Partitioning of ^{14}C-Heroin between Plasma Water and Red Blood Cells—The results of the several studies conducted on the partitioning of ^{14}C-heroin between plasma water and red blood cells (RBC) are summarized in Table II. The heroin in the systems was inhibited from plasmolysis with 86.5 or 104.5 μg of tetraethyl pyrophosphate/ml. The partition coefficient, D, was calculated from (20):

$$D = \frac{[A_{RBC}]}{[A_p^u]} = \left\{ \frac{[A_B]}{[A_p](1-H)(1-f)} - \frac{f}{1-f} - 1 \right\} \frac{1-H}{H} \quad \text{(Eq. 1)}$$

where $[A_{RBC}]$, $[A_B]$, and $[A_p]$ are the ^{14}C-heroin concentrations in the red blood cells, blood, and plasma, respectively, and $[A_p^u]$ is the drug concentration in the plasma that is unbound to plasma proteins. The hematocrit ÷ 100 is H, and f is the fraction of drug in plasma bound to plasma proteins. The value of f was taken as zero in the plasma water systems and as 0.4 in the plasma–red blood cell mixture.

The results of Study A (Table II) showed no significant differences among the partition coefficients, D, within the range of 38–190 ng of ^{14}C-heroin/ml of pseudoblood. Any time of equilibration greater than zero for ^{14}C-heroin in the pseudoblood did not affect significantly the determined partition coefficients (compare Studies A–C in Table II). Also, times of centrifugation, 3 and 10 min, of the plasma water–red blood cell mixtures to separate the plasma water to be assayed did not significantly affect the results. Only Study D appeared to differ slightly from the total results where the average of the averages given in Table II was 1.39 ± 0.15 *SD*.

Study E was performed after separation of the plasma water from Study D, where the red blood cells containing the equilibrated ^{14}C-heroin were reequilibrated with fresh plasma water and subsequently centrifuged to determine the new partition coefficient. Study E was on a synthetic blood, prepared from plasma and red blood cells, and the partition coefficient determined from Eq. 1 for a protein-bound fraction of $f = 0.4$ was the same as that from the plasma water–red blood cell systems. If the fraction bound had been ignored, *i.e.*, $f = 0$, the calculated apparent D would have been 0.814 ± 0.074 *SD*, which differs widely from the plasma water systems since the protein binding makes a fraction of the heroin unavailable for partition into the red blood cells.

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Liquid Chromatography in Pharmaceutical Analysis XI: Determination of Muscle Relaxant–Analgesic Mixtures Using Reversed-Phase and Ion-Pair Techniques

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Abstract □ High-pressure liquid chromatography using reversed-phase and/or ion-pair techniques was used to optimize resolution of aspirin-containing muscle relaxant mixtures as well as other therapeutic agents commonly found in muscle relaxant–analgesic mixtures. The compounds were chromatographed on an octadecylsilane column using methanol–water solvent systems, some of which contained tetrabutylammonium cation as counterion. Mixtures of methocarbamol–aspirin and chlorzoxazone–acetaminophen were selected to demonstrate separation and quantification. The methocarbamol–aspirin mixture was chromatographed with methanol–water (40:60, pH 6.8) containing 0.01 *M* tetrabutylammonium cation at a flow rate of 2.0 ml/min. The chlorzoxazone–acetaminophen mixture was chromatographed with methanol–water (50:50) at a 2.0-ml/min flow rate. The separation and quantitation of each mixture were achieved in approximately 8 min with accuracy in the 2–3% range.

Keyphrases □ Muscle relaxant–analgesic mixtures, various—high-pressure liquid chromatographic analyses of components □ Analgesic–muscle relaxant mixtures, various—high-pressure liquid chromatographic analyses of components □ High-pressure liquid chromatography—analyses, components of various muscle relaxant–analgesic mixtures

Previous high-pressure liquid chromatographic (HPLC) investigations with muscle relaxant–analgesic agents such as meprobamate, methocarbamol, carisoprodol, chlorzoxazone, acetaminophen, aspirin, caffeine, and phenacetin (1) indicated that the aspirin-containing combinations were difficult to chromatograph using nonpolar solvents

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Table I—Effect of the Mobile Phase on Retention Time [a]

