Derivative and Graphical Procedures for Correction of Irrelevant UV Spectrophotometric Absorption in Changeable Matrixes

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Abstract □ The application of direct zero-order UV spectrophotometric and graphical or derivative background correction methods to selected pharmaceutical preparations shows their relative advantages in different situations. The assay of the active components in a changeable matrix is a problem with formulations having a limited shelflife. Although the standard three-point correction procedures can provide accurate data, there are practical problems if the irrelevant absorption band is such that only the derivative approach gives enough resolving power for reliable qualitative and quantitative determinations.

Keyphrases □ Excipients—solutions, suspensions, and suppositories, changeable matrixes and their effect on UV absorbance spectra, comparison of three background correction methods □ Spectroscopy, UV—changeable matrixes of pharmaceutical preparations, comparison of three background correction methods, interference by excipients or degradation products □ Degradation—pharmaceutical preparations, effect on UV absorbance spectra, comparison of three background correction methods, changeable matrixes

The assay of certain drugs or natural products by classical UV spectroscopic techniques usually can be accomplished by simple and direct procedures. These procedures generally involve reading the absorbance of the sample *versus* that of a standard at selected wavelengths, except where the analyte is part of a complex matrix containing other UV-absorbing degradation products and chemicals (1). In such a situation, the spectral overlapping of the irrelevant absorption bands produced by the interfering components can be so strong as to mask completely the band of the compound, making it difficult or impossible to determine not only its concentration but also its presence. Since the irrelevant absorption of multicomponent samples cannot be suppressed efficiently due to the inherent lack of resolution of the classical UV spectroscopic techniques, its effects must be compensated for by often cumbersome sample cleanup and separation procedures. Thus, the convenience of the direct UV readings using a conventional spectrophotometer is lost.

BACKGROUND

The practical limitation derived from variable and nonspecific spectral interferences has been treated by mathematical and graphical methods, ranging from very simple to tedious and lengthy procedures, according to the shape of the irrelevant absorption spectrum (2). The Morton–Stubbs (3) three-point correction method has been used extensively where there is an unknown or variable contribution to the measured absorption. However, the method assumes that the irrelevant absorption is linear over the working wavelength range (4).

Background interferences also have been corrected by the application of absorbance ratios and orthogonal function coefficients (5, 6), which are sensitive to instrumental parameters and difficult to apply if the irrelevant background absorption is similar to the spectral band of the analyte (4). The method of Glenn (5) was applied and evaluated by Wahbi and Abdine (7), who, in spite of considering it useful in many cases, acknowledged that it may give unsatisfactory results if the polynomial, number of points, and wavelength range and intervals are not selected properly. Furthermore, many of these methods require special attention to operational parameters, which detracts from their applicability to many practical problems in pharmaceutical analyses.

On the other hand, the recently introduced commercial systems that can produce a graphic display of the derivative ($dA/d\lambda$ or $d^2A/d\lambda^2$) of the analog signal given by the spectrophotometer provide a different approach to these problems. In many cases, the derivative techniques allow resolution of two spectral bands of very close wavelength so they can be used in the quantitative spectrophotometric assay of complex mixtures of spectrally interfering substances (8).

The derivative spectroscopy technique was introduced (9) as a means of resolving two overlapping spectral bands of almost coincident wavelength. However, the potential of this technique for pharmaceutical analysis has not been studied in detail, although it could be easily inferred from the small, though significant, body of data reported by different laboratories. For instance, derivative recording of UV spectra was applied successfully to steroid structure determination (10) and to the quantitation of mutually interfering rare earth elements and dyes (11) and gases (12, 13). The quantitation of pyrene in the presence of excess anthracene by derivative luminescence also was reported (14), and more recently, the technique was used in biology to determine total urinary porphyrins (15) as well as phenylalanine residues in proteins (16).

A recent communication discussed second derivative UV–visible spectrophotometry for the quantitation of drugs in their dosage forms (17) and reported coefficients of variation of better than 2% for routine quality control. A general review of derivative and wavelength modulation spectroscopy appeared recently, which mentioned other selected applications (18). All of these data support the idea that derivative spectroscopy may have considerable potential in routine quantitative measurements (18, 19).

