Quantitative Determination of Carisoprodol, Phenacetin, and Caffeine in Tablets by Near-IR Spectrometry and Their Identification by TLC

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Abstract ☐ Analytical procedures for the identification and measurement of each individual component in a carisoprodol, phenacetin, and caffeine combination are reported. Identification is confirmed by TLC. Carisoprodol and phenacetin are determined simultaneously in chloroform, while caffeine is isolated from the other two components and measured in carbon tetrachloride. All determinative steps utilize near-IR spectrometry. The validity of the quantitative procedures described is substantiated by data obtained from the analysis of spiked solutions, which shows excellent accuracy and standard deviations for carisoprodol, phenacetin, and caffeine of 1.21, 0.68, and 0.74, respectively. Four commercial tablet preparations were analyzed and the standard deviations for carisoprodol, phenacetin, and caffeine were 1.38, 1.40, and 1.07, respectively. These figures are a reflection of both the manufacturing process and the reproducibility of the methodology.

Keyphrases ☐ Carisoprodol, phenacetin, and caffeine tablets—quantitative near-IR determination of each component, TLC identification ☐ Phenacetin, carisoprodol, and caffeine tablets—quantitative near-IR determination of each component, TLC identification ☐ Caffeine, carisoprodol, and phenacetin tablets—quantitative near-IR determination of each component, TLC identification ☐ IR spectrometry (near)—quantitative determination of components of carisoprodol, phenacetin, and caffeine tablets

The use of drugs in combination for a particular therapeutic advantage makes the analysis for the individual drugs in such dosage forms more complicated. This is certainly true for mixtures of carisoprodol, phenacetin, and caffeine, a useful combination when skeletal muscle relaxation is desirable.

Analytical procedures for the identification and measurement of each individual component in a carisoprodol, phenacetin, and caffeine mixture have been reported. For example, paper chromatographic (1) and TLC (2) methods have been devised for the separation and identification of mixtures of carbamates such as carisoprodol, meprobamate, mebutamate, and hydroxymeprobamate, while individual TLC methods for the identification of meprobamate (3), phenacetin (4), and caffeine (4) have been introduced. As a natural extension, quantitative TLC and GLC methods for carbamates (5–8) have been described. The measurement of phenacetin and caffeine, both individually and in mixtures, has been accomplished using various approaches. The official compendial procedure for phenacetin is a gravimetric analysis in which the unaltered drug is weighed after isolation by repeated solvent extraction and filtration (9). Quantitative IR (10) and visible and UV (11–14) methods are available to measure phenacetin and caffeine after their isolation.

This paper describes a TLC separation and identification scheme for all components in a carisoprodol, phenacetin, and caffeine mixture. It also reports a study of the measurement of these drugs in tablets using their near-IR light-absorbing properties. Although both the TLC and near-IR approaches have been used for the individual drugs, the application to the combination reported here has not been done before. The procedures described in this report are simpler, more rapid, and convenient than previous procedures.

EXPERIMENTAL

All chemicals were reagent grade unless otherwise specified.

Identification of Carisoprodol, Phenacetin, and Caffeine by TLC—Silica gel GF 254 (for TLC according to Stahl, Type 60[1]) 20 × 20-cm plates were used with a standard glass-type tank, equilibrated with ethyl acetate, reagent grade, for 30 min.

A single tablet was taken, placed into a volumetric flask containing some methanol and disintegrated by ultrasonic perturbation[2]. It was dissolved in methanol to contain 4 mg/ml carisoprodol, 3.2 mg/ml phenacetin, and 0.64 mg/ml caffeine and filtered.

Standard Solutions—Individual solutions (in methanol) of 20 mg/ml carisoprodol, 16 mg/ml phenacetin, and 3.2 mg/ml caffeine were prepared. A mixed standard solution was prepared from the individual standard solutions by dilution to contain 4 mg/ml carisoprodol, 3.2 mg/ml phenacetin, and 0.64 mg/ml caffeine.

TLC Procedure—Spot 20 μl each of sample, individual standards, and mixed standard 3–5 cm from bottom of plate and develop the plate for a distance of 15 cm. When the plate has dried, use the appropriate detection procedure.

For the detection of phenacetin and caffeine, use shortwave UV light (253.7 nm) which produces purple-colored spots.

