Journal of Chromatography, 168 (1979) 417–427

CHROM. 11,377

IDENTIFICATION OF DRUGS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY WITH DUAL WAVELENGTH ULTRAVIOLET DETECTION

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(Received July 31st, 1978)

SUMMARY

Using three solvent–column systems, 101 drugs of forensic interest were characterized by their high-pressure liquid chromatographic relative retention times and by the ratio of their absorbances at 254 and 280 nm. Using relative retention times alone, only 9% of the drugs could be distinguished; while when both the retention times and absorbance ratios were used, 95% of the drugs could be distinguished. The compounds were also characterized by comparisons of their retention times on an adsorption column and a reversed-phase column, however this pair of discriminators were less useful than the former techniques.

INTRODUCTION

Since the early 1970's, there has been a very rapid increase in the use of high-pressure liquid chromatography (HPLC) in drug analysis. In fact, HPLC methods are often used more frequently than gas–liquid chromatographic (GLC) methods in the quality control sections of most pharmaceutical firms. However, the routine use of HPLC for the identification of drugs in forensic and toxicology laboratories is much more limited at this time. In part, this may be due to the lower precision of HPLC relative retention times compared to GLC relative retention times and in part due to the difficulty in corroborating the peak identifications by HPLC. A third reason for the lack of utilization of the method in this area is the paucity of HPLC data on narcotics, amphetamines, barbiturates, tranquilizers, and other drugs of interest.

Recently the use of a octadecylsilane reversed-phase HPLC system for the identification of 30 drugs was reported[1]. In addition, a micro-particulate silica system was reported for the identification of a larger list of drugs[2]. Though these were excellent chromatographic systems, only very few drugs could be uniquely identified because of the overlap of retention times.

There are numerous reports of the use of variable wavelength ultraviolet (UV) detectors as an aid in the identification of compounds and this method was recently applied to a small number of drugs and other molecules of biological interest[3,4]. There were two major limitations of this techniques to the problems of routine identifica-

tion. In most cases a stopped-flow method must be used to obtain the UV spectra which made it difficult to obtain precise retention times to serve as the primary means of identification. Secondly, it would have been very difficult to obtain quality UV spectra at the low concentrations encountered in forensic and toxicological applications because of the difficulty in obtaining flat UV spectral baselines at high sensitivity.

The use of two UV detectors in series operating at different wavelengths has long been used as a qualitative aid in the identification of compounds in complex mixtures. Recently it was reported that polynuclear aromatic hydrocarbons could be detected and identified in environmental samples at low levels using dual UV detectors in series[5]. In this report, the initial identifications made on the basis of retention times were corroborated through precise measurements of the peak's absorbance ratio at 254 and 280 nm. The same workers have also applied the technique to the identification of nucleosides in serum samples and it has been shown that the absorbance ratio is very reproducible (1.7% relative standard deviation)[6]. This same basic technique has also been used to identify compounds of biological interests in urine samples[7]. In each of these earlier studies it was found that the absorbance ratio could be easily reproduced and it could be satisfactorily measured in complex samples at low concentrations.

The major objectives of the work reported here were to develop a small number of isocratic HPLC systems that would be useful for the majority of the "drugs of abuse" and to evaluate the absorbance ratio technique as an additional identification discrimination.

EXPERIMENTAL

Instrumentation and calibration

A Waters Assoc. Model 202 chromatograph equipped with a U6K injector and Model M6000 pump was used for the study. The column was connected with 0.009-in. I.D. tubing to a 254-nm detector then in series to a 280-nm detector, both of which were connected to a dual pen strip-chart recorder. The absorbance reading of the two detectors were calibrated in a relative manner by adjusting the gain on the 280-nm recorder channel so that an A_{254}/A_{280} peak height ratio of 1.09 was obtained for a morphine reference standard when chromatographed on system B.

Drug standard and chemicals

The majority of the drugs were obtained from the Theta Corp. (Media, Pa., U.S.A.) or from U.S.P. Reference Standards (Rockville, Md, U.S.A.). A small number of the compounds were obtained directly from various pharmaceutical firms. Methanol and methylene chloride used in the mobile phase was freshly distilled before use. All other chemicals were of reagent grade and used without further purification.

