Simultaneous Multicomponent Drug Determinations with a Vidicon Spectrometer

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Received July 8, 1977, from the *Department of Chemistry, Purdue University, West Lafayette, IN 47907.* Accepted for publication October 6, 1977.

Abstract □ A vidicon spectrometer, useful for absorption spectrophotometry over the 210–800-nm spectral region and capable of scanning a portion of this region at repetition rates of 250 scans/sec, was applied for the simultaneous determination of multicomponent drug formulations without a separation. Results are reported for two drug preparations containing two and four active components. The average of ratios of determined to expected values for the six active components contained in both formulations was 99.2% based on the specified tablet contents. Relative standard errors for the analyses were less than 3%. Other potential applications of the vidicon spectrometer are discussed briefly.

Keyphrases □ Vidicon spectrometer—used in simultaneous determination of multicomponent drug formulations without prior separation □ Spectrophotometry—use of vidicon spectrometer in simultaneous determination of multicomponent drug formulations without prior separation

UV–visible spectrophotometry and spectrofluorometry are used extensively in the pharmaceutical sciences (1). Various applications were reported recently, including quantitative determinations of drugs, metabolites, and degradation products in several sample types; pharmacokinetic studies; determinations of equilibrium constants; determinations of enzyme activities; and separation methods such as extraction and chromatography. Thus, there is good reason to note instrumental developments that improve or extend the capabilities of the methods.

Instruments used currently either operate at a single wavelength or utilize relatively slow mechanical wavelength scanning systems. This report describes an alternative approach to spectrophotometric instrumentation, replacing the mechanical scanning systems with an all-electronic scanning system. The approach uses conventional dispersion optics and a vidicon or television camera tube detector. The detector is useful from about 210 nm to well above 800 nm and can be used to select one or a few wavelengths electronically or to scan segments of the UV–visible region with repetition rates as fast as 250 scans/sec. This paper discusses one type of application where this scanning capability is used to advantage and suggests others.

The analytical problem involves the simultaneous determination of two or more components in pharmaceutical preparations without a separation step. The method utilizes matrix equations to resolve multiwavelength data from the vidicon spectrometer into the concentrations of individual components in each sample. Of the two drug preparations used as illustrative examples, one[1] contained four active components and the other[2] contained two active components and one excipient that absorbed radiant energy in the spectral range of interest. Results are reported for both synthetic mixtures and processed dosage forms.

For the synthetic mixtures, the relative standard errors for individual components ranged from 0.8 to 3%; ratios of found *versus* expected values for the dosage forms ranged from 96 to 104% with an overall average of 99.2%.

INSTRUMENTATION

The word "vidicon" is a generic term that represents a group of devices used as television camera tubes and imaging detectors. The spectrometer developed in this laboratory utilizes a silicon target vidicon. Because the vidicon-based spectrometer is a relatively new development, the concepts and characteristics are discussed briefly so that potential advantages and limitations can be understood.

Figure 1 is a conceptual representation of the vidicon spectrometer, and Fig. 2A represents typical spectral data obtained with the instrument. These spectra were photographed from a display oscilloscope interfaced to a computer that records and processes the data. Each display represents the average of 1000 individual spectra recorded during 10 sec for each solution.

For absorption spectroscopy, polychromatic radiation from a suitable source is passed through the sample cell where different wavelengths, λ, are attenuated, depending on the spectral characteristics of the sample. The emerging radiation is then focused onto the entrance slit of a conventional monochromator. Suitable dispersion optics then separate the attenuated polychromatic radiation into a spectrum, which is focused in a plane usually occupied by the exit slit of the monochromator. In this application, the exit slit is removed and the active surface of the vidicon tube is located in the focal plane so that the different wavelengths illuminate different points along one axis of the active surface of the tube. Thus, a spectrum is displayed along the wavelength axis of the tube.

The circle at the right of the exit focal plane is intended to emphasize the fact that the active surface of the vidicon consists of a two-dimensional array of photodetectors. Although the silicon target vidicon may consist of up to 500,000 individual photodiodes, technical problems limit the number of independent electronic resolution elements to about 40,000, or about 200 along each axis. Therefore, the spectrum displayed across the active surface of the vidicon is resolved into some 200 spectral resolution elements.

