Separation and Determination of Barbiturates in Pharmaceuticals by High Speed Liquid Chromatography

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Abstract □ A high speed liquid chromatographic method for the separation, identification, and quantitative determination of barbiturates in pharmaceutical dosage forms is described. The method is accurate, simple, rapid, and sensitive. The limit of detectability with spectrophotometric detection at 254 nm. is 0.5–1.5 ng. Separation for identification and analysis is achieved on a strong anion-exchange column utilizing an alkaline (0.01 *M* sodium borate + 0.03 *M* sodium nitrate) or acid (0.01 *M* citric acid) aqueous mobile phase. It is possible to predict the inlet pressure, flow rate, and mobile phase ionic strength necessary to achieve a predetermined retention time. High speed liquid chromatographic separations of 16 barbiturates were studied on a strong cation-exchange column and on a permanently bonded partition column—a reversed phase partition system. A study of the response of peak height and area to inlet pressure (flow rate) variations indicated that peak height is disturbed less than area. The response of the UV detector to phenobarbital is described by a simple equation relating peak area and mass of the component.

Keyphrases □ Barbiturates—separation, analysis, high pressure liquid chromatography □ High pressure liquid chromatography—separation, analysis, barbiturates

The utilization of high speed liquid chromatography in pharmaceutical analysis has recently been reported. For example, Henry and Schmit (1) described the separation of some common analgesics as well as the quantitative determination of *N*-acetyl-*p*-aminophenol, Talley (2) reported the separation and determination of several highly polar pyridine isomers, and Henry *et al.* (3) described the separation of various steroids. In addition, Kram (4) described the high speed liquid chromatography behavior of a large number of sulfonamides on a strong anion-exchange column, utilizing an alkaline mobile phase of variable ionic strength. However, the potential applicability of high speed liquid chromatography in this area is yet to be realized.

Although GC (5–8) has been effective in the past for the separation and quantitation of the barbiturates, an important group of drugs, some complications do exist. At times the sample preparation prior to injection has been laborious. For example, when the barbiturates are present as salts, acidification is necessary to obtain the thermally stable acid forms. This is accomplished by addition of an organic acid in acetone (9) or by organic solvent extraction from an aqueous acid (10, 11). Another consideration arises from the nature of the pharmaceutical dosage form, which often makes preparatory steps necessary. When the barbiturates are present in aqueous systems, such as elixirs, they require solvent extraction prior to injection. Finally, the chromatographic behavior is complicated by the polar nature of the acidic imine found in the barbiturates, which causes adsorption on various GC columns (12), resulting in excessive tailing (*e.g.*, phenobarbital) and some irreversible sample loss. The practical consequence of this factor is that quantitative measurement and detection in the submicrogram region using GC is difficult, even with the application of flame-ionization detection (13).

The present work describes the successful separation and determination of a number of barbiturates by high speed liquid chromatography, a technique that permits circumvention of the problems found in GC. Even though the application of liquid chromatography to the separation of four barbiturates was previously reported by Anders and Latorre (14), the conditions used to achieve separation, such as gradient elution and high column temperature (80°), did not appear suitable for extension into pharmaceutical analysis.

This paper has a number of objectives: (*a*) to show that a large number of barbiturates are amenable to separation and analysis by simple liquid chromatography procedures, (*b*) to demonstrate that elution patterns may be altered by effective variation of either the mobile or immobile phase, (*c*) to show that barbiturates can be detected readily at the submicrogram level, and (*d*) to indicate that the effect of pressure (or flow rate) changes on peak heights and areas is orderly and predictable.

EXPERIMENTAL

Apparatus

A liquid chromatograph[1] equipped with a UV detector (254-nm. radiation using a low pressure mercury source, and a detector cell volume of 7 μl.) absorption photometer was used throughout this study. Instrumental features were previously described by Felton (15), and the use of this type of detector for liquid chromatography was discussed in detail by Kirkland (16, 17).

Columns

All columns used are commercially available from the instrument manufacturer and are 1 m. × 2.1 mm. i.d. × 0.6 cm. (0.25 in.) o.d., precision bore stainless steel tubes, containing the following stationary phases: (*a*) Column 1, strong anion exchange (quaternary ammonium substituted methacrylate polymer[2] coated 1% by weight on a controlled porous surface[3]), (*b*) Column 2, strong cation exchange (sulfonated fluorocarbon[4] coated 1% by weight on a controlled porous surface), and (*c*) Column 3, a partition column (an ether stationary phase chemically bonded[5] to a controlled porous surface[3] by means of a Si—O—Si bond, containing approximately 1% stationary phase by weight). The strong anion- and strong cation-exchange columns were operated at ambient temperature (25.0 ± 0.5°), and the partition column was operated at 50°.