Chromatographic system A

A Waters Assoc. 3.9 × 300 mm μBondapak C_{18} column and a mobile phase flow-rate of 2.0 ml/min were used. The mobile phase was prepared by adjusting a 0.025 *M* NaH_2PO_4 in methanol–water (2:3) solution to a pH of 7.0 using 5% aqueous sodium hydroxide solution. Retention times were measured relative to that of phenacetin (6.8 min).

Chromatographic system B

A Waters Assoc. 3.9 × 300 mm μPorasil column and a mobile phase flow-rate of 2.0 ml/min were used. The mobile phase was methanol–2 *N* ammonia–1 *N* ammonium nitrate (27:2:1). Retention times were measured relative to morphine sulfate (3.5 min).

Chromatographic system C

The column and flow-rate used were the same as system B. The mobile phase was prepared by adding 2.0 ml of concentrated ammonia to a closed flask containing 1.0 l of dichloromethane and stirring overnight. Retention times were measured relative to diazepam (4.5 min).

RESULTS AND DISCUSSION

In order for the measurement of any physical parameter of a drug to be useful in its identification, one should have a reasonable estimate of both the short term and long term precision of the measurement. To this end ,a number of drugs were selected on the bases of the diversity of their polarity, acid–base character and UV spectra, and were used as test compounds. The short term relative standard deviation of the HPLC relative retention times (Table I) was found to have an average value of 3.3% which was fairly typical when relative retention times are used. This value was typical of most HPLC studies, but was considerably larger than the relative standard deviation for GLC retention times [0.6% (ref. 8)]. The short term precision of the absorbance ratio measurements was found to be slightly better than the relative retention times measurements. The average value of the relative standard deviation of the absorbance ratio was 1.9%. It was also observed that the relative standard deviation was smallest when the two peaks were of nearly the same size ($A_{254}/A_{280} \approx 1$). If the ratio was very high or very low, the precision of the measurements were satisfactory, but slightly lower.

TABLE I

PRECISION OF RELATIVE RETENTION TIME AND ABSORBANCE RATIO MEASUREMENTS

Drug	*Relative retention time*	A_{254}/A_{280}
Amphetamine	0.519* ± 4.4%**	20.4 ± 3.5%
Phenobarbital	0.862 ± 3.5%	19.7 ± 2.5%
Phenacetin	1.00***	8.42 ± 0.68%
Methaqualone	3.94 ± 1.9%	2.51 ± 0.87%
Average	3.3%	1.9%

* Relative retention time using system A.
** Relative standard deviation.
*** Retention time standard for system A.

The long term reproducibility of the relative retention times and of the absorbance ratio was estimated by repeating the measurements for the drugs listed in Table I over a two month period. It was found that the relative retention times varied

by an average of 3.2% and the absorbance ratio values varied by an average of 21%. In retrospect, it is felt that the variation in the long term reproducibility of the absorbance ratio could be improved by more frequent calibration. Though the long term precision of the absorbance ratio values appeared rather poor, the value was still useful in identifying drugs because of the extremely wide variation of the ratio from drug to drug (Tables II–IV).