The electronic scanning feature results from the fact that individual resolution elements are examined by an electron beam, which is scanned electronically across the surface in a manner analogous to a cathode ray tube. When the electron beam strikes a resolution element, it produces an electric charge proportional to the integrated light intensity on that element since it was last examined. Thus, as the beam moves across the surface, it produces an output signal profile representative of the intensity *versus* wavelength pattern, which is the intensity spectrum. Since the electron beam can be scanned very rapidly across the active surface, the scan rate is limited to the detector response rate; for the silicon target vidicon, this scan rate is limited to slower than 1000 scans/sec for the highest reliability.

While it is not clear from this diagram, an image of the entrance slit for each resolution element is developed along the vertical axis of the detector. Thus, many photodiodes along the vertical axis respond to the same wavelength. The scanning format is such that the signal for each wavelength resolution element represents an average of several hundred detectors along the vertical axis for that wavelength. This feature and the integrating character of the silicon photodiodes contribute signal averaging to each data point.

More complete details of the system were reported elsewhere (2, 3), and only the more salient features are summarized here. The silicon target vidicon has a useful spectral range from about 210 nm to beyond 800 nm; selected segments from this range can be scanned with repetition rates up to 250 scans/sec, with intensity data being recorded at up to 200

[1] Excedrin, Bristol-Myers Co., New York, N.Y.
[2] Fedahist, Dooner Laboratories, Haverhill, Mass.

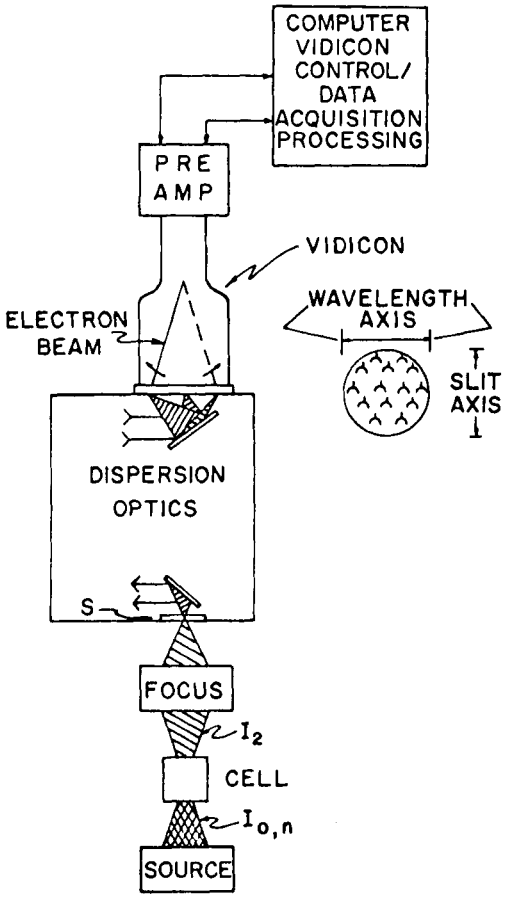

Figure 1—*Schematic representation of vidicon spectrometer.*

wavelengths during each scan. The spectrometer exhibits an uncertainty of about 0.001 absorbance unit throughout this range and has a linear dynamic range of at least four orders of magnitude of intensity (3). Because the spectral scans are performed electronically and involve no mechanical motion, wavelength resolution elements are selected very reproducibly. This latter feature is important for signal averaging applications and for the multiwavelength applications discussed.

The system has some potential limitations. Because there is only a finite number of resolution elements, the user must exercise a tradeoff between spectral resolution and spectral range. Furthermore, the silicon target vidicon is a unity gain detector and does not have the spectral sensitivity of a photomultiplier. Finally, because the sample is illuminated with polychromatic radiation, any tendency toward photodecomposition or fluorescence could represent more severe problems in this type of instrument than in conventional spectrophotometers where the sample is illuminated with monochromatic radiation attenuated by the monochromator.

All of these limitations are real, but none has proven serious. Photodecomposition has been a problem only in some preliminary work with a reaction involving sodium nitroprusside.

EXPERIMENTAL

Reagents and Standards—All solutions were prepared in distilled water that had been further purified by being passed through a cation–anion-exchange resin column.