[1] Du Pont model 820, equipped with a model 410 UV detector, E. I. du Pont de Nemours and Co., Wilmington, Del.
[2] SAX, du Pont.
[3] Zipax, du Pont. The characteristics of Zipax were described by Kirkland (18).
[4] SCX, du Pont.
[5] Permaphase-ETH, du Pont.

Table I—Retention Time in Minutes (Relative to Methanol) for Some Barbiturates

Mobile Phase	Column 1[a] 0.01 *M*[d]	Column 1[a] 0.01 *M* Sodium Borate + 0.03 *M*[d]	Column 1[a] 0.05 *M*[d]	Column 1[a] 0.01 *M* Citric Acid	Column 2[b] 0.01 *M* Citric Acid	Column 3[c] Water
Barbituric acid	1.96	0.59	0.55	0.87	0.01	−0.24[e]
Barbital	2.58	0.75	0.70	0.75	0.01	0.39
Metharbital	5.25	1.55	1.27	1.38	—	—
Vinbarbital	6.02	1.78	1.48	4.21	0.91	1.69
Allobarbital	6.41	1.91	1.56	2.44	0.28	0.98
Aprobarbital	8.05	2.41	1.88	3.39	0.79	1.61
Butethal	10.2	3.09	2.42	5.39	1.06	2.20
Butabarbital	10.7	3.22	2.66	5.24	1.50	2.28
Butalbital	10.7	3.25	2.66	5.87	1.26	2.91
Hexobarbital	11.8	3.66	2.81	6.65	2.76	3.82
Heptabarbital	14.1	4.28	3.44	11.8	2.40	5.00
Talbutal	17.9	5.59	4.38	9.25	1.89	3.58
Phenobarbital	19.5	5.75	4.69	9.57	0.16	2.44
Amobarbital	20.2	6.25	4.92	11.4	2.36	4.69
Pentobarbital	24.1	7.50	6.02	13.3	3.03	5.00
Mephobarbital	32.3	10.5	8.21	14.8	2.91	6.34
Secobarbital	39.6	12.7	10.0	21.3	3.86	7.99
Methanol	3.21	3.21	3.21	5.40	3.70	3.74
Pressure at inlet, psig.	500	500	500	600	700	300
Flow rate, ml./min.	0.48	0.48	0.48	0.36	0.40	0.40
Column temperature	25 ± 0.5°	25 ± 0.5°	25 ± 0.5°	25 ± 0.5°	25 ± 0.5°	50.0°

[a] Strong anion-exchange column (SAX, du Pont). [b] Strong-cation exchange column (SCX, du Pont). [c] Reversed-phase partition column (ETH Permaphase, du Pont). [d] Molarity, sodium nitrate (aqueous). [e] Elutes prior to methanol emergence.

Mobile Phases

The following mobile phases were utilized in this study: (*a*) 0.01 *M* sodium borate + sodium nitrate (variable), (*b*) 0.01 *M* citric acid, and (*c*) water. Solid reagents were ACS certified grade. Prior to use, the mobile phases were purged with nitrogen to remove dissolved oxygen.

Solvent and Standards

Methanol was a satisfactory solvent for both the barbituric acids and their sodium salts. Chromatographic retention of methanol was minimal and, therefore, was considered as nonretained. Barbiturates were all laboratory working standards, with prior assay by conventional methods at 99.0% purity or higher. The concentrations of the barbiturates used for the collection of retention data were varied: 0.2 mcg./μl. for the sodium borate mobile phase, 0.1 mcg./μl. for the aqueous mobile phase, and 2.0 mcg./μl. for the citric acid mobile phase. Samples of 5 μl. of standard solution were injected, using a 10-μl. Hamilton microsyringe, while the mobile phase was flowing.

Quantitative Analysis

In this study, barbiturates (acids and salts) were quantitatively determined in commercial preparations using the strong anion-exchange column (ambient temperature) in both alkaline and acid mobile phase systems. Since each mobile phase system has a different preparation as well as UV detection characteristics, each will be discussed separately. However, the alkaline mobile phase is most applicable and will receive the most attention. Although two methods, labeled A and B, are offered as guides, such variables as ionic strength or inlet pressure (flow rate) may be changed to meet a specific need.

Method A: Alkaline Mobile Phase—*Chromatographic Conditions*—The mobile phase is 0.01 *M* sodium borate and 0.03 *M* sodium nitrate (may be varied). The inlet pressure and flow rate are 500 psig.[6] and 0.48 ml./min. (may be varied), respectively. The UV detector (aufs)[7] setting is variable.