Mobile Phase [b]	Meprobamate	Methocarbamol	Carisoprodol	Chlorzoxazone	Acetaminophen	Aspirin	Caffeine	Phenacetin
A	120	110	232	160	88	80	104	130
	(14)[c]	(16)	(20)	(16)	(14)	(16)	(14)	(16)
B	192	152	532	300	100	84	120	220
	(20)	(26)	(52)	(36)	(12)	(26)	(14)	(24)
C	392	245	1580	580	116	88	168	392
	(36)	(31)	(520)	(60)	(14)	(16)	(20)	(32)
D	124	108	212	172	72	101	104	136
	(12)	(20)	(20)	(20)	(14)	(12)	(12)	(20)
E	177	144	464	312	96	125	116	200
	(20)	(16)	(28)	(28)	(14)	(20)	(16)	(24)
F	324	217	1206	660	116	151	148	352
	(34)	(20)	(111)	(52)	(16)	(20)	(20)	(28)
G	116	120	224	188	88	105	104	144
	(12)	(10)	(24)	(20)	(10)	(16)	(14)	(16)
H	164	148	448	292	96	126	112	216
	(16)	(20)	(42)	(24)	(10)	(16)	(12)	(20)
I	296	226	1080	532	104	184	134	355
	(24)	(28)	(88)	(28)	(10)	(24)	(16)	(48)

[a] Retention time expressed as seconds measured as elapsed time between injection and the chromatographic peak maximum. The eluted peaks were monitored using refractive index detection. [b] A, B, and C are 60:40, 50:50, and 40:60 mixes of methanol and water, respectively. D, E and F are 60:40, 50:50, and 40:60 mixes of methanol and water, respectively, containing 0.01 *M* tetrabutylammonium cation and 0.01 *M* dibasic sodium phosphate and buffered to pH 8 with phosphoric acid. G, H, and I are 60:40, 50:50, and 40:60 mixes of methanol and water, respectively, containing 0.01 *M* tetrabutylammonium cation and 0.01 *M* dibasic sodium phosphate and buffered to pH 6.8 with phosphoric acid. [c] Base peak width expressed as seconds.

and normal phase chromatography. Generally, aspirin eluted close to the solvent front in approximately 50% of the solvents tested or had extremely long retention times in about 25% of the solvents.

Earlier experience with ion-pair chromatography (2, 3) suggested that resolution of acidic components from nonpolar constituents of these muscle relaxant–analgesic mixtures by this technique would be a more successful approach. Therefore, ion-pair formation with tetrabutylammonium cation was investigated, especially with mixtures containing aspirin. The use of tetrabutylammonium cation as a suitable counterion in HPLC was investigated previously (4–6). Ion-pair formation also was utilized (7–9) as a tool for effecting chromatographic analysis of selected organic ions. Included in the present study were investigations of HPLC separations of nonaspirin muscle relaxant–analgesic mixtures using reversed-phase chromatography without ion-pair formation.

The chromatographic data can be applied readily to the separation of selected multidrug dosage forms. This paper reports the separation and quantitation at unit-dose levels of methocarbamol–aspirin using ion-pair and reversed-phase chromatography and of chlorzoxazone–acetaminophen using reversed-phase chromatography.

EXPERIMENTAL[1]

Reagents and Chemicals—Powdered samples of meprobamate[2], methocarbamol[3], carisoprodol[2], chlorzoxazone[4], acetaminophen[5], aspirin[6], caffeine[5], and phenacetin[5] were used. All other chemicals and solvents were the highest purity of the commercially available materials.

Mobile Phases—The mobile phases, consisting of varying proportions of absolute methanol–distilled water, were prepared fresh daily. Certain mobile phases (D–I in Table I) were made 0.01 *M* with respect to the tetrabutylammonium cation in the following manner. An appropriate quantity of tetrabutylammonium iodide[6], calculated to give the desired molar concentration of tetrabutylammonium cation in the final solvent mix, was dissolved in absolute methanol and stirred at room temperature for 1 hr with an equivalent quantity of finely divided silver oxide. The silver oxide was removed by filtration, and a quantity of the methanolic solution was mixed with distilled water to give the desired solvent ratio.

Dibasic sodium phosphate also was added to the distilled water prior to mixing such that its concentration in the final solvent mix was 0.01 *M*. Then the pH of Solvents D–F and G–I was adjusted to 8 and 6.8, respectively, with concentrated phosphoric acid.