Buffers—A pH 10, 0.1 *M* ammonia buffer was prepared by dissolving 4.08 g of ammonium chloride and 21.6 ml of concentrated ammonium hydroxide in water and diluting to 4 liters. A pH 4.7, 0.1 *M* acetate buffer was prepared by dissolving 16.4 g of sodium acetate and 11.4 ml of concentrated acetic acid in water and diluting to 4 liters.

Standards—A stock solution of each active ingredient in the four-component sample was prepared by dissolving 0.0408 g of salicylic acid[3], 0.0203 g of acetaminophen[4], 0.0208 g of caffeine[4], and 0.0402 g of salicylamide[4] in pH 10 ammonia buffer and diluting each solution to 100 ml with the same buffer. Standard solutions (4–40 mg of salicylic acid and salicylamide/liter and 2–20 mg of caffeine and acetaminophen/liter) were prepared by diluting appropriate amounts of stock solutions with ammonia buffer. The synthetic mixtures (M1–M7 in Table I) were prepared by diluting appropriate amounts of stock solutions to 100 ml with ammonia buffer.

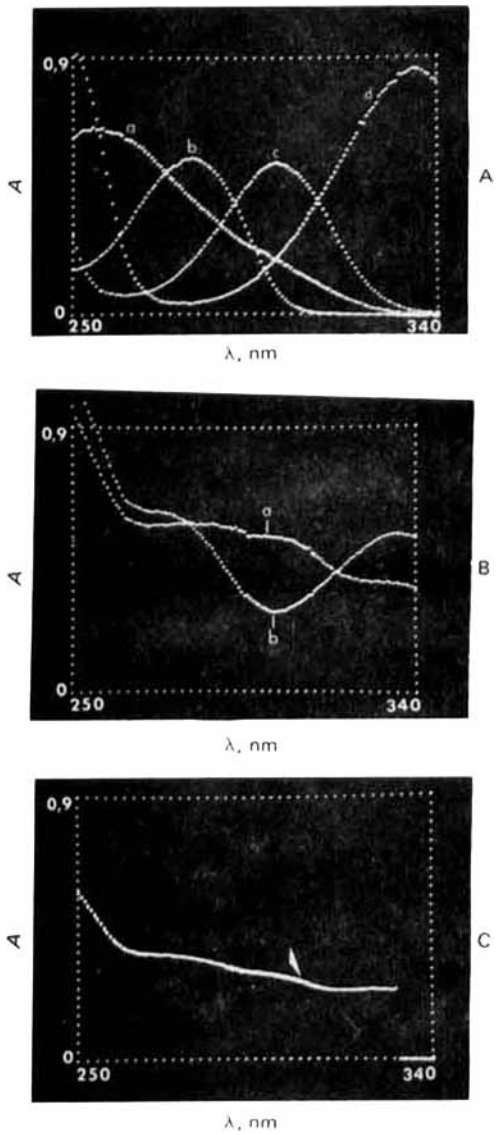

Figure 2—*Spectra for the four-component sample. A. Pure components: a, acetaminophen (10 mg/liter); b, caffeine (10 mg/liter); c, salicylic acid (20 mg/liter); and d, salicylamide (20 mg/liter). B. Synthetic mixtures containing 6, 4, 12, and 8 mg/liter (a) and 6, 6, 0, and 12 mg/liter (b), respectively, of the four components in A. C. Superimposed spectra of a tablet extract and a mixture of 4.0, 2.8, 6.1, and 5.3 mg/liter, respectively, of the four components in A.*

A stock solution of each active ingredient in the two-component sample was prepared by dissolving 0.0320 g of chlorpheniramine maleate[5] and 0.4813 g of pseudoephedrine hydrochloride[4] in pH 4.7 acetate buffer and diluting to 100 ml with the same buffer. Standard solutions containing 3–22 mg of chlorpheniramine maleate/liter and 48–340 mg of pseudoephedrine hydrochloride/liter were prepared by diluting appropriate amounts of each stock solution with acetate buffer. The synthetic mixtures (M8–M16 in Table I) were prepared by diluting appropriate amounts of stock solutions to 100 ml with acetate buffer.

Procedure—Each four-component tablet was crushed and equilibrated with ammonia buffer for 10–15 min, after which the supernate

[3] J. T. Baker Co., Phillipsburg, N.J.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Dow Chemical Co., Indianapolis, Ind.