Sample Solution—A sample of the powder (*e.g.*, a composite of tablets or capsules) is accurately weighed or the liquid (*e.g.*, an elixir) is accurately measured so as to contain an estimated 8–70 mg. of barbiturate and it is quantitatively transferred to a 100-ml. volumetric flask. If the sample is a solid, methanol is added to achieve solution.

To act as an internal standard, an alternative barbiturate is chosen (as described subsequently), and an appropriate sample size (as described subsequently) is accurately weighed and quantitatively transferred to the 100-ml. volumetric flask containing the sample. Dilute the solution to volume with mixing using methanol. Although the choice of internal standard is arbitrary, as a rule the most suitable choices are those having retention ratios (defined as internal standard absolute retention time divided by sample absolute retention time) between 0.6 and 0.8 (elution before the sample) or 1.2 and 1.4 (elution after the sample). This value provides for closeness of elution while ensuring quantitative separability. The quantity of internal standard to be used may be conveniently calculated using Eq. 1:

$$M_1 = \frac{t_{r_1} M_2}{t_{r_2}} \qquad \text{(Eq. 1)}$$

where M_1 is the weight (milligrams) of internal standard to be added; M_2 is the weight (milligrams) of barbiturate estimated in the sample for analysis; and t_{r_1} and t_{r_2} are the absolute retention times for the internal standard and the sample barbiturate, respectively.

The absolute retention times (adjusted retention time plus the solvent emergence) may be obtained from Table I.

Standard Solution—A standard solution, containing internal standard and sample barbiturate in the same quantities chosen for the sample solution, is prepared concurrently.

Chromatography—Make preliminary injections of the standard solution to determine the UV detector (in terms of aufs) and sample volume to give a 50–70% full-scale response. When the instrumental settings and sample volume have been satisfactorily determined, inject a number of sample volumes for quantitative analysis. Measure the peak heights or areas of both the internal standard and sample barbiturate.

Calculations—The total quantity of sample barbiturate taken for analysis may be calculated using Eq. 2:

$$M_1 = \frac{R_1 R_3 M_2 M_4}{R_2 R_4 M_3} \qquad \text{(Eq. 2)}$$

where R represents peak height or peak area; M represents weight (milligrams); and subscripts 1, 2, 3, and 4 represent the sample barbiturate, the standard barbiturate, the internal standard in the standard solution, and the internal standard in the sample solution, respectively.

Calculation of the quantity of barbiturate per dosage unit can be achieved by introduction of appropriate factors into Eq. 2.

Method B: Acidic Mobile Phase—*Chromatographic Conditions*—The mobile phase is 0.01 *M* citric acid. The inlet pressure is 1000 psig. (may be varied). The UV detector (aufs) setting is variable.

Sample Solution—An accurately measured sample of the solid or liquid containing the barbiturate is quantitatively transferred to a 50-ml. volumetric flask and dissolved in methanol. Since the molar absorptivities of the barbiturates in acidic aqueous media vary con-

[6] Pounds per square inch gauge.
[7] Absorbance units full scale.

siderably, the weights of the sample and internal standard must be determined experimentally. For example, 40 mg. phenobarbital, 100 mg. aprobarbital, and 140 mg. butabarbital are suitable sample and internal standard weights and will give equivalent peak heights under the stated conditions. After the internal standard is added and dissolved, methanol is added to bring the solution to volume.

Standard Solution—A standard solution, containing the internal standard and sample barbiturate in the same quantities chosen for the sample solution, is prepared concurrently.

Chromatography and Calculations—These are carried out as in *Method A*.

RESULTS AND DISCUSSION

Column and Mobile Phase Variation—The chromatographic behavior of the barbiturates studied is summarized in Table I. This tabulation includes the use of three different chromatographic columns and five different mobile phases. Adjusted retention times (retention time relative to the solvent) are listed in preference to absolute retention times (retention time from injection) for more effective comparison of mobility behavior. The latter can be calculated from available data.

Most of the data was obtained using a strong anion-exchange column with an alkaline sodium borate (pH 9.0) mobile phase and variations in ionic strength (Table I: columns 2, 3, and 4). As is also true in conventional ion-exchange chromatography, an effective way to alter mobility is to vary ionic strength. This response is noted in the data for the barbiturates: the greatest degree of separation is at the lowest sodium nitrate molarity (0.01 *M*) but the retention time may be shortened by increasing the mobile phase ionic strength. This finding is consistent with the concept of competition for anion bonding sites between the nitrate and the barbiturate anion, the latter being present in the alkaline mobile phase at concentrations dependent on the acid strength of the barbiturate. For the alkaline medium, the overall order of retention times is independent of the mobile phase ionic strength, behavior that might be expected from a family of compounds. Other studies (1, 4) have been based on the use of a similar chromatographic system—*viz.*, a strong anion-exchange column and an alkaline mobile phase of adjustable ionic strength.