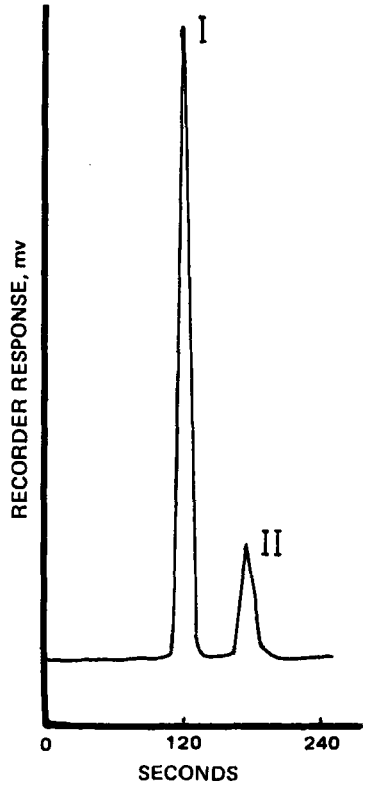

Figure 1—*Liquid chromatogram of simulated meprobamate–aspirin dosage form using refractive index detection on an octadecyl column with absolute methanol–water (50:50, pH 8.0) containing 0.01* M *tetrabutylammonium cation. Concentrations of meprobamate and aspirin were 15 and 25 mg, respectively, dissolved in 25 ml of the mobile phase. A 50-μl injection was made. Key: I, aspirin; and II, meprobamate.*

[1] A Waters Associates liquid chromatograph (model ALC 202), equipped with an M-6000 pump, a U6K injector, UV (254 nm) and differential RI detectors, a Spectra-Physics autolab minigrator with digital printout, and a Waters packed column, 4 mm i.d. × 30 cm, was used.

[2] Wallace Laboratories, Cranbury, N.J.

[3] A. H. Robins, Richmond, Va.

[4] McNeil Laboratories, Fort Washington, Pa.

[5] Eastman Chemicals, Rochester, N.Y.

[6] Aldrich Chemicals, Milwaukee, Wis.

Table II—Typical Calibration Data for Standard Drug Solutions

Compound	Final Concentration, mg[a]	*D/IS* Ratio[b]	Slope	Intercept	$r \pm s_{y \cdot x}$[c]
Methocarbamol	4.0	0.1157 ± 0.0006[d]	0.0303	−0.0041	0.9999 ± 0.0026
	8.0	0.2405 ± 0.0076			
	16.0	0.4802 ± 0.0056			
Aspirin	3.25	0.1816 ± 0.0034	0.0560	0.0011	0.9999 ± 0.0026
	6.50	0.3670 ± 0.0052			
	13.00	0.7281 ± 0.0006			

[a] Total mg/25 ml of solution. [b] Data represent three replicate injections of standard solutions; *D/IS* is the ratio of the integrated area of the drug at some concentration divided by the integrated area of phenacetin at a concentration of 1 mg/25 ml. [c] Standard error of the estimate of *y (D/IS)* on *x* (concentration). [d] Confidence limits at $p = 0.05$.

Drug Solutions—Solutions of each drug (5–10 mg/ml) were prepared by dissolving each powder in the appropriate mobile phase.

Internal Standard Solutions—For methocarbamol–aspirin, 100 mg of phenacetin was dissolved in 100 ml of absolute methanol. For chlorzoxazone–acetaminophen, 49 mg of phenacetin was dissolved in 25 ml of absolute methanol–distilled water (50:50).

Stock Solutions for Calibration Curves—*For Methocarbamol–Aspirin*—Separate stock solutions of methocarbamol (200 mg/100 ml) and aspirin (162.5 mg/100 ml) were prepared in absolute methanol. Accurately pipetted volumes of 2.0, 4.0, and 8.0 ml of each stock solution were placed in 25-ml volumetric flasks. The internal standard stock solution, 1 ml, was added to each flask, followed by the addition of absolute methanol to volume. The three concentrations of each drug were subjected to regression analysis, and the slope and intercept were calculated (Table II).

For Chlorzoxazone–Acetaminophen—Separate stock solutions of chlorzoxazone (62.8 mg/25 ml) and acetaminophen (75.0 mg/25 ml) were prepared in absolute methanol–distilled water (50:50). Accurately pipetted volumes of 0.5, 1.0, and 2.0 ml of each stock solution were placed in 5-ml volumetric flasks. The internal standard stock solution, 1 ml, was added to each flask, followed by the addition of absolute methanol–distilled water (50:50) to volume. The three concentrations of each drug were subjected to regression analysis and the slope and intercept were calculated (Table III).