Table I—Synthetic Mixtures of Pure Components Analyzed by the Matrix Method

Mixture	Concentration, mg/liter			
	Salicylic Acid	Caffeine	Salicylamide	Acetaminophen
M1	12	4	8	6
M2	12	6	12	0
M3	0	6	12	6
M4	16	6	12	2
M5	8	12	8	4
M6	14	3	8	6
M7	8	4	0	8
	Pseudoephedrine Hydrochloride		Chlorpheniramine Maleate	
M8	0		16.0	
M9	241		0.0	
M10	241		16.0	
M11	289		3.2	
M12	144		6.4	
M13	337		9.6	
M14	48.1		12.8	
M15	193		19.2	
M16	96.3		22.4	

was removed by filtration and diluted to 250 ml with ammonia buffer. Then 1.00 ml of the diluted solution was again diluted to 100 ml with ammonia buffer, and this latter solution was used in the measurement step.

Each two-component tablet was crushed and equilibrated for 10–15 min with acetate buffer, after which the supernate was removed by filtration and diluted to 250 ml with acetate buffer. This diluted solution was used in the measurement step.

Because the vidicon-based spectrometer is a single-beam instrument, each intensity measurement must be corrected for dark current. This step is done for each set of runs by storing a dark current reading for each resolution element in the computer memory and then subtracting that dark current value from the 100% T and sample intensities at each wavelength before computing percent T and/or absorbance.

A scan repetition rate of 100 scans/sec was used. For each standard, synthetic mixture, and real sample, 1000 spectral scans were averaged. The average absorbances at selected wavelengths were used to establish absorptivity matrixes from standards and to compute concentrations in synthetic mixtures and real samples. For the two-component sample, five standard solutions containing known amounts of each of the pure components were used, and absorbance data at 15 approximately equally spaced wavelengths between 250 and 280 nm were used to establish the absorptivity matrix. For the four-component sample, six standards and 16 approximately equally spaced wavelengths between 250 and 340 nm were used to establish the absorptivity matrix. Absorbance data for each synthetic mixture and sample at the same wavelengths for which absorptivity data were evaluated were used to compute sample composition.

Mathematical Formulation—The computational procedure was presented previously (4) and will be discussed only briefly. Beer's law is used in matrix form:

$$A = KC \qquad \text{(Eq. 1}a\text{)}$$

where A is a k by m matrix of absorbances of m mixtures at k analytical wavelengths, K is a k by n matrix of absorptivities of n components at k analytical wavelengths, and C is an n by m matrix of concentrations of n components in each m mixture. The absorptivity matrix is evaluated as:

$$K = AC^T(CC^T)^{-1} \qquad \text{(Eq. 1}b\text{)}$$

where C^T is the transpose matrix of C. When the absorptivity matrix has been determined, unknown concentrations are computed as:

$$C_u = QA_u \qquad \text{(Eq. 2)}$$

where C_u is a column matrix of concentrations of each n component, $Q = (K^TK)^{-1}K^T$ is an n by k matrix, A_u is a column matrix of absorbances of the unknown at the k analytical positions, and K^T represents the transpose of the absorptivity matrix.

This calculation gives the least-squares multiple regression fit of the component spectral curves to the data. The net result of the calculations is a set of concentrations of the n components in the sample mixture that gives the best fit to the spectral data.

RESULTS AND DISCUSSION

Results are reported for two pharmaceutical preparations. In each case, analyses were performed both on synthetic mixtures prepared from pure components and on tablets treated as described. The synthetic mixture data are included to give some indication of the dynamic range of the method and to provide statistical data against which results for real samples can be compared.

All uncertainties are reported at ±1 *SD*.

Four-Component Sample—The four active components were: acetaminophen, 97 mg; caffeine, 65 mg; aspirin, 195 mg; and salicylamide, 130 mg. Synthetic mixtures and tablets were processed as described and treated as four-component mixtures.

Pure Components and Synthetic Mixtures—At pH 10, aspirin is converted to salicylic acid and is treated as such in this discussion. Curves a–d in Fig. 2A represent the absorption spectra of acetaminophen (10 mg/liter), caffeine (10 mg/liter), salicylic acid (20 mg/liter), and salicylamide (20 mg/liter). All spectra overlapped, but there were significant differences among them.