For the detection of carisoprodol, spray the plate with 5% vanillin in concentrated sulfuric acid following the UV detection of phenacetin and caffeine. An easily seen yellow-colored spot results.

The R_f values obtained were: caffeine, 0.13; phenacetin, 0.59; and carisoprodol, 0.82.

Simultaneous Quantitation of Carisoprodol and Phenacetin—*Instruments and Reagents*—A ratio recording spectrophotometer[3] was used with the following settings: sensitivity dial, 2.00; gears, 10 nm/cm; scanning time switch, 20; time constant, 0.1; range selector, 0–100 (0–1); and photomultiplier, PbS cell mode.

Alumina[4] (basic, 80–200 mesh) was activated daily by heating for 2 hr at 300°. Chloroform, water and alcohol free (15), was prepared daily as follows. Extract the alcohol by passing about 500

[1] EM Reagents, EM Laboratories, Inc., Elmsford, NY 10523
[2] Cole-Parmer Instruments, Chicago, IL 60648
[3] Beckman DK-2A.
[4] Fisher No. A-540 or equivalent.

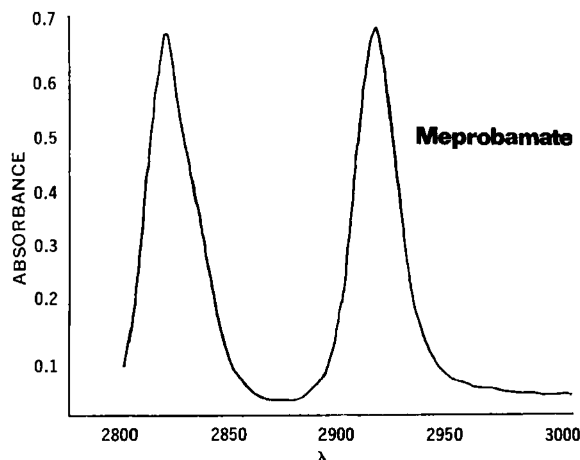

Figure 1—*Near-IR spectrum of meprobamate (64 mg/100 ml in chloroform); λ = wavelength (nanometers).*

ml of chloroform through three 1000-ml separators, each containing 50–75 ml of water. Pack two chromatographic columns (glass, 20 × 300 mm) half-full with alumina to remove the water. Mount one column above the other and pass the chloroform through both columns. If more than 500 ml of chloroform is needed, repeat the purification with fresh alumina. Since alcohol-free chloroform is prone to the formation of phosgene, it should not be stored longer than 1 week. The solvent should be kept in low-actinic glassware and transferred to avoid picking up water.

Prepare the carisoprodol standard solution as follows. Weigh about 50 mg of carisoprodol standard accurately (previously dried at 60° under vacuum for 3 hr) and transfer quantitatively to a 100-ml volumetric flask. Dissolve in and dilute to volume with alcohol-free chloroform. Filter through dry Whatman No. 1 paper containing about 2 g of anhydrous sodium sulfate. Discard the first 20–25 ml of filtrate and collect the remainder in a dry glass-stoppered erlenmeyer flask.

To prepare the phenacetin standard solution, weigh about 100 mg of phenacetin standard accurately and proceed as directed for the carisoprodol standard solution starting with "(previously dried. . . .

Preparation of Sample—Weigh and finely pulverize 20 tablets until a homogeneous powder is obtained. Accurately weigh an amount of sample composite equivalent to about 50 mg of carisoprodol and transfer quantitatively to a 100-ml volumetric flask. Dissolve in alcohol-free chloroform by shaking for 15 min and dilute to volume with the same solvent. Filter through dry Whatman No. 1 paper containing about 2 g of anhydrous sodium sulfate. Discard the first 20–25 ml of filtrate and collect the remainder in a dry glass-stoppered erlenmeyer flask.

Determination of Carisoprodol and Phenacetin—Filter a portion of alcohol-free chloroform through dry Whatman No. 1 paper containing about 2 g of granular anhydrous sodium sulfate. Discard the first few milliliters of filtrate and collect the remainder in a dry glass-stoppered erlenmeyer flask. Zero the instrument at 2914 nm with this chloroform in two matching near-IR silica cells. Scan the near-IR spectra of sample and standard solutions against chloroform between 3000 and 2750 nm. Measure the baseline absorbance values at 2910 and 2820 nm from straight lines drawn between the minima at 3000 and 2870 nm and at 2870 and 2790 nm. Subtract baseline values from peak absorbances at 2910 and 2820 nm to obtain net absorbance.