Chromatographic Separation and Quantification—The degassed mobile phase was pumped through an octadecylsilane[7] column at a flow rate of 2.0 ml/min (2000 psig) at room temperature until a stable baseline was obtained. Replicate 20- and 40-μl injections of sample and standard solutions for the methocarbamol–aspirin and chlorzoxazone–acetaminophen studies, respectively, were made using a 100-μl syringe[8]. The chart recorder provided a record of drug elution from the column as peaks on a chromatogram. In all cases, the solute was measured by electronic integration of the peak area[1].

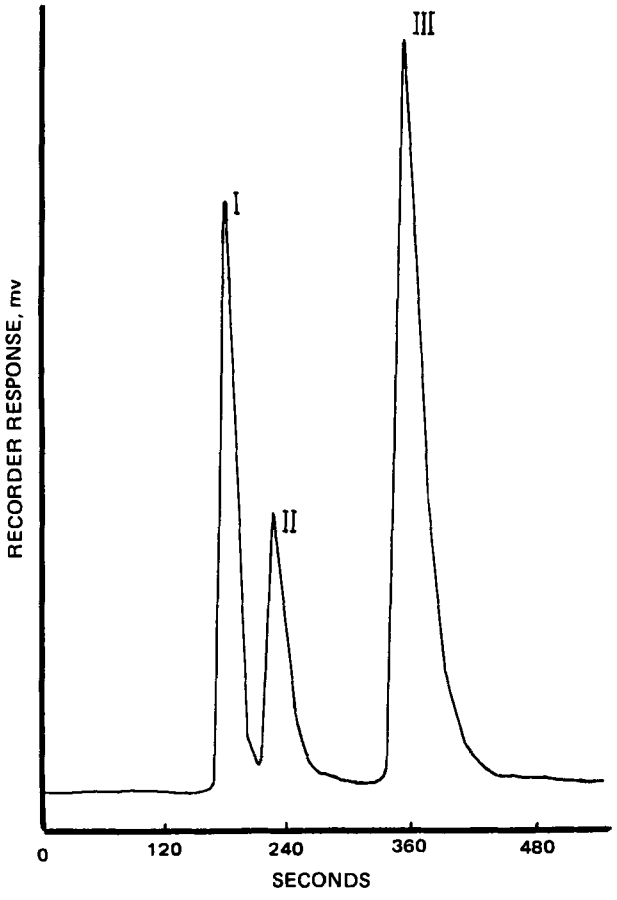

Figure 2—*Liquid chromatogram of simulated methocarbamol–aspirin dosage form at 254 nm on an octadecyl column with absolute methanol–water (40:60, pH 6.8) containing 0.01 M tetrabutylammonium cation. Concentrations of methocarbamol and aspirin were 8 and 6.5 mg, respectively, dissolved in 25 ml of the mobile phase. A 20-μl injection was made. The phenacetin concentration was 1 mg/25 ml. Key: I, aspirin; II, methocarbamol; and III, phenacetin (internal standard).*

RESULTS AND DISCUSSION

Optimization of resolution of the therapeutic agents most commonly found in muscle relaxant–analgesic mixtures necessitated: (*a*) examination of variation in solvent composition combining absolute methanol with changing concentrations of distilled water, and (*b*) addition of tet-