TABLE II

DATA FOR DRUGS CHROMATOGRAPHED ON SYSTEM A

Drug	*Relative retention time*	A_{254}/A_{280}	A_{254}***	$N^{\S}$
Barbituric acid	0.21*	19.1	2.9	—
Sulfanilamide	0.23	5.25	2.7	4,520
Phenylephrine	0.27	1.73	0.049	2,680
Theobromine	0.28	2.25	0.23	3,190
Acetaminophen	0.30	8.23	1.8	3,750
Aspirin	0.31	0.32	0.029	3,750
Hydroxyamphetamine	0.32	0.94	0.043	3,750
Phenylpropanolamine	0.34	16.0	0.0088	1,420
Theophylline	0.34	3.24	0.46	4,660
Barbital	0.35	6.93	0.0078	416
Dimenhydrinate	0.38	3.67	0.16	5,320
Oxymorphone	0.41	2.80	0.046	7,580
Ephedrine	0.41	17.1	0.0083	1,150
Mescaline	0.43	5.41	—	1,600
Caffeine	0.48	2.15	1.3	2,330
Ectylurea	0.50	17.9	0.013	260
Procaine	0.51	3.0	0.61	1,250
Amphetamine	0.52	20.4	0.019	630
Salicylamide	0.53	0.83	0.094	2,680
Nikethamide	0.58	16.5	0.15	1,480
Phenacemide	0.59	33.5	0.0055	110
Oxycodone	0.60	2.1	0.0026	230
Morphine	0.62	1.67	0.011	950
Dichloralphenazone	0.66	6.0	0.026	240
Mephenoxalone	0.67	1.79	0.0035	240
Allobarbital	0.70	12.0	0.0049	260
Methocarbamol	0.72	1.43	0.0035	190
Dimethyltryptamine	0.75	1.89	—	750
Methylamphetamine	0.75	6.50	0.0021	230
Metharbital	0.77	9.75	0.0032	340
Tetrahydrozoline	0.77	9.46	0.0054	360
Phenmetrazine	0.80	13.3	0.0021	670
Dihydrocodeine	0.81	1.08	0.0043	270
Hydromorphone	0.84	2.0	0.0070	460
Nicotine	0.85	14.4	0.056	990
Phenobarbital	0.86	19.7	0.026	1,950
Aprobarbital	0.87	10.4	0.042	550
Mephentermine	0.88	7.67	0.0020	450
Codeine	0.90	2.47	—	940
Bromural	0.95	14.4	0.0059	710
Naloxone	0.96	2.21	0.0077	600
Hexobarbital	0.97	9.35	0.015	1,140

TABLE II *(continued)*

Drug	*Relative retention time*	A_{254}/A_{280}	A_{254}***	$N^{\S}$
Phenacetin	1.00**	8.42	0.49	2,620
Heroin	1.11	1.75	—	1,190
Lobeline	1.11	8.52	0.0028	1,920
Mephenesin	1.11	3.23	0.0092	880
Naphazoline	1.11	1.14	0.019	500
Butabarbital	1.14	9.67	0.0023	1,840
Fluorescein	1.15	2.12	0.21	2,220
Cyclobarbital	1.22	9.58	0.019	1,920
Butalbital	1.41	11.3	0.0037	1,110
Methylphenidate	1.46	17.0	0.0018	320
Mephobarbital	1.56	10.1	0.013	1,800
Nylidrine	1.59	1.76	0.0025	480
Hydrocodone	1.66	1.94	0.0029	400
Ethylmorphine	1.69	2.18	0.0057	660
Levorphanol	1.78	0.35	0.0006	370
Chlordiazepoxide	2.02	3.69	0.045	1,180
Pentazocine	2.03	0.46	0.0009	360
Diphenylhydantoin	2.07	16.4	0.0091	630
Glutethimide	2.12	12.0	0.0039	1,350
Pentobarbital	2.16	9.33	0.0023	1,660
Phencyclidine	2.16	19.3	—	250
Amobarbital	2.25	11.5	0.0019	1,250
Levallorphan	2.35	0.44	0.0007	650
Phenaglycodol	2.90	16.5	0.0026	860
Doxylamine	2.95	11.4	0.0093	340
Flurazepam	3.21	3.76	0.016	690
Secobarbital	3.28	10.0	0.0008	1,580
Thiopental	3.55	0.33	0.013	3,150
Oxymethazoline	3.62	1.00	0.0014	650
Methaqualone	3.94	2.51	0.049	920
Phenazocine	4.05	0.72	0.0011	780
Oxazepam	4.05	6.16	0.061	1,510
Thiamylal	4.58	0.31	0.016	2,370
Methohexital	4.80	9.0	0.0015	2,600
Papaverine	7.06	3.0	0.029	2,440
Diazepam	9.56	6.04	0.10	2,650

* The column void volume was slightly less than 0.21.

** Phenacetin used as standard, retention time 6.8 min.

*** Absorbance of a 10-μl injection of a 1.0 mg/ml solution of the drug.

§ Number of theoretical plates.