Calculate the carisoprodol concentration using absorbance at 2820 nm as follows:

$$\text{mg carisoprodol/tablet} = \frac{A_{2820}}{A_{cs\,2820}} \times C \times \frac{T}{W} \times 100 \qquad \text{(Eq. 1)}$$

where:

A_{2820} = absorbance of sample solution at 2820 nm
$A_{cs\,2820}$ = absorbance of carisoprodol standard solution at 2820 nm
C = concentration of carisoprodol standard (milligrams per milliliter)
T = average tablet weight (milligrams)
W = weight of sample composite (milligrams)

Calculate the phenacetin concentration as follows:

$$\text{mg phenacetin/tablet} = \frac{A_{2910} - A_{c2910}}{A_{p2910}} \times C \times \frac{T}{W} \times 100 \qquad \text{(Eq. 2)}$$

where:

A_{2910} = absorbance at 2910 nm for carisoprodol plus phenacetin in sample solution
$A_{c\,2910}$ = absorbance at 2910 nm for carisoprodol alone in sample solution
$A_{p\,2910}$ = absorbance at 2910 nm for phenacetin in standard solution

and:

$$A_{c2910} = \frac{A_{c2820}\,A_{cs2910}}{A_{cs2820}} \qquad \text{(Eq. 3)}$$

where:

$A_{c\,2820}$ = absorbance at 2820 nm for carisoprodol in sample solution
$A_{cs\,2910}$ = absorbance at 2910 nm for carisoprodol in standard solution

Spiked Solutions: Carisoprodol and Phenacetin—A sample composite similar to the one already described was accurately weighed and transferred quantitatively to a 100-ml volumetric flask. Then 50 mg of carisoprodol and 50 mg of phenacetin were accurately weighed and added to the sample flask. The resulting sample spike was dissolved in and diluted to volume with alcohol-free chloroform and then filtered as described previously. Then 25 ml of the resultant solution was diluted to exactly 50 ml with the same solvent. Phenacetin and carisoprodol standard solutions containing about 1 and 0.5 mg/ml, respectively, in alcohol-free chloroform were prepared as described. Near-IR spectra were obtained for the sample and standard solutions. The recoveries of carisoprodol and phenacetin were calculated based on assay values for the samples previously determined.

Quantitation of Caffeine—*Reagents*—The following were used: chloroform[5], ACS grade, water washed just prior to use; ether[6], reagent grade, water washed just prior to use; carbon tetrachloride[7], reagent grade, shaken with anhydrous sodium sulfate just prior to use; and diatomaceous earth[8], acid washed.

To prepare the caffeine standard solution, dry a caffeine reference standard for 4 hr at 80° under vacuum. Weigh about 15 mg accurately and transfer quantitatively to a 10-ml volumetric flask. Add about 7 ml of carbon tetrachloride, stopper the flask, and place in the sonic bath for 10 min to dissolve. Cool to room temperature and dilute to volume with the same solvent.

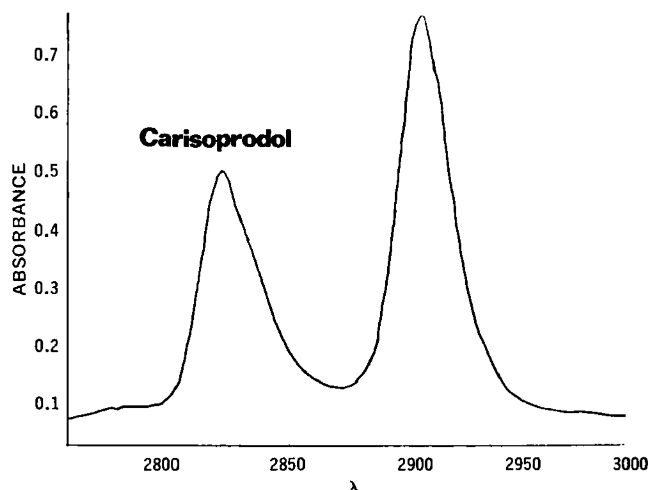

Figure 2—*Near-IR spectrum of carisoprodol standard solution (100 mg/100 ml in chloroform); λ = wavelength (nanometers).*

[5] Allied Chemical Co. or equivalent.
[6] Ethyl ether, Mallinckrodt Chemical Works or equivalent.
[7] Fisher Scientific Co. or equivalent.
[8] Celite 545, Johns-Manville.