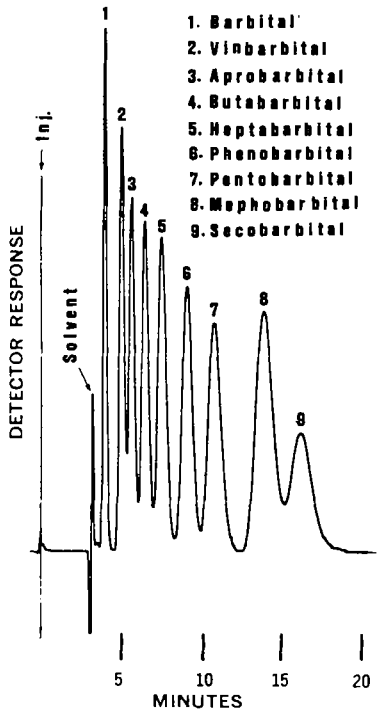

Figure 1—*Liquid chromatogram of nine barbiturates on a strong anion-exchange column (Column 1) using a 0.01* M *sodium borate + 0.03* M *sodium nitrate mobile phase. Instrumental conditions were: inlet pressure and flow rate, 500 psig. and 0.48 ml./min., respectively; UV detector sensitivity, 254 nm., 0.08 aufs; and column temperature, 25.0 ± 0.5°.*

A liquid chromatogram of nine barbiturates, using the 0.01 *M* sodium borate + 0.03 *M* sodium nitrate mobile phase system, is presented in Fig. 1. Although baseline separation is not achieved and some overlapping occurs, the compounds are quantitatively separated owing to the symmetrical nature of the peaks. Since column efficiency increases as the flow rate decreases, the barbiturates can be further resolved. Most of the barbiturates in this chromatogram are listed in the official compendia (11, 19) and are frequently found in therapeutic combinations. Amobarbital (absolute retention time 9.46 min.), which is not included in Fig. 1, is well separated from secobarbital, a compound with which it is often formulated.

The nine-barbiturate standard mixture used in obtaining the liquid chromatogram in Fig. 1 was studied using additional ionic strength mobile phase systems under the same conditions reported in Table I. Figure 2 is a plot of the adjusted retention time *versus* sodium nitrate molarity. A sharp increase in the adjusted retention time is seen at low ionic strengths. This plot presents the data in a continuous manner, thereby allowing prediction of adjusted retention times over a range of selected conditions of ionic strength.

Since the adjusted retention ratio of any pair of barbiturates at any particular ionic strength is a constant, and since a reference curve is available, the adjusted retention times of all barbiturates may be determined at a selected ionic strength. The adjusted retention ratios may be calculated from the data available in Table I (column 2, 3, or 4).

Further studies were made of the behavior of the barbiturates in a 0.01 *M* citric acid mobile phase on both strong anion- and cation-exchange chromatographic columns (Table I: columns 5 and 6). At this pH, it is expected that the predominant barbiturate species will be the undissociated acid with some small quantity of anion, depending upon the pertinent pKa. No barbiturate-containing cation species can form. The retention times listed in Table I for the citric acid studies indicate that, as expected, the anion-exchange column is more effective in separating the barbiturates. The cation-exchange column, on the other hand, does not provide as wide a range of retention times, the maximum being 3.86 min. The elution order of these systems differs from the results of the studies made with an alkaline mobile phase. In fact, there are three exceptions when the anion-exchange resin is used and seven variations with the cation-exchange system. In the latter case the phenomena responsible for the migrations are probably nonelectrostatic and depend more strongly on individual structural features. This would account for

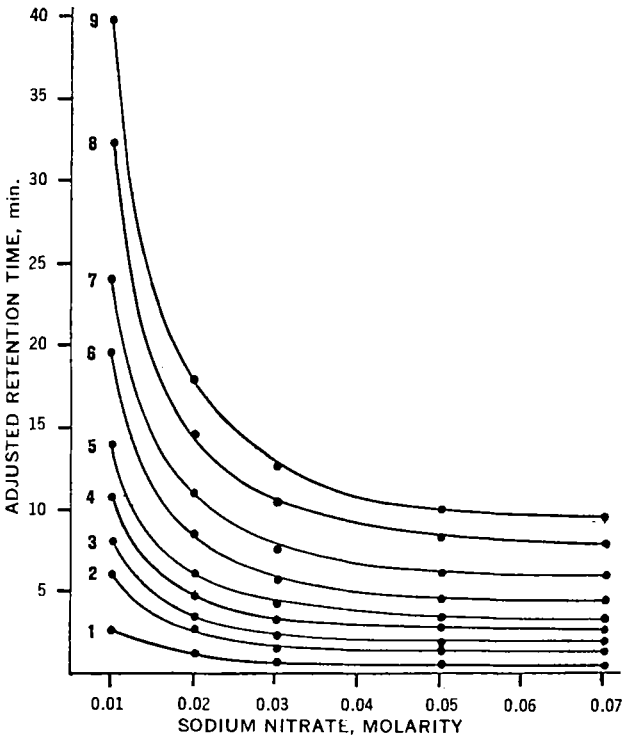

Figure 2—*Effects of ionic strength of an alkaline mobile phase on mobility. Barbiturates and instrumental conditions are the same as those in Fig. 1.*