The retention times of the drugs using system A (Table II) were found to correlate very well with those reported for a much more limited series of drugs chromatographed with a similar system[1]. Because of minor impurities in the samples, the correct chromatograph peak assignments were occasionally in doubt. In an effort to verify the chromatographic peak assignments, the A_{254}/A_{280} values of each drug was compared with the UV spectral data of the drug obtained with similar solvents[9,10]. In a more limited number of cases where the correct assignment of the drug was still in question, the fractions of individual peaks were collected, and their UV spectra and thin-layer chromatograms were compared to those obtained for the original drug.

TABLE III

DATA FOR DRUGS CHROMATOGRAPHED ON SYSTEM B

Drug	*Relative retention time*	A_{254}/A_{280}	A_{254}***	N§
Noscapine	0.53*	0.61	0.035	—
Phenacetin	0.53	0.84	0.051	—
Naloxone	0.56	0.82	0.025	—
Papaverine	0.56	1.06	0.070	—
Benzphetamine	0.58	2.10	0.069	—
Piminodine	0.58	3.02	0.16	—
Cocaine	0.61	0.86	—	—
Phenazocine	0.61	0.24	0.0081	—
Procaine	0.61	0.44	0.048	—
Nylidrin	0.61	0.75	0.013	—
Levallorphan	0.64	0.12	0.0026	—
Methylphenidate	0.67	9.50	0.0062	—
Pentazocine	0.67	0.16	0.0049	—
Phendimetrazine	0.67	8.00	0.010	—
Ethinamate	0.70	1.00	0.0020	—
Phenmetrazine	0.72	31.0	0.040	—
Meperidine	0.75	30.7	0.0030	710
Quinine	0.75	0.62	0.0042	1,700
Promethazine	0.76	2.21	0.14	710
Diphenhydramine	0.77	90.0	0.059	1,600
Methapyrilene	0.77	1.89	0.13	750
Phenylpropanolamine	0.78	65.0	0.0021	—
Heroin	0.80	0.64	—	1,800
Methadone	0.83	1.57	0.072	1,140
Phencyclidine	0.83	22.0	—	1,900
Thioridazine	0.83	2.08	0.20	840
Amphetamine	0.86	60.0	0.0039	940
Oxymorphone	0.86	1.17	0.0044	110
Doxylamine	0.89	17.7	0.0017	2,330
Ethylmorphine	0.92	1.15	0.020	2,440
Hydroxyamphetamine	0.92	0.38	0.011	1,080
Propylhexedrine	0.92	4.0	0.0005	290
Oxycodone	0.92	1.13	0.0029	100
Codeine	1.00	0.88	—	450
Morphine	1.00**	1.09	0.016	470
Dimethyltriptamine	1.09	0.77	—	3,190
Methamphetamine	1.19	31.0	0.0034	1,862
Ephedrine	1.20	52.0	0.0034	1,050
Phenylephrine	1.22	0.50	0.0045	466
Hydrocodone	1.28	0.93	0.0085	1,170
Ethoheptazine	1.31	27.4	0.0022	1,510
Mescaline	1.31	2.93	—	2,140
Xylometazoline	1.33	8.67	0.0034	2,290
Mephenteramine	1.36	36.3	0.0038	1,370
Dihydrocodeine	1.36	0.53	0.0065	1,290
Oxymetazoline	1.36	0.31	0.012	1,370
Tetrahydrozoline	1.42	16.1	0.038	1,460
Hydromorphone	1.43	1.09	0.0091	970
Strychnine	1.54	3.22	0.0045	370
Dextromethorphan	1.56	0.14	0.0022	1,750
Naphazoline	1.61	0.49	0.062	1,210
Levorphanol	1.64	0.12	0.0013	1,210

* The column void volume was slightly less than 0.53.

** Morphine was used as standard, retention time 3.5 min.

*** Absorbance of a 10-μl injection of a 1.0 mg/ml solution.

§ Number of theoretical plates.