Table I—Analysis of Mixed Carisoprodol and Phenacetin Spiked Solutions

Sample	Carisoprodol Added, mg	Carisoprodol Found, mg	Carisoprodol Recovery, %	Phenacetin Added, mg	Phenacetin Found, mg	Phenacetin Recovery, %
1	50.6	51.3	101.4	45.8	45.9	100.2
2	53.2	53.9	101.3	51.7	51.4	99.4
3	50.3	50.7	100.8	51.8	51.1	98.6
4	49.6	49.0	98.8	48.2	48.1	99.8
Average			100.6			99.5
Standard deviation, %			1.21			0.68
Relative standard deviation, %			1.21			0.69

Sample Preparation—Weigh an amount of sample composite equivalent to about 75 mg of caffeine into a 50-ml glass-stoppered erlenmeyer flask. Add about 25 ml of chloroform, stopper, and shake for 15 min. Filter through Whatman No. 40 paper, previously saturated with chloroform, into a 50-ml volumetric flask. Wash the erlenmeyer flask several times with chloroform and filter the washings into the volumetric flask. Wash the filter paper and dilute to volume with chloroform.

Pack a pledget of glass wool in a 24 × 240-mm glass chromatographic column. Intimately mix 2 ml of 4 *N* H_2SO_4 with 3 g of diatomaceous earth in a beaker and pack on top of the glass wool. Overlay the diatomaceous earth with a second pledget of glass wool. Pipet 10 ml of sample solution into a 100-ml beaker, dilute with 50 ml of water-washed ether, and pour onto the column. Wash the beaker with four successive 50-ml portions of water-washed ether to ensure quantitative transfer of the sample solution to the column. Wash the tip of the column with chloroform and discard the ether eluate. Place a 200-ml beaker under the column and elute the caffeine with 150 ml of water-washed chloroform. Evaporate the chloroform eluate on a steam bath under a stream of air to a volume of about 5 ml, transfer quantitatively to a 25-ml glass-stoppered erlenmeyer flask, and evaporate to dryness as before. Dry the residue for 4 hr at 80° under vacuum. Pipet 10.00 ml of carbon tetrachloride into the flask, stopper tightly, place in a sonic bath for 10 min, and cool to room temperature.

Determination of Caffeine—Zero the instrument at 3390 nm with carbon tetrachloride in two matching near-IR silica cells. Scan the near-IR spectra of sample and standard solutions against carbon tetrachloride between 3500 and 3200 nm. Measure the baseline absorbance value at 3390 nm from the straight line drawn between 3500 and 3150 nm. Subtract the baseline absorbance value from the peak absorbance at 3390 nm to obtain the net absorbance.

Calculate the caffeine concentration using the absorbances at 3390 nm as follows:

$$\text{mg caffeine/tablet} = \frac{A_{3390}}{A_{\text{caf}3390}} \times C \times \frac{T}{W} \times 50 \quad \text{(Eq. 4)}$$

where:

A_{3390} = absorbance of sample at 3390 nm
$A_{\text{caf}3390}$ = absorbance of caffeine standard solution at 3390 nm
C = concentration of caffeine standard solution (milligrams per milliliter)
T = average tablet weight (milligrams)
W = sample weight (milligrams)

Spiked Solutions: Caffeine—A sample composite equivalent to about 37.5 mg of caffeine was accurately weighed and transferred quantitatively to a 50-ml glass-stoppered erlenmeyer flask. About 37.5 mg of caffeine, 258 mg of carisoprodol, and 188 mg phenacetin were accurately weighed and transferred quantitatively to the same flask. The resultant mixture was then analyzed for caffeine as directed in the *Determination of Caffeine* section. The recoveries of caffeine were calculated based on assay values for the samples previously determined.

Table II—Analysis of Caffeine Spiked Solutions

Sample	Added, mg	Found, mg	Recovery, %
1	39.7	39.5	99.5
2	37.4	37.5	100.3
3	38.2	38.7	101.3
4	40.2	40.4	100.5
Average			100.4
Standard deviation, %			0.74
Relative standard deviation, %			0.74

RESULTS AND DISCUSSION

The described TLC system is useful in establishing the presence of each of the three therapeutic elements in the dosage form. Since the solvent is a single chemical entity, no solvent preparation is necessary. The R_f values indicate that a satisfactory resolution may be expected. Since the study was designed to find the drugs at therapeutic levels, the limits of detection of the described TLC procedure were not determined. However, these limits could easily be established if it were necessary to use the procedure to detect lower drug levels.