Figure 2B represents spectra for two different mixtures of three or more of these components. The lower absorbance of curve a near 340 nm is a clear indication that there was less salicylamide in this solution than in that represented by curve b. On the other hand, the dip in curve b is a clear indication that there was less salicylic acid in the solution represented by curve b than in that represented by curve a. These differences in shape are utilized by the matrix equations to resolve mixture spectra into the compositions of individual components.

Figure 2C represents the overlapped spectra of a solution of the soluble components from a tablet and a standard mixture containing the nominal amount of each component in the tablet. As indicated earlier, the matrix program resolves the unknown spectrum into the amounts of the four components that give the best fit to the spectrum.

Six synthetic mixtures were analyzed by the proposed procedure. Regression equations (found *versus* added) were $y = 0.98x + 0.07$ mg/liter for acetaminophen, $y = 1.01x - 0.04$ mg/liter for caffeine, $y = 1.0x + 0.03$ mg/liter for salicylic acid, and $y = 1.01x - 0.03$ mg/liter for salicylamide. More complete statistical data are presented in Table II. The correlation coefficients suggest good linearity, and the slopes and intercepts suggest good agreement between computed and added values.

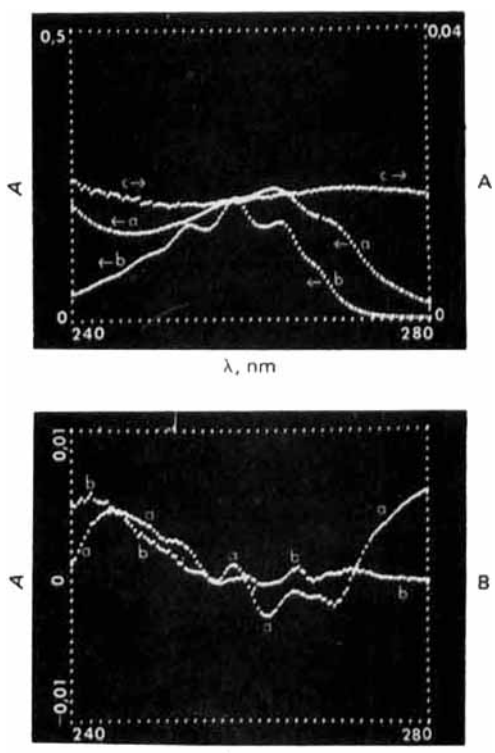

Figure 3—*Spectral data for the two-component sample. A. Spectra of pure components: a, chlorpheniramine maleate (16.0 mg/liter); b, pseudoephedrine hydrochloride (241 mg/liter); and c, lactose. B. Tablet regression residuals for two-component treatment (a) and three-component treatment (b).*

Table II—Statistics for the Regression of Found *versus* Added Concentrations of Drugs in Synthetic Mixtures

Drug	Slope ± *SD*	Intercept ± *SD*, mg/liter	*SE* of Estimate, mg/liter	Correlation Coefficient
Acetaminophen	0.980 ± 0.019	0.07 ± 0.05	0.106	0.9974
Caffeine	0.998 ± 0.008	−0.04 ± 0.03	0.076	0.9995
Salicylic acid	0.998 ± 0.010	0.03 ± 0.05	0.101	0.9994
Salicylamide	1.014 ± 0.004	−0.03 ± 0.02	0.003	0.9999
	Two-Component Treatment			
Chlorpheniramine maleate	1.014 ± 0.004	−0.10 ± 0.06	0.09	0.9999
Pseudoephedrine hydrochloride	0.990 ± 0.007	0.03 ± 1.54	2.39	0.9996
	Three-Component Treatment			
Chlorpheniramine maleate	1.005 ± 0.007	0.12 ± 0.09	0.14	0.9997
Pseudoephedrine hydrochloride	0.977 ± 0.007	1.35 ± 1.5	2.39	0.9996

Tablets—The dilution factor used for the tablets corresponds to nominal concentrations of 3.9, 2.6, 6.0, and 5.2 mg/liter for acetaminophen, caffeine, salicylic acid, and salicylamide, respectively. Nine individual tablets processed *via* the proposed procedure yielded average values of 3.8 ± 0.2, 2.5 ± 0.1, 6.1 ± 0.4, and 5.2 ± 0.3 mg/liter for the four components, respectively. The differences between expected and found results compared favorably with the standard estimates of error reported in Table II for the synthetic mixtures. Additional information related to these data are summarized in Table III.