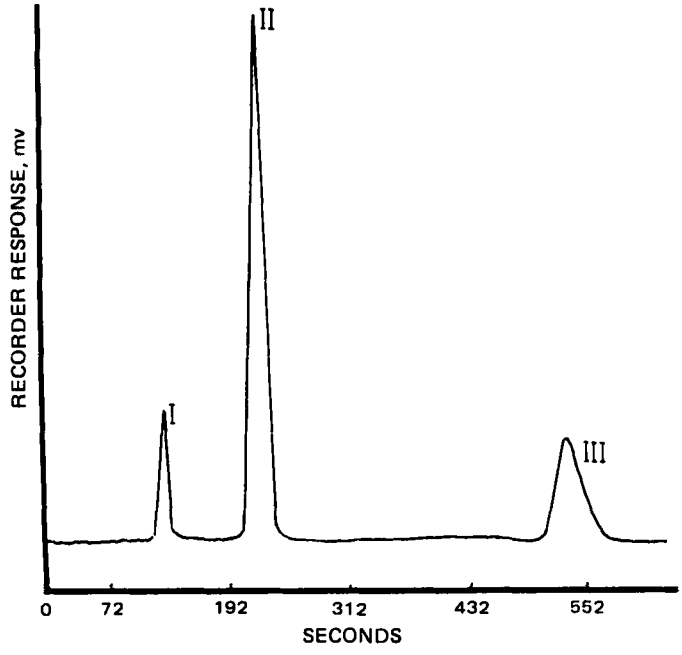

Figure 3—*Liquid chromatogram of simulated carisoprodol–phenacetin–caffeine dosage form using refractive index detection on an octadecyl column with absolute methanol–water (50:50). Concentrations of carisoprodol, phenacetin, and caffeine were 20, 16, and 3.2 mg, respectively, dissolved in 5 ml of the mobile phase. A 10-μl injection was made. Key: I, caffeine; II, phenacetin; and III, carisoprodol.*

[7] μBondapak C_{18}, <10 μm, Waters Associates, Milford, Mass.
[8] Model B-110, Precision Sampling Corp., Baton Rouge, La.

Table III—Typical Calibration Data for Standard Drug Solutions

Compound	Final Concentration, mg[a]	*D/IS* Ratio[b]	Slope	Intercept	$r \pm s_{y,x}$[c]
Chlorzoxazone	1.26	0.5534 ± 0.0430[d]	0.4295	0.0136	0.9999 ± 0.0027
	2.51	1.0939 ± 0.0146			
	5.02	2.1692 ± 0.0547			
Acetaminophen	1.5	0.6402 ± 0.0360	0.4086	0.0322	0.9999 ± 0.0093
	3.0	1.2656 ± 0.0136			
	6.0	2.4816 ± 0.0304			

[a] Total mg/5 ml of solution. [b] Data represent three replicate injections of standard solutions; *D/IS* is the ratio of the integrated area of the drug at some concentration divided by the integrated area of phenacetin at a concentration of 1.96 mg/5 ml. [c] Standard error of the estimate of *y (D/IS)* on *x* (concentration). [d] Confidence limits at $p = 0.05$.

rabutylammonium cation to the mobile phase to effect ion-pair formation, especially in mixtures containing aspirin. The stationary phase was limited to octadecylsilane chemically bonded to a porous silica support.

The effects of these parameters on retention times and base peak widths of the drugs are shown in Table I. The eluted peaks were monitored using refractive index detection since many of the muscle relaxants were aliphatic compounds and did not possess satisfactory UV absorption. For pH stabilization, dibasic sodium phosphate was added to mobile phases containing tetrabutylammonium cation.