Thus, considering the present state of the art with regard to UV determinations of a complex matrix in pharmaceutical preparations, comparative assessment was desired for the applicability of simple, direct, graphical–algebraical and derivative UV measurements in cases where: (*a*) the background absorption of excipients or other products may not be constant due to manual batch-to-batch variations, and (*b*) the corresponding excipient absorbance is either strong (samples with large excipient concentrations) or weak compared to that of the substance assayed (samples with few excipients and a large amount of active drug). Four examples representative of the various cases usually encountered in pharmaceutical analysis were considered.

EXPERIMENTAL

Apparatus—A double-beam ratio recording, scanning, grating spectrophotometer[1] with a variable bandpass from 0.2 to 4.0 nm was used. This spectrophotometer was equipped with a derivative spectrum attachment[2] consisting of an analog differentiating circuit that can record optionally the first or second derivative spectrum and the original (zero-order) spectrum.

The spectra were obtained at a bandpass of 1 nm and a scanning speed of 240 nm/min. The recorder response was set at medium, and the scan speed was set to 12 cm/min at a span of 1 or 2 and a mode of 0.5.

Pharmaceutical Preparations—*Suppositories*—The active ingredients in the suppositories were: dipyrone (1 g), anisotropine methylbromide (0.020 g), and phenobarbital (0.015 g). The suppositories also contained 2.204 g of saturated fatty acid glycerides as excipients and had a total weight of 3.24 g.

Solutions—One solution contained 1.58 g of oxyfedrine hydrochloride (a sympathomimetic agent) and 113.2 g of excipients brought to a total volume of 150 ml with distilled water. Estradiol benzoate (10 mg/ml) was

[1] Perkin-Elmer model 200.
[2] Perkin-Elmer model 200-0628.

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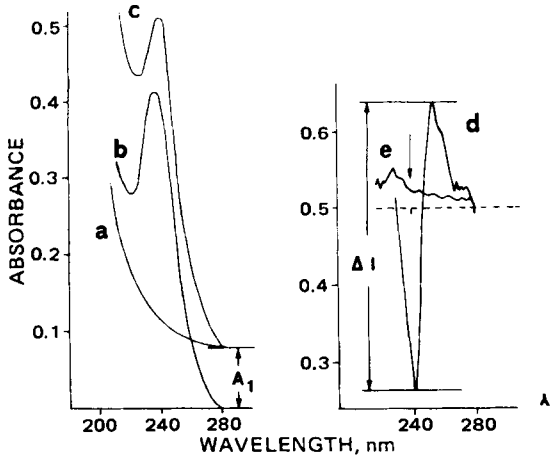

Figure 1—*Curve a, zero-order UV spectrum of a sample containing all of the pharmaceutical preparation components, except phenobarbital; curve b, zero-order UV spectrum of a phenobarbital standard; curve c, zero-order UV spectrum of an extract of the commercial formula; curve d, second derivative UV spectrum of the extract of the commercial formula; and curve e, second derivative UV spectrum of the extract of the excipients without phenobarbital.*

contained in a suspension of lecithin (8 mg/ml) and chlorobutanol (3 mg/ml). An excipient solution (100 ml) contained 0.2 g of progesterone.

All preparations except the progesterone-containing formula were commercial preparations. For the three solutions, the only sample preparation needed prior to the actual determinations was simple dilution to a predetermined volume with a suitable solvent. However, since phenobarbital was present in a ratio of 1 part to 216 parts of excipients, the suppositories first were subjected to a cleanup procedure to remove a significant proportion of the unwanted materials.

Cleanup Procedure for Phenobarbital in Suppositories—The sample to be assayed (three suppositories, equivalent to 45 mg of phenobarbital) was placed in a 250-ml erlenmeyer flask and dissolved in ~200 ml of ether. The solution was filtered[3], and the filtrate was collected in a 500-ml volumetric flask and taken to dryness on a rotary-film evaporator.

The dry residue was extracted with 200 ml of 10% NH_4OH in ethanol at ~50°. The filtrate was cooled in an ice bath under agitation, filtered[3] again under vacuum, and collected in a 250-ml flask. The residue on the filter paper was washed, and the filtrate was brought to volume with the ammonium hydroxide–ethanol solution. A 5-ml aliquot was diluted to 100 ml with ammonium hydroxide in ethanol to obtain a final phenobarbital concentration of 9 μg/ml.