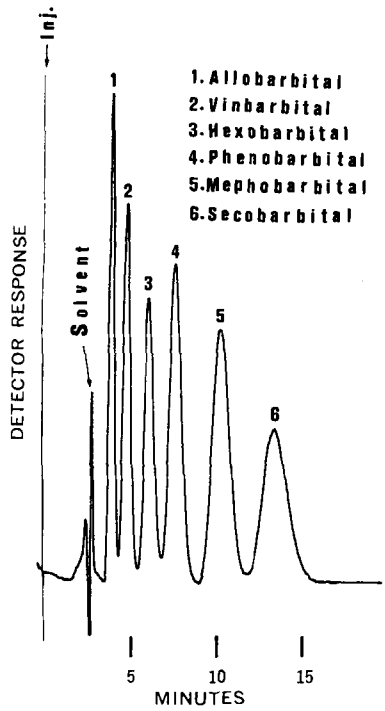

Figure 3—*Liquid chromatogram of six barbiturates on a strong anion-exchange column (Column 1) using a 0.01* M *citric acid mobile phase. Instrumental conditions were: inlet pressure and flow rate, 1000 psig. and 0.60 ml./min., respectively; UV detector sensitivity, 254 nm., 0.08 aufs; and column temperature, 25.0 ± 0.5°.*

the variation from the patterns established for the system whose separations appear to depend on pH-influenced species distribution. A striking result is observed in the very rapid migration of phenobarbital when compared to the other chromatographic systems.

Figure 3 shows the liquid chromatography separation of six barbiturates on the strong anion-exchange column using the 0.01 *M* citric acid mobile phase. Allobarbital and vinbarbital, although having different retention times in the alkaline mobile phase system, could not be quantitatively separated. However, as noted on this chromatogram, these two barbiturates are separated and, in addition, reversed in elution order. Mephobarbital is shown to be further resolved from secobarbital.

This group of barbiturates was also studied on the partition column, a reversed phase liquid chromatography separation with the application of an aqueous mobile phase. The separations (Table I: column 7) were run at 50° to increase column efficiency and resolution and to prevent overloading. The molar absorptivity of the barbiturates in water is satisfactory for detection purposes. Since the basis for these separations is *partition*, the regularity established in the separations involving the anion-exchange–alkaline mobile phase is not present. In fact, barbituric acid elutes before the solvent in this case. Amobarbital and phenobarbital are quantitatively separable on this column.

Pressure and Flow Rate Variation—A variable that is potentially useful is column pressure or mobile phase flow rate. Although the mobility data for the barbiturates in an alkaline medium at varying ionic strength in Table I and Fig. 2 were gathered at one inlet pressure (flow rate), it is possible to predict behavior at other predetermined pressures. When the same mobile phase system has been used, it has been observed that the adjusted retention volume (*i.e.*, the mobile phase volume required to elute a barbiturate after the solvent is eluted) is a constant, independent of the flow rate. Since the adjusted retention volume (V_r') equals the adjusted retention time (t_r') multiplied by the flow rate (F), Eq. 3 follows:

$$t_{r_1}'F_1 = t_{r_2}'F_2 \qquad \text{(Eq. 3)}$$

where t_{r_n}' is the adjusted retention time for compound n, and F_n is the flow rate set for the study of compound n. Solving for F_2 yields Eq. 4:

$$F_2 = \frac{t_{r_1}'F_1}{t_{r_2}'} \qquad \text{(Eq. 4)}$$

Because inlet pressure (P_i) is linearly related to flow rate, substitution in terms of pressure results in Eq. 5:

$$P_{i_2} = \frac{t_{r_1}'P_{i_1}}{t_{r_2}'} \qquad \text{(Eq. 5)}$$

where P_{i_n} is the pressure (in psig.) used in separating compound n. The very simple relationship defined by Eq. 5 allows calculation of the inlet pressure necessary to attain a desired adjusted retention time if a known adjusted retention time and pressure are available. For example, secobarbital (Table I: column 2) shows t_r' to be 39.6 min. at 500 psig. If it were desired to bring about more rapid migration in that mobile phase system, *e.g.*, 10 min., Eq. 5 predicts that a pressure of 1980 psig. should be necessary. Equations 4 and 5, in combination with the continuous retention data available in Fig. 2, have been found useful in fixing approximate conditions prior to injection.