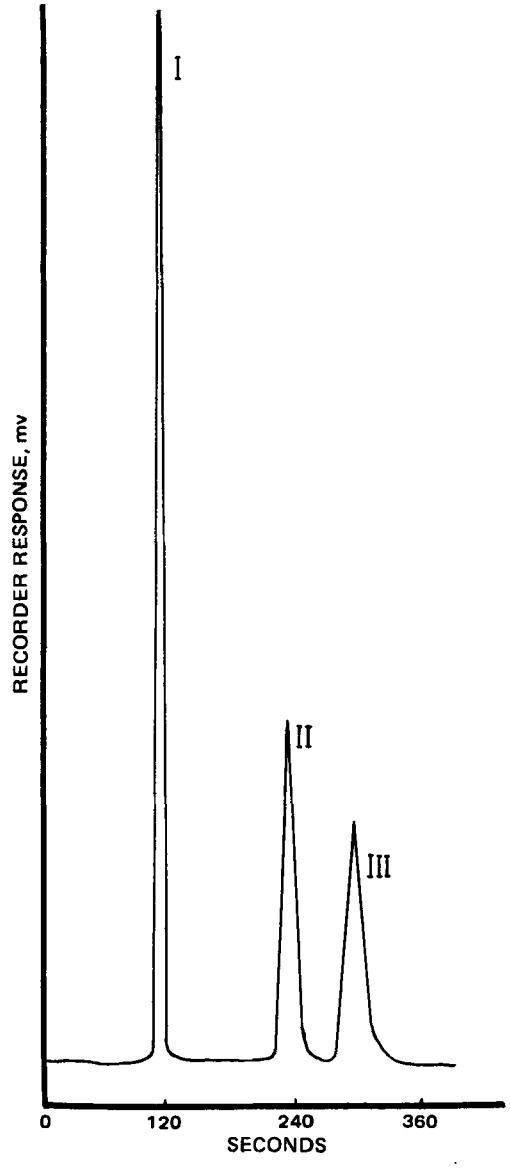

Figure 4—*Liquid chromatogram of simulated chlorzoxazone–acetaminophen dosage form using refractive index detection on an octadecyl column with absolute methanol–water (50:50). Concentrations of chlorzoxazone and acetaminophen were 2.51 and 3.0 mg, respectively, dissolved in 5 ml of mobile phase. A 40-μl injection was made. The phenacetin concentration was 1.96 mg/5 ml. Key: I, acetaminophen; II, phenacetin (internal standard); and III, chlorzoxazone.*

Reasonably high concentrations of methanol were used instead of the pentanol-saturated mobile phase recommended previously (5, 6). This substitution, especially in studies involving tetrabutylammonium cation, desensitizes the mobile and stationary phases to fluctuations in ambient temperature, which could have a large effect on pentanol saturation levels (5, 6). In addition, routine column cleanup procedures can be performed without a prolonged reequilibration step. The stripping of pentanol from a reversed-phase stationary support was documented (6).

The following generalizations can be inferred from the data in Table I. Retention times for all drugs increased as the mobile phase polarity increased. The noncharged solutes such as meprobamate, carisoprodol, and methocarbamol had a wide range of retention times in methanol–water systems ($k' = 0.2$–18). Increasing the polarity of the mobile phase (Solvents A → C) predictably increased the k' value for any selected neutral solute. The addition of tetrabutylammonium counterion to the mobile phase (*e.g.*, Solvent C *versus* Solvent F or I) should have further increased the mobile phase polarity, leading to an additional increase in retention time. This increase was not observed. The retention times for the neutral solutes in tetrabutylammonium-containing solvents were the same as or, in many cases, shorter than those observed with methanol–water. This anomaly in the observed retention time can be cited as evidence for the association of tetrabutylammonium counterion with the hydrophobic stationary phase, which renders this phase more polar than when the solvent contains only methanol–water. As a result, the neutral solutes have a lower affinity for the stationary phase and elute in shorter retention times.

Meprobamate was eluted using all solvents employed. A meprobamate–aspirin mixture was separated with any solvent system except G. However, in Solvents A–C, aspirin traveled close to the solvent front and was difficult to distinguish from other components present at the front. Figure 1 illustrates a separation of meprobamate–aspirin using Solvent E, which contained the tetrabutylammonium cation.

All solvent systems were also satisfactory for the elution of methocarbamol. A typical mixture of methocarbamol and aspirin was separated with Solvents A–C, F, and I (Fig. 2).

All solvents were useful for the elution of carisoprodol on the octadecylsilane column. The mixture carisoprodol, phenacetin, and caffeine was separated by any of the mobile phases listed in Table I (Fig. 3).

Chlorzoxazone was satisfactorily eluted on the octadecylsilane column using Solvents A–I. A chlorzoxazone–acetaminophen mixture was separated using any of the solvent systems (Fig. 4).

The elution of acetaminophen was best accomplished with Solvents B, C, F, and I. The retention time for the drug in the remaining solvents was at or near the solvent front, thus rendering it difficult to distinguish clearly between acetaminophen and other components present at the front.

Aspirin traveled at or near the solvent front in the methanol–water systems containing no tetrabutylammonium ion. Addition of tetrabutylammonium ion to the mobile phase caused retention of aspirin on the column, with the retention time increasing as the polarity of the mobile phase increased. There were slight differences in retention time for aspirin in the pH 8 *versus* 6.8 mobile phases.

Caffeine and phenacetin were eluted successfully with all solvent systems. A typical aspirin, phenacetin, and caffeine mixture was satisfactorily separated by Solvents A–C and I.