Two-Component Sample—The two-component sample contained chlorpheniramine maleate, 4 mg, and pseudoephedrine hydrochloride, 60 mg. Results are reported for synthetic mixtures and processed tablets, treated as two- and three-component mixtures. The third component was a water-soluble excipient (lactose) having measurable absorbance.

Pure Components and Synthetic Mixtures—Curves a and b in Fig. 3A represent absorbance spectra of 16 mg of chlorpheniramine maleate/liter and 241 mg of pseudoephedrine hydrochloride/liter in pH 4.7 buffer. There is no wavelength at which both components can be determined independently of the other. The multiwavelength method was applied to eight mixtures containing known amounts of each component. Regression equations (found *versus* added) were $y = 1.014x - 0.10$ mg/liter for chlorpheniramine maleate and $y = 0.99x + 0.03$ mg/liter for pseudoephedrine hydrochloride. More complete statistical data are included in Table II. The correlation coefficients, slopes, and intercepts suggest good linearity and good agreement between expected and found values.

Tablets—When individual tablets are dissolved and diluted to 250 ml with buffer, they should yield nominal concentrations of 16 mg of chlorpheniramine maleate/liter and 240 mg of pseudoephedrine hydrochloride/liter. When eight tablets were prepared and treated as two-component samples, the average results obtained were 17.9 and 217 mg/liter, respectively, for the two components, corresponding to so-called recoveries of 112 and 90.5%. These results are not satisfactory for this determination.

The reason for the problem can be illustrated with curve a in Fig. 3B. This curve represents the residuals (or difference spectrum) between the average of the spectra for the tablets and the absorbance spectrum predicted by the matrix program. The wavelength range from 250 to 280 nm is the range fit by the matrix equation. The residual plot deviates from zero by as much as 0.005 absorbance unit near 280 nm, suggesting that the matrix program has not been able to fit the data to within the uncertainty limits of 0.001 absorbance unit imposed by the instrument.

These data suggested another absorbing species, which was not being accounted for. Subsequent inquiries identified an excipient in the sample as lactose. Curve c in Fig. 3A represents the absorption spectrum of lactose (note expanded scale). The important feature of this spectrum is the relatively high absorbance near 280 nm where absorbances for both active components are lowest. With this information, the tablets were then treated as three-component unknowns including lactose; values of 16.7 and 230 mg/liter were obtained and compared to expected values of 16 and 240 mg/liter, respectively. Curve b in Fig. 3B represents the residuals obtained from this fit of the data. Between 250 and 280 nm, the fit was close to the 0.001 absorbance unit uncertainty of the instrument.

Table III—Analyses of Tablet Preparations

Component	Concentration, mg/liter Expected	Found (*SD*)	Ratio × 100 (Found/Expected)
	Four-Component Tablets		
Acetaminophen	3.9	3.8 (0.2)	97.4
Caffeine	2.6	2.5 (0.1)	96.2
Salicylic acid	6.0	6.1 (0.4)	101.6
Salicylamide	5.2	5.2 (0.3)	100.0
		Average	98.8
	Two-Component Tablets		
Chlorpheniramine maleate	16	16.7 (0.8)	104
Pseudoephedrine hydrochloride	240	230 (1.7)	96
		Average	100

The data for the active components based on three-component treatment are summarized in Table III. When the data for the synthetic mixtures were treated by taking into consideration the possible interference, the regression equations of found *versus* added drugs were $y = 1.005x + 0.12$ mg/liter for chlorpheniramine maleate and $y = 0.98x + 1.35$ mg/liter for pseudoephedrine hydrochloride. More complete statistics are included in Table II.

Although the three-component treatment is necessary to account for the absorbing excipient in the two-component tablets, this treatment might be expected to degrade the results of synthetic mixtures because none of the excipient was added to these samples. That this does occur is apparent from the statistical data presented in Table II for the two- and three-component treatments.