RESULTS AND DISCUSSION

Phenobarbital in Suppositories—Standard solutions of phenobarbital and of the excipient mixture of the commercial preparations were run individually on the UV spectrophotometer and gave the absorption spectra shown in Fig. 1 (peaks a and b). The corresponding absorption spectrum of a commercial product extract (phenobarbital plus excipients) is also illustrated in Fig. 1 (peak c). In this case, the spectral band was displaced upward due to the background interference of the coextracted excipients, which was clearly reflected in the absorbance value (A_1) at 280 nm (Fig. 1, peak c). Quantitatively, this spectral interference significantly affected the direct measurement of the absorbance value at the peak maximum. Consequently, the relative error obtained by direct UV determinations of the known phenobarbital amounts added to the residual excipient matrix remaining after extraction of the suppositories was +14.3% (Table I).

In contrast, the peak-to-peak (ΔI) measurement of the second derivative spectra of the same samples (Fig. 1, peak d) reduced the relative error to −4.2%. Accordingly, the dispersion of these comparative data indicates that the applicability of the procedure is neither dependent on nor limited by the extraction efficiency of the active drug but instead by the method used to quantitate the UV absorbance curves. As will be demonstrated with other examples, this case is often true in the application of UV spectrophotometric techniques for the quantitation of pharmaceutical preparations.

[3] W-41 filter paper, Whatman Inc., Clifton, N.J.

Table I—Determination of Phenobarbital Content of Standard Solutions [a]

	Direct Reading of UV Absorbance	Second Derivative Readings (ΔI)
Average, %[b]	114.3	95.8
Relative error,	+14.3	−4.2
SD, %	4.0	0.6

[a] Heavy spectral interferences, due to relatively large quantities of excipients, were removed partially by preextraction of reconstituted samples (n = 5) lacking phenobarbital but prepared according to the manufacturing process. These excipient samples were extracted according to the procedure outlined in the text. A known amount of phenobarbital (equivalent to that contained in the commercial formulas) was next added to the extracts, which thus were certified to contain the specified amount of active drug. [b] n = 5.

Table II—Determination of Phenobarbital Content in Suppositories [a]

	UV Absorbance			
	Direct Reading		Second Derivative	
Sample	A_{242}	Found, mg	ΔI (n = 5)	Found, mg
1	0.486	17.81	9.33	14.99
2	0.454	16.63	8.97	14.43
3	0.455	16.67	8.76	14.10
4	0.457	16.74	8.74	14.07
5	0.506	18.54	9.75	15.66
6	0.454	16.63	8.59	13.83
$\bar{x}$		17.17		14.51
SD, %		4.73		4.75
Relative error, %		+14.46		−3.2

[a] Recently prepared commercial suppositories with a declared value of 15 mg/suppository.

The experimental data proved that phenobarbital was extracted efficiently from the suppositories in a 97% yield (average of five derivative determinations, *SD* 1.6%). Nevertheless, to discard the possibility of a loss due to faulty extraction or to any other unexplained causes, the active drug was added to the final ethanol solution of the extract, thus ensuring that each test sample contained exactly the specified amount of phenobarbital. By removing this possible sampling error, it was expected that the values obtained from the different spectroscopic measurements would allow for a true and representative assessment of the UV background correction methods.

An application of the direct UV reading and second derivative procedures (Fig. 1, peak d) to the control assay of suppositories randomly selected from a single recent batch gave the results shown in Table II. The relative errors from both methods represent the kind of data consistently obtained in this laboratory since the introduction of second derivative UV spectroscopic methods for routine control work on complex changeable matrixes. Moreover, the −3–−4% error of these second derivative determinations could be a consequence of the second derivative UV absorption band of the blank sample (no phenobarbital added) (Fig. 1, peak e).

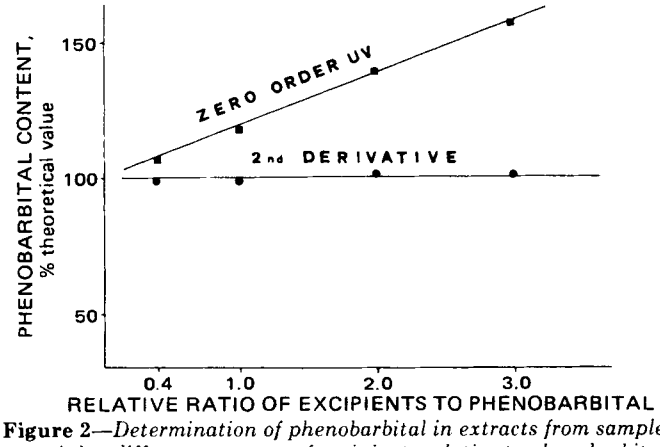

Figure 2—*Determination of phenobarbital in extracts from samples containing different amounts of excipients relative to phenobarbital.*