TABLE IV

DATA FOR DRUGS CHROMATOGRAPHED ON SYSTEM C

Drug	*Relative retention time*	A_{254}/A_{280}	A_{254}***	N§
Disulfiram	0.39	1.21	0.29	3,750
Phenaglycodol	0.55	2.90	0.0059	1,160
Benzphetamine	0.57	8.11	0.047	6,400
Propoxyphene	0.61	5.36	0.012	2,220
Methaqualone	0.74	1.40	0.055	2,330
Chlordiazepoxide	0.76	1.80	0.042	2,680
Piminodine	0.76	2.27	0.022	870
Glutethimide	0.79	7.70	0.013	2,440
Diphenoxylate	0.88	7.00	0.0065	2,140
Naloxone	0.89	3.75	0.0049	3,750
Phenylpropanolamine	0.89	2.50	—	2,600
Flurazepam	0.91	2.00	0.0010	1,660
Phenazocine	0.96	2.00	0.029	1,330
Diazepam	1.00**	3.90	0.075	4,340
Noscapine	1.02	0.88	0.054	4,820
Papavarine	1.04	1.81	0.059	420
Fentanyl	1.06	6.00	0.0052	1,020
Procaine	1.61	0.87	0.047	2,330
Promethazine	1.89	5.10	0.12	2,590
Phenacetin	2.71	4.61	0.088	400
Salicylamide	3.20	0.37	0.0078	1,100

* The column void volume was slightly less than 0.39.

** Absorbance of a 10-μl injection of a 10 mg/ml solution.

§ Number of theoretical plates.

The correlation between the lipophilic nature of the barbitutates and their retention time on system A was very high (Fig. 1). The octanol–water partition coefficients were not experimentally obtained, but were calculated using the methods developed by Hansch and co-workers[11,12]. It should also be noted that these partition coefficients were calculated for the non-ionized form while actually 20 to 40% of the

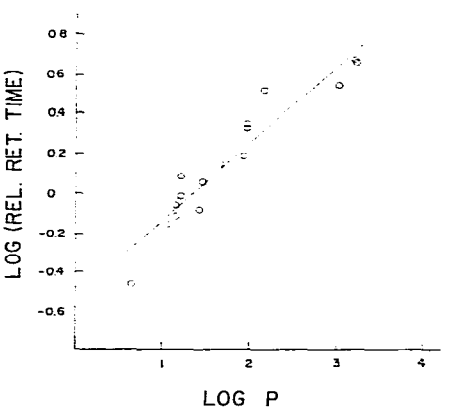

Fig. 1. Correlation of the retention time of barbiturates on system A and their calculated octanol–water partition coefficients.

barbiturate would be in the ionic form at the pH of 7.0 used for system A. Considering that the slight variations in the pK_a values of the barbiturates were not compensated for, the correlation coefficient of 0.95 that was observed was rather high. The slope of the curve in Fig. 1 was found to be 0.39 which indicated that the stationary mobile phase partition coefficient does not increase nearly as rapidly as the octanol–water partition coefficient in response to an increase in the lipophilic character of the drug. Recently very extensive studies on hydrophobic interactions in the μBondapak C_{18} column have been reported[13]. These studies showed that the slope of the long k' *vs.* carbon number (of a series of homologous alkanes or carboxylic acids) decreased markedly as the mole fraction of methanol in water increased. If one were to extrapolate these findings to conditions used for system A, one would have expected a slope of 0.47 for Fig. 1. Thus it would appear that the low value observed for the slope in this study was not related to the partial ionization of the barbiturates or any other property unique to the barbiturates, but was simply the result of decreased hydrophobic interactions in the mobile phase because of the methanol content.

Other examples of correlations between drug lipophilicity and retention time on system A were observed. For example, the retention times of the amphetamine series: methamphetamine > amphetamine > ephedrine > phenylpropanolamine. A similar ordering was also observed for the opiates: naloxone > codeine > hydromorphone > dihydrocodeine > morphine > oxycodone > oxymorphine. In the case of systems B and C (both μPorasil columns), it was generally observed that the retention times increased with the polarity of the drug in a general manner, but they were not nearly as well correlated with calculated partition coefficients as wes the reverse phase column.

The A_{254}/A_{280} values were found to vary over a very wide range (Tables II–IV). The primary value of this parameter was in the identification of individual drugs in a purely empirical manner, but it was also useful in the identification of various classes of drugs. For example, the majority of the morphine analogs run on system A were observed to have A_{254}/A_{280} values in the 1.7 to 2.5 range: most amphetamines, 36 to 56; and most barbiturates, 7 to 12.

