and 3 show the separation of analgesics, barbiturates, and antihistamines. Figure 4 shows the separation of mixed classes of drugs by this polar–nonpolar type of column.

Retention times on the 4% HI-EFF-8BP column (Column B) in some cases are considerably different from those by the mixed column; thus, some drugs which are not separated by one can often be assayed and identified by the other. Figure 5 shows the separation of drugs by this column.

Relative retention times for 50 drugs were obtained and are listed in Table II. Some of these drugs were not determined quantitatively; they did, however, give good response and symmetrical peaks by one or both columns, indicating that quantitation would be possible.

No study was made of the stability of the drugs on the gas chromatographic columns used. This proce-

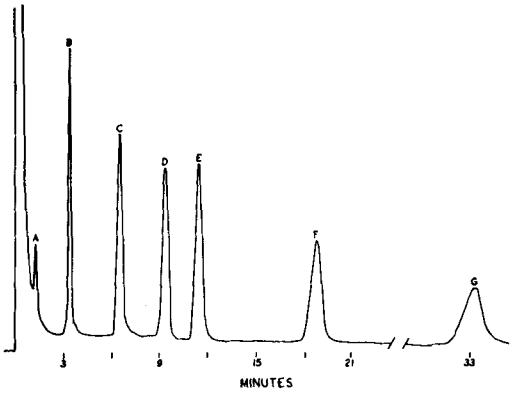

Fig. 3—Separation of some analgesics on 1.8-m. (6-ft.) 1% HI-EFF-8BP + 10% SE 52 column on Gas Chrom Q 80–100 mesh at 220°. Key: A, *aspirin;* B, *acetanilide;* C, *salicylamide;* D, *phenacetin;* E, *phenylsalicylate;* F, *caffeine; and* G, *acetaminophen.*

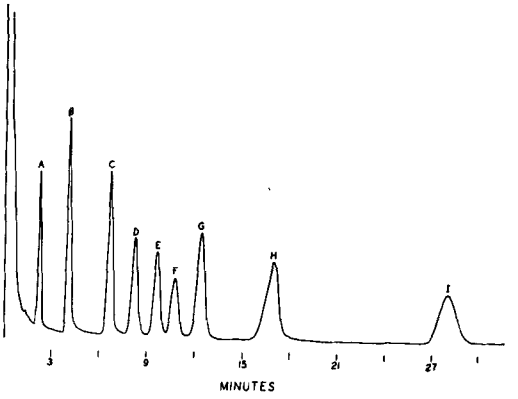

Fig. 4—Separation of mixed drugs using a 1.8-m. (6-ft.) 1% HI-EFF-8BP + 10% SE 52 column at 220°. Key: A, *acetanilide;* B, *salicylamide;* C, *phenacetin;* D, *amobarbital;* E, *caffeine;* F, *orphenadrine citrate;* G, *methapyrilene;* H, *acetaminophen;* I, *pyrilamine maleate.*

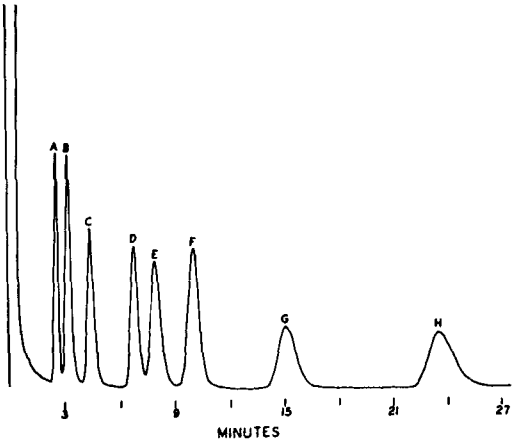

Fig. 5—Separation of mixed drugs using a 1.8-m. (6-ft.) 4% HI-EFF-8BP column on Gas Chrom Q 80–100 mesh at 210°. Key: A, *acetanilide;* B, *pheniramine maleate;* C, *orphenadrine citrate;* D, *salicylamide;* E, *methapyrilene HCl;* F, *phenacetin;* G, *amobarbital;* H, *pyrilamine maleate.*

TABLE II—RELATIVE RETENTION TIMES OF 50 DRUGS RELATIVE TO PENTOBARBITAL AT 220° C.