Satisfactory quantitation of a multicomponent dosage form depends on adequate component resolution. The theoretical parameter, R_s (10), is a reasonable measure of the separation of two species on a column. It can be approximated for any two drugs included in this study from the

Table IV—Analysis of Methocarbamol–Aspirin and Chlorzoxazone–Acetaminophen in Known Mixtures

Mixture	Components	Amount Added, mg	Amount Found[a], mg	Accuracy, %
A	Methocarbamol	8.00[b]	8.19 ± 0.12[c]	2.38
	Aspirin	6.50	6.67 ± 0.13	2.62
B	Acetaminophen	3.00[d]	3.08 ± 0.08[c]	2.67
	Chlorzoxazone	2.51	2.59 ± 0.05	3.19

[a] Based on four replicate determinations of the known mixture. [b] Total mg/25 ml of solution. [c] Confidence limits at $p = 0.05$. [d] Total mg/5 ml of solution.

data contained in Table I[9]. Simulated dosage form mixtures containing methocarbamol–aspirin[10] and chlorzoxazone–acetaminophen[11] were selected for quantitation to exemplify this situation with Solvents I and B, respectively. Both solvent systems allowed good overall resolution with reasonable retention times.

Typical chromatograms of the drug mixtures are shown in Figs. 2 and 4. Various concentrations of stock solutions of each drug dissolved in the appropriate mobile phase (see *Experimental*) were chromatographed using the octadecylsilane column. Phenacetin was added to each solution as the internal standard. The area under the curve for each peak on the chromatograms was determined with an electronic integrator. The ratio of each drug peak area to the area of the internal standard was calculated for each chromatogram. Regression analysis of these data at the various concentrations of each drug gave the slope, intercept, and correlation coefficient for each calibration curve (Tables II and III).

Solutions containing known quantities of methocarbamol–aspirin and/or chlorzoxazone–acetaminophen in ratios equivalent to those found in the commercial dosage forms were chromatographed, and the ratios of drug peak areas to internal standard peak areas (*D/IS*) were calculated for each drug. The slope and intercept data from the regression analysis for each drug (Tables II and III) were used to solve for drug concentration [*D/IS* = (slope × concentration) + intercept][12] in these simulated dosage form mixtures.

The data in Table IV demonstrate the quantitative results obtained for the mixtures. The utility of HPLC in the analysis of methocarbamol–aspirin and chlorzoxazone–acetaminophen mixtures is clearly demonstrated with accuracy in the 2–3% range.

[9] It is possible to calculate the approximate resolution, R_s, of two components by the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are retention times and w_1 and w_2 are base peak widths of Compounds 1 and 2, respectively. In past experience, two components with an R_s value >1.50 showed satisfactory resolution for quantification by this analytical technique if the peak areas were approximately equal. A significant difference in peak areas for two components may require an R_s value of 2 or greater.

[10] Robaxisal, A. H. Robins, Richmond, Va.

[11] Parafon-Forte, McNeil Laboratories, Fort Washington, Pa.

[12] Olivetti-Underwood programma 101.

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Formation of a Thio Analog of Noracronine

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Abstract □ Acronine was heated with tetraphosphorus decasulfide in benzene to give a mixture of products, the major component (A) being identified tentatively as an unstable product derived from four molecules of noracronine with one molecule of tetraphosphorus decasulfide. Treatment of Compound A with various solvents and heat converted it into a maroon solid (B), which was shown to be 7-thionoracronine.

Keyphrases □ 7-Thionoracronine—synthesized from acronine and tetraphosphorus decasulfide □ Noracronine thio analog—7-thionoracronine synthesized from acronine and tetraphosphorus decasulfide □ Prodrugs, potential—7-thionoracronine synthesized from acronine and tetraphosphorus decasulfide

The alkaloid acronine[1] (I) has a wide spectrum of anticancer activity (1) but the disadvantage of low aqueous solubility. Various attempts have been made (2) to improve the formulation of I, including the preparation of molecular complexes of the alkaloid with povidone and gentisic acid. A prodrug (II) that regenerates acronine quantitatively and shows improved water solubility was prepared but suffers from the disadvantage of being hydrolyzed too rapidly under physiological conditions (3). Reaction of II with aniline gave a rapid quantitative yield of anil[2], and the hydrolysis rates were similar for a series of analogs of II in which the acetyl group was replaced by other acyl functions (2).

Recent studies revealed that hydrolysis in water of II

[1] Previously referred to as acronycine (NSC 403169).

[2] B. Kreilgard, Royal Danish School of Pharmacy, Copenhagen, Denmark, unpublished data.

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