The success of the quantitative analysis for carisoprodol and phenacetin is dependent upon the existence of absorption bands in the 3100–2800-nm region for dilute solutions of amides in nonpolar solvents. As an example of the carbamate class, meprobamate, a dicarbamate, gives rise to two medium intensity absorption bands in the near-IR region at 2820 and 2910 nm. The band at 2820 nm is due to the asymmetric stretching mode of the NH_2 (amide) group, and the band at 2910 nm is due to the corresponding symmetric stretching mode (16).

The near-IR spectrum of meprobamate is, therefore, characterized by two equivalent intensity absorption maxima of direct importance to this work (Fig. 1). The carisoprodol molecule, which incorporates both an unsubstituted and substituted amide group, exhibits a near-IR spectrum characterized by an absorption maximum at 2820 nm due to the asymmetric stretching mode of the NH_2 (amide) group and a stronger maximum at 2910 nm ascribable to the corresponding symmetric stretching of the unsubstituted amide plus the weaker intensity vibrational change of the substituted NH (amide) group (Fig. 2). The near-IR absorption

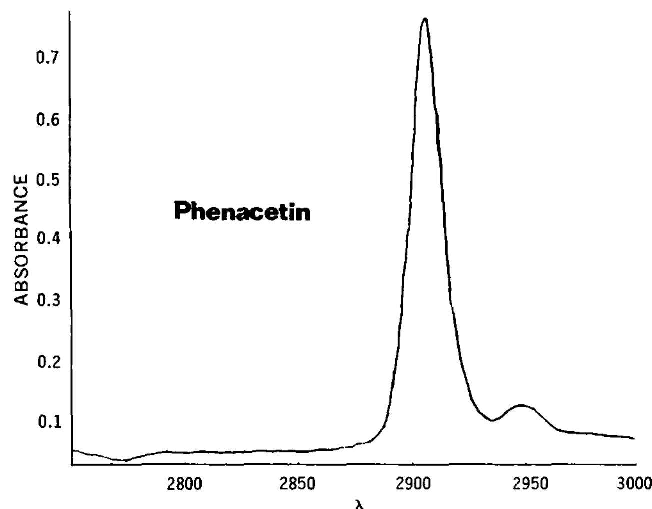

Figure 3—*Near-IR spectrum of phenacetin standard solution (150 mg/100 ml in chloroform); λ = wavelength (nanometers).*

Table III—Complete Analysis of Commercial Tablets Containing Carisoprodol, Phenacetin, and Caffeine

Sample Lots	Carisoprodol Declared, mg	Carisoprodol Found, mg	Phenacetin Declared, mg	Phenacetin Found, mg	Caffeine Declared, mg	Caffeine Found, mg
1	200	188.4	160	157.9	32	30.4
2	200	186.2	160	159.0	32	30.4
3	200	185.6	160	159.3	32	32.3
4	200	188.1	160	156.2	32	32.2
Mean		187.1		158.1		31.3
Standard deviation		1.38		1.40		1.07
Relative standard deviation		0.74		0.89		3.41
Average recovery, % of declared		93.6		98.7		97.9

spectrum for phenacetin also exhibits a maximum at 2910 nm due to the vibrational transition of the NH (amide) group (Fig. 3). These absorption bands were used for the simultaneous determination of carisoprodol and phenacetin in a tablet combination (Fig. 4).

The small peak appearing at 2950 nm in the phenacetin spectrum, although not used analytically, was identified as the double-frequency overtone of the "amide I" fundamental band of —C(=O)—NHAr group (17, 18). Its presence did not affect the accuracy of the near-IR procedure.

The near-IR properties of carisoprodol and phenacetin provided a convenient method for the simultaneous spectrophotometric analysis of both drugs. Caffeine could not be measured at the same time but, fortunately, did not interfere. A caffeine standard solution did not exhibit any absorption at either 2910 or 2820 nm, the analytical wavelengths.