Table III—Comparison of Phenobarbital Content (Percent) in Aged Commercial Preparations by Different Methods

Sample	HPTLC	Direct UV[a]	Second Derivative UV	UV with Morton–Stubbs Three-Point Correction[a]
D-1 (1969)	79	108.0 (29)	79.3 (0.3)	82.5 (3.5)
D-2 (1969)	91.1	127.7 (36.6)	88.3 (−1.8)	90.7 (−0.4)
F-1 (1971)	94.7	115.9 (21.2)	94.8 (0.1)	—
F-2 (1971)	84.2	106.8 (22.6)	79.7 (−4.5)	82.3 (−1.9)

[a] The differences relative to the HPTLC determinations are given in parentheses (8).

The small positive signal at 242 nm (indicated by the arrow) has a subtracting effect on the total amplitude of the ΔI measured. These results are in agreement with the errors calculated theoretically for a similar case. According to the data reported previously (20) using the same peak-to-peak (ΔI) measurement of the second derivative band, theoretical systematic errors of −2–−4.3% and a random error of 1.8% were found, which compare favorably with the data in this report.

The potential of the derivative data for quantitation is demonstrated in Fig. 2, which shows that the increase in the excipient concentration relative to that of phenobarbital affected the zero-order UV determinations of this active drug with relative errors of up to 58%, without much effect on the derivative measurements.

Although the example given proves in principle the higher reliability of the derivative UV data, it also could be argued that a three-point Morton–Stubbs (3) background correction often still could compete favorably with the derivative procedures. In pharmaceutical product analysis, the procedure is applicable, providing that the excipients give a uniform background and can be used as a kind of "blank" (1). However, even in such a case, the irrelevant background absorption should be constant in each sample, which may be difficult to achieve due to the usual variations between excipient batches. This factor is especially important in aged commercial preparations that also contain undefined amounts of unknown degradation products. Furthermore, the operating conditions must remain perfectly reproducible. And the corresponding absorbance must be weak in comparison to that of the active drug (1), a condition that often cannot be met without lengthy concentration or cleanup procedures (4), as was done in this study to increase the ratio of phenobarbital to excipients (initially, 1:216).

For these reasons, the results of the direct, derivative, and three-point UV methods were compared for the quantitative determination of phenobarbital in aged commercial preparations. These samples were considered to be representative of cases in which the background absorption changes with time due to the formation of unknown degradation products (*i.e.*, no reference samples available as "blanks"). The results (Table III) demonstrate that when a derivative UV spectrophotometer is unavailable, the Morton–Stubbs correction provides comparable data; in this case, the amount of phenobarbital remaining undegraded in the four samples was determined by separating it from the excipients and degradation products using high-performance TLC (HPTLC). These samples then were quantitated spectrophotometrically (8).

Oxyfedrine Hydrochloride Solution—The UV absorption spectrum of a standard oxyfedrine solution as the hydrochloride (Fig. 3a) showed two maxima in the 350–220 range, one centered at 310 nm and the other at 252 nm. The absorption spectrum of the excipient mixture was characterized by a peak at 270 nm with an appreciable shoulder at 310 nm (Fig. 3b). This comparison represents an interesting practical case since the difference in absorption maxima of the active drug and the excipient blank (252 *versus* 270 nm) was such that the inflection point in the composite spectrum (at the position indicated by the arrow in Fig. 3c) in many cases was obliterated completely. The result was a continuous straight slope that precluded any meaningful qualitative or quantitative work without isolation of the active product from the excipient mixture or the application of background correction methods.