Name of Drug	Column A: 1% HI-EFF-8BP + SE 52	Column B: 4% HI-EFF-8BP
Acetanilide	0.26	0.19
Acetaminophen	1.86	2.63
Aminopyrine	1.27	0.62
Amobarbital	0.90	0.90
Antipyrine	1.35	1.01
Aspirin	0.11	...
Atropine sulfate	3.14	1.78
Barbituric acid	0.48	0.53
Benzocaine	0.46	0.33
Butabarbital	0.82	0.84
Caffeine	1.06	0.98
Chlorpheniramine maleate	1.43	0.49
Chlorpromazine	7.0	...
Codeine phosphate	5.23	4.00
Dextromethorphan	1.99	0.73
Diallylbarbital	0.69	...
Dihydrocodeinone bitartrate	6.22	5.79
Diphenhydramine HCl	0.83	0.24
Ephedrine sulfate	0.19	0.09
Glyceryl guaiacolate	0.62	0.55
Hexobarbital	1.24	0.88
Lidocaine	1.02	0.41
Lobeline HCl	0.86	0.42
Menadione	0.33	0.15
Mephenesin	0.48	0.33
Mephobarbital	1.51	1.32
Meprobamate	0.31	0.23
Methenamine	0.05	0.12
Methapyrilene HCl	1.36	0.51
Nicotinamide	0.38	0.40
Orphenadrine citrate	1.05	0.30
Pentobarbital	1.00	1.00
Phenacetin	0.73	0.60
Phenindamine tartrate	2.41	0.99
Pheniramine maleate	0.73	0.22
Phenobarbital	3.86	5.19
Phenylpropanolamine HCl	0.19	0.10
Phenylsalicylate	0.91	0.29
Pilocarpine	3.51	3.83
Procaine	1.98	1.42
Pyrilamine maleate	3.05	1.36
Salicylamide	0.44	0.44
Salicylic acid	0.25	0.25
Secobarbital	1.19	1.23
Tetracaine	3.47	1.90
Theophylline	4.00	...
Thenyldiamine	1.28	0.55
Theobromine	1.91	2.13
Thiamine	0.17	0.09
Trifluoperazine	10.6	...
Tripelennamine	1.36	0.44

dure is not recommended for the isolation of a pure drug prior to additional analysis (such as infrared or mass spectrophotometry).

CONCLUSION

The quantitative and qualitative determination of 25 different drugs in 19 commercial preparations and seven synthetic samples was accomplished by a fast, reasonably accurate method. The quantitative results of these analyses are shown in Table I. Recoveries from the synthetic samples ranged from 96 to 106%, with most recoveries between 98 and 103%. Analysis of commercial preparations containing one active ingredient by ultraviolet spectrophotometric methods showed good agreement with GLC results.

REFERENCES

(1) Celeste, A., and Turczan, J., *J. Assoc. Offic. Agr. Chemists*, **46**, 1055(1963).
(2) Celeste, A., and Polito, M. C., *ibid.*, **49**, 541(1966).
(3) Fontan, C. R., Smith, W. C., and Kirk, P. L., *Anal. Chem.*, **35**, 591(1963).
(4) Jain, N. C., and Kirk, P. L., *Microchem. J.*, **12**, 242 (1967).
(5) MacDonald, A., Jr., and Pflaum, R. T., *J. Pharm. Sci.*, **53**, 887(1964).
(6) Cieplinski, E. W., *Anal. Chem.*, **35**, 256(1963).
(7) Gudzinowicz, B. J., and Clark, S. J., *J. Gas Chromatog.*, **3**, 147(1965).
(8) Jain, N. C., and Kirk, P. L., *Microchem. J.*, **12**, 249 (1967).
(9) Martin, H. F., and Driscoll, J. L., *Anal. Chem.*, **38**, 345(1966).
(10) Parker, K. D., and Kirk, P. L., *ibid.*, **33**, 1378(1961).
(11) Parker, K. D., Fontan, C. R., and Kirk, P. L., *ibid.*, **35**, 418(1963).
(12) Brochmann-Hanssen, E., and Fontan, C. R., *J. Chromatog.*, **19**, 296(1965).
(13) Jain, N. C., and Kirk, P. L., *Microchem. J.*, **12**, 229 (1967).
(14) Massingill, J. L., Jr., and Hodgkins, J. E., *Anal. Chem.*, **37**, 952(1963).
(15) Erney, D. R., Food and Drug Administration, U. S. Department of Health, Education, and Welfare, private communication, 1965.
(16) Condal-Bosch, L., *J. Chem. Ed.*, **41**, A235(1964).

Keyphrases

Quantitative determination—single, multiple components
Extraction—single, multiple component dosage forms
GLC—analysis
Retention times, 50 drugs—relative to pentobarbital