In the extensive study of similar reverse phase systems by Twitchett and Moffat[1], it was noted that basic drugs exhibited lower theoretical plate counts than acidic or neutral drugs. In the present study that was made with a larger number of drugs (Table II), it was also observed that basic drugs did tend to have a lower column efficiency. However, this was only a general trend and numerous examples of the converse relationship could also be cited. Other than the trend for basic drugs to show a low column efficiency on system A, no other common structural characteristic could be discerned among the compounds showing a low plate count.

Chromatographic system B was found to have a much higher column efficiency for the basic drugs than system C. In a direct comparison of basic drugs that had been run on both systems, the theoretical plate count was on the average 196% higher on system B than on system A . Chromatographic system C was also found to have satisfactory column efficiency for most of the basic drugs and the values were fairly typical of what would be obtained for neutral compounds.

From a forensic or toxicological applications viewpoint, a major consideration in the evaluation of a method is the usefulness of the technique in uniquely identifying a drug in relatively complex mixtures. The primary objective of the present study was

to evaluate the usefulness of accurate absorbance ratio measurements as an additional discrimination in the identification of drugs by relative retention times. As would be expected, there was very little correlation between the A_{254}/A_{280} value and the retention time of a given drug (Fig. 2). It is not an uncommon practice to identify a drug on the basis of GLC retention times on two different liquid phases. In such applications, there is usually a very high correlation between the retention time of a given compound on the two phases, thus the addition of the second discriminator adds little to one's ability to identify a given drug. When HPLC system A was used (Table II), a fairly large number of drugs had relative retention times between 0.7 and 1.0 which made it impossible to identify even one drug using retention times alone. However, the differences in the absorbance ratio values in this group was large enough to permit most of the drugs to be uniquely identified from other members of the group.

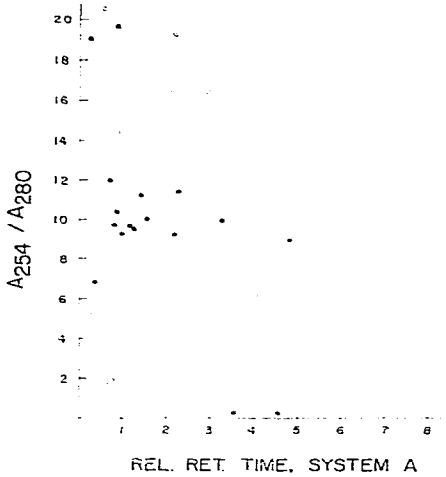

Fig. 2. Variation of the UV absorption ratio with the relative retention time of the drugs on system A. Closed circles represent the barbiturates.

In order to determine if each drug could be uniquely identified it would have been necessary to have standard deviations of the relative retention time and absorbance ratio value of each drug, then compare the two means to each of the other drugs in the data set. As a means of reducing this task to a more reasonable scale, it was assumed that standard deviation of the two parameters was the same as the average values that had been determined from a smaller group of drugs and that have been discussed in the first part of this section (3.3% for the relative retention times, 1.9% for the absorbance ratios). A given drug was then considered identifiable using only the retention time parameter if the difference between the retention time of the drug in question and all other drugs in the data set was greater than the sum of the standard deviation of the drug and the standard deviation of each of the other drugs in the data set. Thus when only the retention time of the drugs run on system A were used, only 9% of the drugs could be uniquely identified (Table V). A drug was considered to be identifiable using both parameters if either the value for the retention time or the absorbance ratio of the given drug differed by more than the sum of the two standard deviations of each of the other drugs in the data set. Thus when the retention time and

TABLE V

IDENTIFICATION OF DRUGS USING MULTIPLE PARAMETERS

First parameter	*Second parameter*	*Number of compounds*	*Identifications using first parameter*	*Identifications using both parameters*
Retention time, system A	A_{254}/A_{280}	78	9%*	95%**
GLC retention time***	Response index***	71	41%	85%
Retention time, system A	GLC retention time***	51	12%	100%
Retention time, system A	Retention time, system B	35	23%	83%

* Percent of drugs uniquely identifiable in the group using only the first parameter. See text for computation method.