This system is recognized as a simple binary mixture (carisoprodol and phenacetin) with one member (phenacetin) absorbing at one analytical wavelength only. Thus, in a mixture of phenacetin and carisoprodol, the total absorbance at 2910 nm, A'_{2910}, is described by:

$$A'_{2910} = A'_{c2910} + A'_{p2910} \quad \text{(Eq. 5)}$$

where:

A'_{c2910} = absorbance at 2910 nm for carisoprodol standard solution
A'_{p2910} = absorbance at 2910 nm for phenacetin standard solution

The value for A'_{c2910} may be calculated from the Beer's law behavior of carisoprodol at 2820 nm, whereas Eq. 6 provides the basis for evaluating A'_{p2910}:

$$A'_{p2910} = A'_{2910} - A'_{c2820}\frac{a_{c2910}}{a_{c2820}} \quad \text{(Eq. 6)}$$

where:

A'_{c2820} = absorbance at 2820 nm for carisoprodol standard solution
a_{c2910} = absorptivity of carisoprodol at 2910 nm (0.662)
a_{c2820} = absorptivity of carisoprodol at 2820 nm (0.377)

The absorbances of a series of carisoprodol standard solutions were measured, and adherence to Beer's law was observed at both maxima (Fig. 5). A similar series of phenacetin standard solutions was also analyzed and found to obey Beer's law (Fig. 5). Finally, a series of mixed carisoprodol–phenacetin standard solutions was analyzed and exhibited linearity.

The actual analytical system is relatively simple. The 2820-nm line for carisoprodol in Fig. 5 provides the basis for carisoprodol measurement. For phenacetin determination, Eq. 6 is needed to yield A'_{p2910}. Since A'_{2910} and A'_{c2820} are direct readings and a_{c2910} and a_{c2820} are obtained as the slopes of the straight lines in Fig. 5, the calculation may be easily accomplished.

The validity of the simultaneous quantitative procedure for phenacetin and carisoprodol was substantiated by the data obtained from the analysis of spiked solutions (Table I), which show the relative standard deviations for carisoprodol and phenacetin to be 1.21 and 0.69%, respectively.

The quantitative procedure described for caffeine was inferred from an IR method reported for its determination in a multicomponent tablet mixture (19). The IR absorption spectrum of a chloroform solution of caffeine presented in the method revealed two small bands at about 3400 and 3350 nm. Chloroform is an unacceptable solvent for near-IR determinations in this region, since the radiation is absorbed by this solvent and a reference energy cannot be obtained. Carbon tetrachloride was a satisfactory substitute.

The determination of caffeine was complicated by interference from carisoprodol, a much stronger absorber at 3390 nm. The latter was removed in the chromatographic step by elution with 250 ml of ether, while caffeine was retained on the acidified column and easily eluted with 150 ml of chloroform. Although a chromatogram is not included, these volumes were found to be ideal. Evaporation of the chloroform eluate was followed by drying to remove water absorbed by caffeine from the solvents. Failure to do this would yield high results due to the light-absorbing properties of water in the near-IR region being considered.

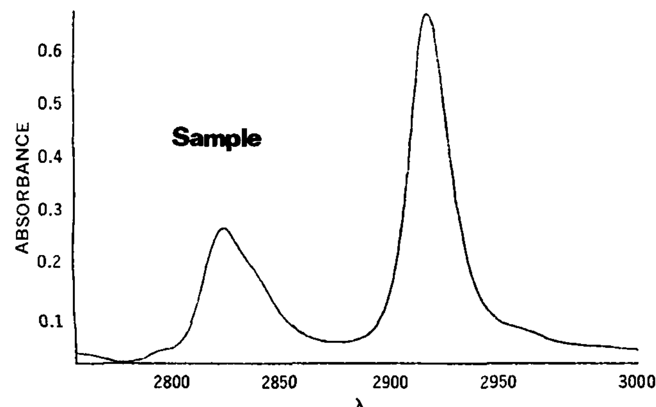

Figure 4—*Near-IR spectrum of a carisoprodol and phenacetin solution in a tablet combination (50 mg/100 ml carisoprodol and 40 mg/100 ml phenacetin in chloroform); λ = wavelength (nanometers).*