The effect of pressure on chromatographic peaks is demonstrated in Fig. 4, a presentation of the chromatograms of four barbiturates at two ionic strength levels in an alkaline medium under the influence of a fourfold pressure change. As is noted, the sensitivity in terms of peak height varies inversely with pressure and retention time and directly with ionic strength.

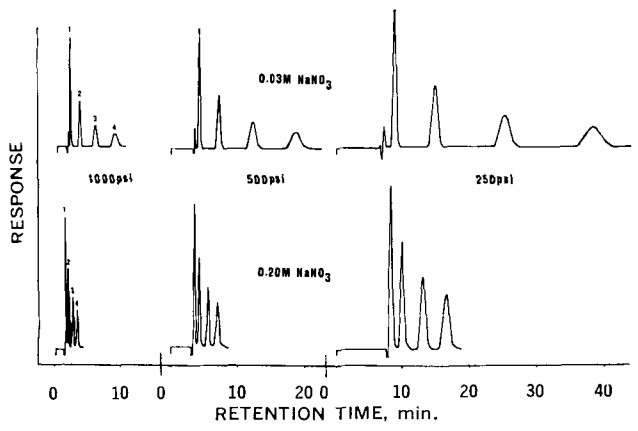

Figure 4—*Liquid chromatograms of four barbiturates [(1) barbital, (2) butabarbital, (3) pentobarbital, and (4) secobarbital] on a strong anion-exchange column (Column 1) using an alkaline mobile phase at two sodium nitrate levels and at three different pressures. UV detector sensitivity was 254 nm., 0.32 aufs, and column temperature was 25.0 ± 0.5°.*

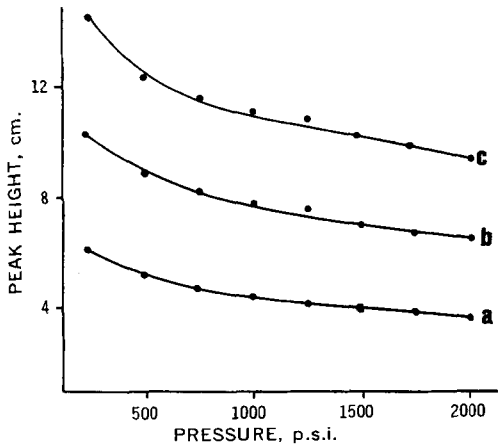

Figure 5—*Effect of pressure on the peak height of phenobarbital. Injection mass was: (a) 0.34, (b) 0.58, and (c) 0.82 mcg. Conditions were: column, strong anion exchange (Column 1); mobile phase, 0.01* M *sodium borate + 0.05* M *sodium nitrate; column temperature, 25.0 ± 0.5°; flow rate, 0.24 ml./min. at 250 psig. (linearly related to pressure); and UV detector sensitivity, 254 nm., 0.08 aufs.*

Further studies involving pressure changes were carried out. The effects of pressure on peak height and peak area are reported in Figs. 5 and 6, respectively, using phenobarbital as the test component. As shown, pressure affects peak area dramatically, particularly at low pressures, whereas the peak height is much less sensitive to pressure change. The reason that peak height increases as the inlet pressure (or flow rate) decreases is that the column is becoming more efficient, as evidenced by a marked increase in theoretical plates. Although the peak area is generally taken to be a more acceptable means of relating response to quantity (1, 2), the measurement of peak height is more rapid and convenient, especially when the peaks are symmetrical. It is good practice, in any case, to choose an internal standard relatively close in elution order for quantitation, since the pressure does change somewhat along the length of the column.

Response of UV Detector—The detector used in this work is a standard UV unit with light at 254 nm. Like the thermal conductivity detector in GC, the UV unit is also a concentration response unit. Figure 7 is a plot of area *versus* retention time (absolute) for three different concentrations of phenobarbital. Each straight line is described by the following equation:

$$A = 1.55 \pm 0.01 m t_r \qquad \text{(Eq. 6)}$$

where A is the peak area, m is the total mass (injected) of phenobarbital, and t_r is the absolute retention time. Since retention time is inversely related to the flow rate, Eq. 6 follows the form of the equation presented by McNair and Bonelli (20) to describe the response of the thermal conductivity detector. The constant 1.55 relates the UV detector response to the phenobarbital under the experimental conditions.