Applications of a *t*-test with the standard deviations of slopes and intercepts for synthetic mixtures in Table II indicated that none of the slopes was statistically different from unity and that none of the intercepts was statistically different from zero at the 95% confidence level. The standard error of estimate is an important statistic because it indicates by how much the experimental values can be expected to deviate from the least-squares line for the data. The 95% confidence intervals were 0.27, 0.20, 0.26, and 0.085 mg/liter for acetaminophen, caffeine, salicylic acid, and salicylamide, respectively, and 0.33 and 5.6 mg/liter for chlorpheniramine maleate and pseudoephedrine hydrochloride, respectively, by the three-component treatment.

Applications of a *t*-test with standard deviations for tablets in Table III indicated that "found" results for all components except psuedoephedrine hydrochloride were within the 95% confidence limits. The 95% confidence limits for pseudoephedrine hydrochloride ($n = 8$) were ±4.0 mg/liter, and the difference between expected and found values exceeded this range. This apparent discrepancy cannot be explained at this time.

While comparison data for the components determined in this work are not available, the agreement between expected and found values for both the two- and four-component samples is comparable to results reported recently for other two-component mixtures analyzed with the aid of a liquid chromatographic separation prior to the measurement step (5). The uncertainties that range between 1 and 4% are consistent with those generally expected when more involved separation methods are applied to real samples.

Projections—This work demonstrated one type of application in which the rapid scanning capability of a vidicon detector can be used advantageously. This same instrument system has been used effectively as a multiwavelength detector for liquid chromatography (6), offering several advantages in comparison with single-wavelength or mechanically scanned detectors. Other workers performed multicomponent determinations with other array detectors used for derivative spectroscopy (7) and as fluorescence detectors (8). All of these and other possible applications exhibit real potential for pharmaceutical analyses.

REFERENCES

(1) C. A. Janicki, R. K. Gilpin, E. S. Moyer, R. H. Almond, Jr., and R. H. Erlich, *Anal. Chem.*, **49**, 110R (1977).

(2) M. J. Milano, H. L. Pardue, T. E. Cook, R. E. Santini, D. W. Margerum, and J. M. T. Raycheba, *ibid.*, **46**, 374 (1974).
(3) M. J. Milano and H. L. Pardue, *ibid.*, **47**, 25 (1975).
(4) J. Sustek, *ibid.*, **46**, 1676 (1974).
(5) R. M. Rocco, D. C. Abbott, R. W. Giese, and B. L. Karger, *Clin. Chem.*, **23**, 705 (1977).
(6) A. E. McDowell and H. L. Pardue, *Anal. Chem.*, **49**, 1171 (1977).
(7) M. J. Milano and K. Kim, *ibid.*, **49**, 555 (1977).
(8) I. M. Warner, J. B. Callis, E. R. Davidson, and G. D. Christian, *Clin. Chem.*, **22**, 1483 (1976).

ACKNOWLEDGMENTS

Supported in part by Research Grant GM 13326-11 from the National Institutes of Health.

The authors thank Mr. Jim Audibert, Dooner Laboratories, for supplying information about Fedahist tablets.

Fluorometric TLC Determination of Free and Conjugated Propranolol, Naphthoxylactic Acid, and *p*-Hydroxypropranolol in Human Plasma and Urine

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Received July 29, 1977, from the *Biopharmacy Laboratory, Pharmacy Research and Development Division, Ayerst Laboratories, Inc., Rouses Point, NY 12979.* Accepted for publication October 13, 1977.

Abstract □ Sensitive, specific, and reproducible TLC methods are described for the determination of propranolol and its major metabolites in humans, conjugated propranolol, free and conjugated naphthoxylactic acid, and free and conjugated *p*-hydroxypropranolol. The drug or metabolites are extracted from plasma or urine with ether and applied to TLC plates of silica gel or microcrystalline cellulose. After development, the plates are scanned in a spectrodensitometer equipped to measure fluorescence in the UV and blue regions of the light spectrum. Quantitation is achieved by comparing the areas under the peaks obtained from the unknowns to those obtained from standards applied to the same plate. Limits of quantitation in plasma are: free propranolol, 2 ng/ml; free *p*-hydroxypropranolol, 10 ng/ml; conjugated propranolol, 15 ng/ml; total (free and conjugated) naphthoxylactic acid, 25 ng/ml; and conjugated *p*-hydroxypropranolol, 50 ng/ml. These methods were used to obtain plasma level data in a volunteer after one single dose of propranolol and in patients under propranolol therapy. The R_f values of some known metabolites of propranolol obtained in various TLC developing systems are also presented.