Furthermore, the inapplicability of the uncorrected measurements for quantitative purposes is demonstrated in the plot of Fig. 4, where even at a low ratio of excipients to oxyfedrine (0.5), the zero-order data included a 20% error. This situation deteriorated further as the matrix changed due to increased concentrations of those excipients responsible for the irrelevant background absorption relative to the concentration of the active drug. As shown, at a relative ratio of two, the uncorrected error was 70% relative to the true 100% value. The corresponding values obtained with UV derivative techniques or the Morton–Stubbs three-point correction (Fig. 4) stayed within a 12% margin of error. A good

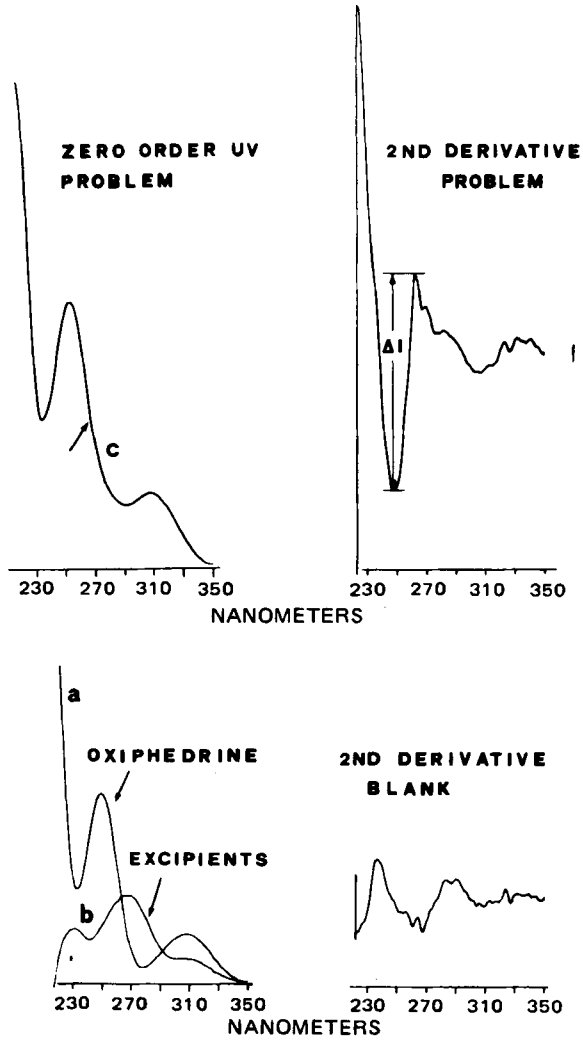

Figure 3—*Zero-order and second derivative UV spectra of oxyfedrine-containing formulation. Key: a, UV spectrum of oxyfedrine alone; b, UV spectrum of the excipients; and c, UV spectrum of the mixture.*

correlation between both correction methods thus is illustrated by this practical application. However, as the next two examples indicate, this case is not always true.

Progesterone in Solution—Figure 5 shows the results obtained with a noncommercial progesterone-containing product according to the zero-order and second derivative UV spectroscopic methods. The UV determination of progesterone alone does not pose any special difficulties. However, in these mixtures, it gave a spectrum in which the absorbance

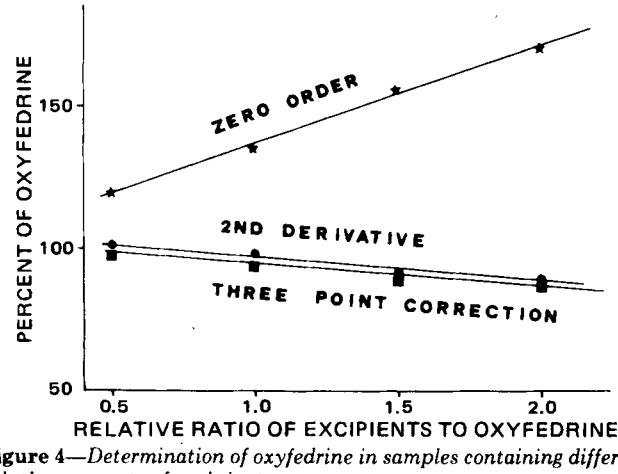

Figure 4—*Determination of oxyfedrine in samples containing different relative amounts of excipients.*