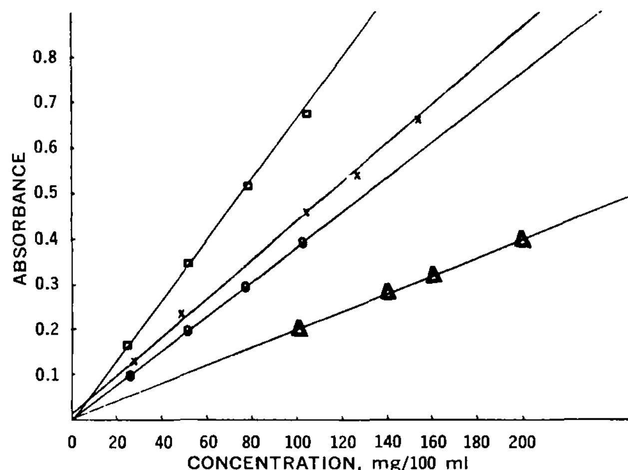

Figure 5—*Beer's law plots for individual carisoprodol, phenacetin, and caffeine standard solutions. Key: □, carisoprodol at 2910 nm in chloroform; O, carisoprodol at 2820 nm in chloroform; X, phenacetin at 2910 nm in chloroform; and Δ, caffeine at 3390 nm in carbon tetrachloride.*

The need for the separation of caffeine from carisoprodol can be understood by studying the IR-absorbing properties of both molecules. The near-IR spectrum of caffeine in the 3500–3200-nm region is characterized by a strong intensity absorption maximum at 3390 nm, attributable to the asymmetric CH-stretching mode of its three methyl groups, and a weaker maximum at 3340 nm, attributable to the corresponding symmetric CH-stretching mode (21). The carisoprodol molecule, incorporating four methyl groups and four CH_2 groups, absorbs strongly at three contiguous wavelengths (3390, 3420, and 3500 nm) due to asymmetric CH-stretching and moderately at 3340 nm due to symmetric CH-stretching. These spectral similarities and, more important, the much greater absorption intensities of the carisoprodol demonstrate the need for an isolation procedure for caffeine.

A series of caffeine standard solutions in carbon tetrachloride was studied spectrophotometrically, and the Beer's law plot exhibited linearity (Fig. 5, absorptivity 0.193). The data in Table II obtained from the analysis of spiked solutions, which shows an excellent solute recovery in terms of the mean and a relative standard deviation of 0.74%, verify the validity of the quantitative procedure for caffeine.

Four different commercial lots of tablets were assayed for carisoprodol, phenacetin, and caffeine. The results (Table III) indicate that the near-IR procedures described are reproducible with standard deviations of 1.38, 1.40, and 1.07 mg for carisoprodol, phenacetin, and caffeine, respectively. Since each sample lot is a true independent sample, the uncertainties experienced reflect both the variations inherent in the manufacturing process and the statistical perturbations possessed by the proposed near-IR spectrophotometric procedure. When the recovery data in Tables I and II are compared with those of Table III, the agreement obtained is reasonable. The results presented in Table III are considered satisfactory.

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Colorimetric Analysis of Procaine Hydrochloride

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Abstract □ A colorimetric assay procedure was developed for the quantitative analysis of procaine hydrochloride. The method is based on the interaction of procaine hydrochloride with *p*-dimethylaminocinnamaldehyde in the presence of trichloroacetic acid in absolute methanol to form a red Schiff base which can be quantitated spectrophotometrically at 547.5 nm. The Beer-Lambert law was adhered to over the 0.1–7-μg/ml range. Best accuracy was attained for solutions containing 0.4–4 μg/ml. The color was stable for at least 70 min. The method was applied to procaine hydrochloride injections, both with and without epinephrine, without prior separation of the drug. The results were comparable to those obtained by the official procedures.

Keyphrases □ Procaine hydrochloride—colorimetric analysis using *p*-dimethylaminocinnamaldehyde □ *p*-Dimethylaminocinnamaldehyde—colorimetric reagent for determination of procaine hydrochloride □ Colorimetry—determination, procaine hydrochloride, using *p*-dimethylaminocinnamaldehyde

The USP XVIII (1) assay procedure for procaine hydrochloride is time consuming, since it is based on a diazotization titration of the compound with standard sodium nitrite at low temperatures using starch iodide paper as the external indicator. This method is subject to variations between individuals in terms of determining the end-point. The official assay method (2) for procaine hydrochloride injection is also lengthy, since a prior isolation of the compound by solvent extraction is required before the compound can be determined. These reasons prompted this laboratory to develop an assay method offering