Keyphrases □ Propranolol—and major metabolites, fluorometric TLC analyses in human plasma and urine □ Fluorometric TLC—analyses, propranolol and major metabolites in human plasma and urine □ TLC fluorometry—analyses, propranolol and major metabolites in human plasma and urine □ Cardiac depressants—propranolol and major metabolites, fluorometric TLC analyses in human plasma and urine

In addition to β-adrenergic blockade, propranolol (I) has been reported to have antihypertensive, antianxiety, anticonvulsant, and antianginal effects (1–5). Over 95% of an orally administered dose of propranolol is metabolized to several different substances in humans (6–11). The major metabolites in humans were identified as conjugated propranolol (II), *p*-hydroxypropranolol (III), and naphthoxylactic acid (IV) in their free and conjugated forms.

GLC methods were reported for propranolol and *p*-hydroxypropranolol in biological fluids (12–14). For the routine analysis of unchanged propranolol, wet fluorometric procedures are commonly used (15–18). Recently, a high-pressure liquid chromatographic (HPLC) method was reported (19). The present report describes simple, sensitive, and highly specific methods to assay I, II, and free and conjugated III and IV in human plasma and urine.

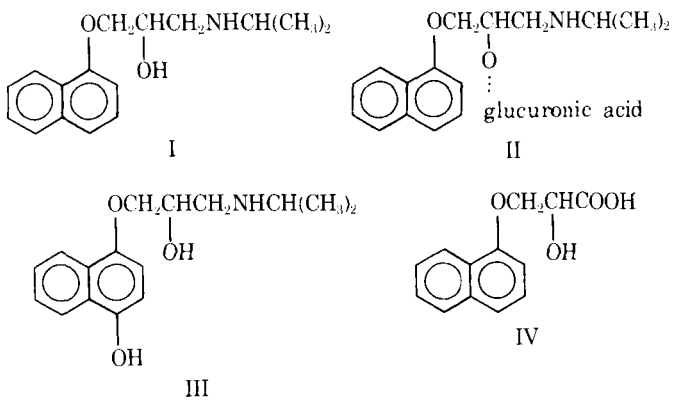
EXPERIMENTAL

Materials—All reagents and solvents were analytical reagent grade. TLC plates of silica gel or microcrystalline cellulose, 10 × 20 cm, were used. The plates were divided into 10 equal channels, 1 cm wide, with a scoring device[1]. Samples were applied to the plates with 25- or 50-μl microdispensers[2].

Acetate buffer, 0.1 *M*, pH 4.7, was prepared by dissolving 0.37 g of sodium acetate in 100 ml of distilled water and adjusting to pH 4.7 with acetic acid. Enzyme solutions[3] were prepared in distilled water. Standard solutions of I hydrochloride[4], III hydrochloride[4], and IV[4] were prepared in methanol. Ether[5], refluxed for 30 min in the presence of lithium aluminum hydride and distilled in an all-glass apparatus, was used for extracting III.

Determination of Free Propranolol—Human plasma or urine, 5 ml, was made basic with 1.0 ml of 1 *N* NaOH and shaken for 15 min with 40 ml of ether. After centrifugation, the organic phase was transferred and dried with 12 g of sodium sulfate. A 25-ml aliquot of the ether extract was transferred to a clean conical tube and evaporated under nitrogen[6] at 45°. The inside of the tube was washed with 1 ml of ethanol, and the solution was evaporated to dryness.

The dry residue was dissolved in 100 μl of ethanol, and aliquots of 25 μl were applied to a prescored TLC plate of silica gel 60[7]. Standard solutions of propranolol also were spotted on the same plate. The plate was



[1] SDA 320, Schoeffel Instrument Corp.
[2] Dialamatic, Drummond Scientific Co.
[3] Glusulase, Endo Laboratories.
[4] Imperial Chemical Industries, Great Britain.
[5] Mallinckrodt, Fisher, or Matheson, Coleman and Bell.
[6] N-Evap, Organomation Associates.
[7] E. Merck.