Equation 6 provides the basis for quantitative analysis of components when the UV detector is used. For example, if two solutions are chromatographed, one containing an unknown quantity of solute and the second containing a known concentration of the same compound in the role of a pure reference standard, then, since each analysis may be described by Eq. 6, it is clear that the unknown concentration or, directly, the mass may be calculated from Eq. 7 (the symbols for which are presented following Eq. 8):

$$M_1 = \frac{A_1 t_{r_2}}{A_2 t_{r_1}} M_2 \qquad \text{(Eq. 7)}$$

In the situation where an internal standard is used, two solutions are analyzed. Each contains the compound undergoing analysis, in one case at a known and in the other at an unknown concentration, as well as the internal standard compound. Since each solution component response may be described by Eq. 6, keeping in mind that the slope differs for each different compound, the four resultant equations may be solved for the unknown mass (or concentration since the volume injected is known) and result in Eq. 8:

$$M_1 = \frac{A_1 A_3 t_{r_2} t_{r_4} M_2 M_4}{A_2 A_4 t_{r_1} t_{r_3} M_3} \qquad \text{(Eq. 8)}$$

where A indicates area; t_r indicates retention time; M indicates mass; and subscripts 1, 2, 3, and 4 correspond to the unknown, the standard, the internal standard in the standard, and the internal standard in the unknown, respectively.

However, careful experimental maintenance of constant pressure would make $t_{r_1} = t_{r_2}$ and $t_{r_3} = t_{r_4}$, thereby simplifying Eq. 8. Equation 2 is of the same form as Eq. 8 and provides for the use of peak heights, which have been found to be proportional to the mass of a compound injected for analysis.

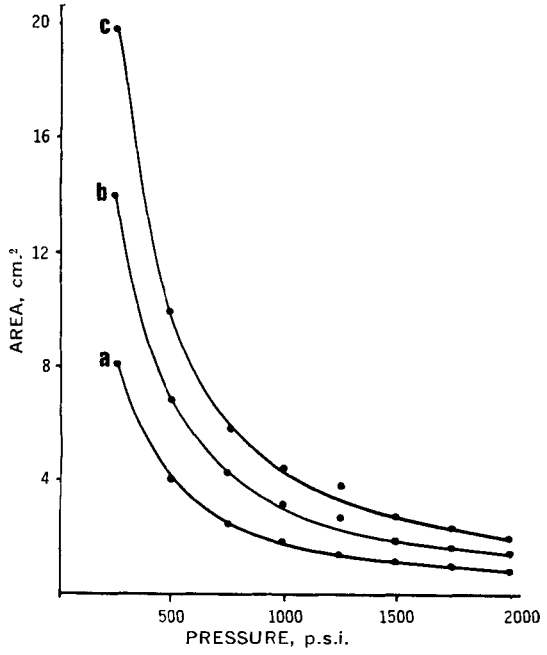

Figure 6—*Effect of pressure on the area of phenobarbital. All conditions are the same as in Fig. 5. Area was calculated as:* bh/2.

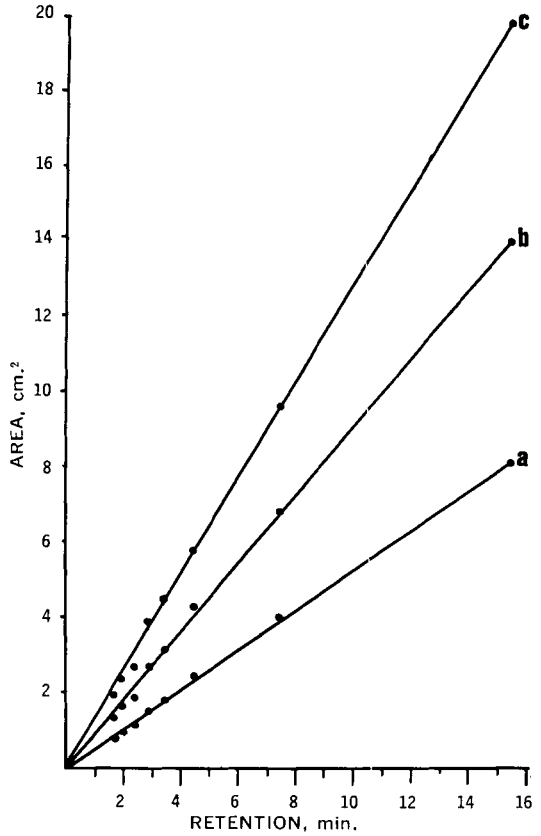

Figure 7—*Plot of area* versus *absolute retention time of phenobarbital. All conditions are the same as in Fig. 5*

Identification and Quantitative Analysis[8]—The work carried out in these laboratories has resulted in the development of both qualitative and quantitative schemes for barbiturate analysis. For both kinds of determinations, the anion-exchange column with an alkaline sodium borate and variable sodium nitrate or the citric acid mobile phase proved useful.