** Percent of drugs uniquely identifiable in the group using both of the parameters.

*** Data taken from ref. 8.

absorbance ratio values were used, 95% of the drugs run on system A could be identified.

The HPLC retention time–A_{254}/A_{280} system was at least on a par or slightly better than a GLC dual detector system in the identification of the drugs (Table V). If the HPLC retention times on system A were paired with the GLC retention times of the drugs, the identifiability of the drugs appeared to be even greater, but the increase in the value was largely due to the reduction in the number of drugs in the sample. Only a limited number of drugs were run on both HPLC system A (reversed-phase) and system B (normal adsorption), however, it was clear that the use of these two columns was inferior to the use of one column and the absorbance ratio (Table V, Fig. 4).

In a homologous series of compounds such as the barbiturates, it is often extremely difficult to identify each individual member of the series because of isomeric relationships. Because of the similarity of the UV spectra of all of the barbiturates[9,10] and because of the similarity of the lipophilicities of the isomeric compounds, one would have anticipated that HPLC retention times on a reversed-phase system paired with UV absorbance ratio measurements would not have been very useful in the identification of the compounds (Fig. 2). However, if the barbiturates were considered as a separate group, each of the compounds could be identified. If HPLC retention time was used as the first discriminator and the absorbance ratio as the second (Fig. 2), there was a higher dispersion of the data points for the barbiturates than when GLC retention time was used as the second discriminator (Fig. 3). Since the lipophilicity of the barbiturate and its vapor pressure would closely relate to the length of the side chain of the barbiturate, one might then expect a high covariance between the retention time on HPLC system A and the GLC retention times.

In conclusion, it was found that the use of HPLC relative retention times paired with accurate measurements of the A_{254}/A_{280} value was slightly more useful in the identification of drugs than other commonly paired techniques. This is not to suggest that the other techniques should be abandoned however, but that the method would be a relatively simple and inexpensive way of adding to the certainty of the identification of a specific drug that may have a retention time similar to other drugs. Even in cases where the chromatographic peak was well resolved from other drugs, the absorbance ratio technique would be useful in distinguishing the drug from other compounds present in the sample.

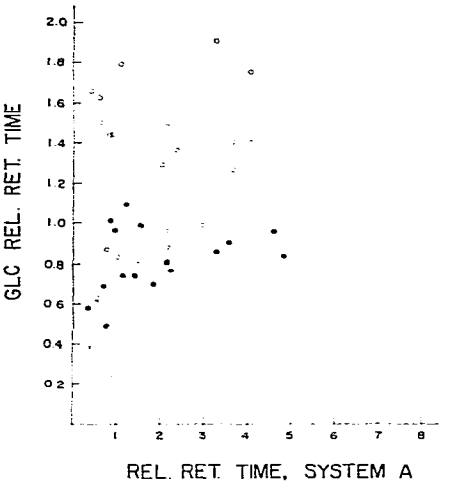

Fig. 3. Variation of the relative retention time of the drug on an OV-17 GLC column (ref. 8) with the relative retention time of the drug on system A. Closed circles represent the barbiturates.

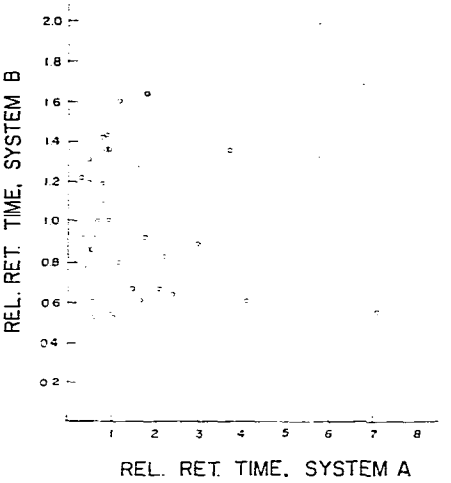

Fig. 4. Variation of the relative retention time of the drug on system B with the relative retention time on system A.

ACKNOWLEDGEMENT

Parts of this investigation were supported by the Research Institute of Pharmaceutical Sciences, University of Mississippi.

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