The data of Table I suggest a useful identification scheme for barbiturates. Since both base and acid forms of the barbiturate absorb in the UV region (254 nm.) with different characteristic molar absorptivities, the following approach is recommended.

After a chromatographic study on the anion-exchange column, both with the alkaline and the acid mobile phases under defined conditions, the ratio of the molar absorptivities of the two peaks (thought to be the same barbiturate) is calculated in terms of peak height responses of the same quantity of drug. This ratio should be a constant and characteristic for each barbiturate. Thus, the retention times together with the peak height ratio help to establish the identity of the barbiturate under study.

[8] The Permaphase-ETH du Pont column was not investigated for quantitative analysis.

Table II—Analysis of Commercial Samples[a]

Sample	Declared	—High Speed Liquid Chromatography— Base[b]	Acid[c]	Percent of Declared Independent Method
Phenobarbital elixir	16.2 mg./5 ml.	93.3	92.9	92.2[d]
Aprobarbital elixir	40 mg./5 ml.	102	101	103[e]
Sodium butabarbital elixir	32.4 mg./5 ml.	96.9	96.3	95.1[f]
Phenobarbital tablets	16.2 mg./tablet	96.3	—	96.3[f]
Aprobarbital elixir	40 mg./5 ml.	99.3	—	100[e]
Amobarbital capsules	50 mg./capsule	105	—	103[f]
Sodium amobarbital capsules	25 mg./capsule	109	—	110[g]
Sodium secobarbital capsules	25 mg./capsule	97.6	—	100[g]
Sodium butabarbital elixir	15 mg./5 ml.	103	—	—
Sodium phenobarbital elixir	15 mg./5 ml.	94.7	—	—
Sodium pentobarbital elixir	15 mg./5 ml.	100	—	—
Sodium secobarbital elixir	15 mg./5 ml.	107	—	—

[a] Column was strong anion exchange (Column 1); mobile phases were basic (0.01 *M* borax + 0.03 *M* $NaNO_3$) and acid (0.01 *M* citric acid). Temperature was 25.0 ± 0.5°.

[b]

Internal Standards	Samples
Butabarbital	1, 4, 6, 7
Hexobarbital	2, 5
Phenobarbital	3
Heptabarbital	8

[c]

Aprobarbital	1, 3
Phenobarbital	2

[d] GLC. [e] NF XIII (p. 63). [f] UV. [g] NF XIII (p. 659).

In anticipation of some potential interferences, some of the analgesics formulated with the barbiturates have been studied and they do not interfere with the separation when the alkaline mobile phase system is utilized. Other compounds should be screened if they are to be expected in multidrug dosage forms.

A point of interest to both qualitative and quantitative analyses is that of detection limits or sensitivity. When using the strong anion-exchange column with a 0.01 *M* sodium borate and 0.2 *M* sodium nitrate mobile phase at a pressure of 250 psig., results indicate that barbiturate levels between 0.5 and 1.5 ng. are detectable, depending on elution order. In contrast, the limit for detection by GC using flame ionization was reported for several barbiturates as 2–10 ng. (21). Since larger sample volumes, *e.g.*, 50 μl., may be injected into the instrument, lower practical detection limits (based on concentration) are possible. Phenobarbital was detected at the concentration level of 0.1 ng./μl. of solution in our work.

Either peak height or area can be used for quantitative analysis. Both measurements were found to be linear with sample size. At 250 psig., the peak heights *versus* sample size exhibits a slope of 17.60 cm./mcg., whereas a similar plot of area has a slope of 24.40 $cm.^2$/mcg. Thus, area is somewhat more sensitive. Since peak height is easier to measure and is less sensitive to pressure changes (while still changing linearly with sample size), it is used in the reported quantitative analyses.

The quantitative analyses of eight different dosage forms containing barbiturates are reported in Table II. The various internal standards used are indicated. In addition to single-component samples, two multicomponent samples are included. In general, the results are in good agreement with those by independent methods of analysis.

In summary, the high speed liquid chromatography technique seems particularly suitable for the rapid separation, identification, and analysis of barbiturates in pharmaceuticals and permits the rapid detection of these compounds at the submicrogram level.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 16, 1972, from the *Food and Drug Administration, Department of Health, Education and Welfare, Brooklyn, NY 11232*

Accepted for publication July 26, 1972.

Presented in part at the 85th Annual Meeting of the Association of Official Analytical Chemists (Abstract 132), October 11–14, 1971.

The author thanks Dr. Thomas Medwick, Science Advisor, Food and Drug Administration, New York District, and Professor of Pharmaceutical Chemistry, Rutgers University, New Brunswick, N. J., for his help in the preparation of this paper; he also thanks Martin J Finkelson, FDA, New York, N. Y., for his assistance in the preparation of the figures.