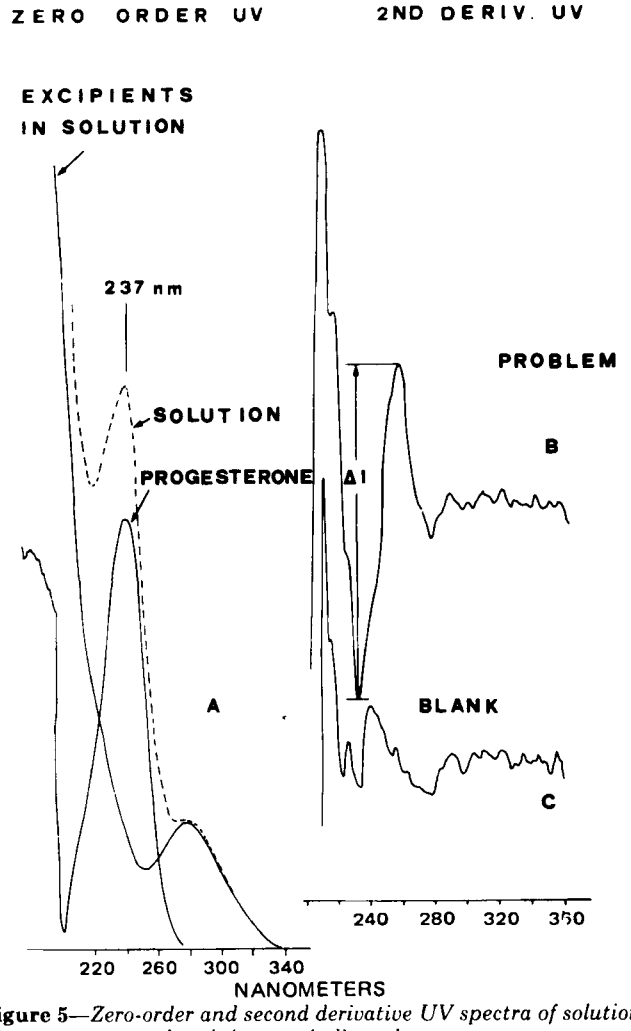

Figure 5—*Zero-order and second derivative UV spectra of solutions of progesterone and excipients as indicated.*

band with its characteristic 237-nm maximum became a shoulder. On the first determinations carried out on this preparation, it was observed that at a progesterone concentration of 10 μg/ml, corresponding to a progesterone to excipients ratio of one, the direct zero-order UV measurements produced relative errors of +37 and 45% for replicate sample determinations while the second derivative and three-point correction methods gave errors of −5.4 and +11.9%, respectively. In contrast with the previous two cases, the relative error calculated by the three-point method was significantly higher than the value computed from the second derivative (ΔI in Fig. 5). Therefore, it was necessary to analyze different solutions characterized by values of the progesterone to excipients below or above the usual ratio of one. The results obtained are plotted in Fig. 6. In this case, since the level of the irrelevant background absorption was kept constant while the amount of the active drug was increased, the points on the graph were distributed, as expected, along curves asymptotically approaching the theoretical value.

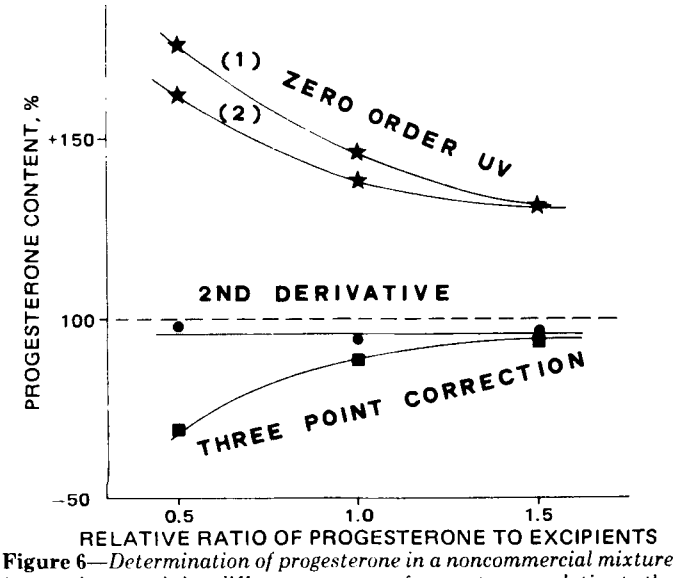

Figure 6—*Determination of progesterone in a noncommercial mixture in samples containing different amounts of progesterone relative to the excipients. The two zero-order UV curve determinations were carried out on different samples. As indicated in Table IV, the points of curve 2 are the average values obtained from the UV readings of two solutions from the same sample.*

Table IV—Replicate Determination of Progesterone in Samples with a Progesterone to Excipient Ratio of 1:2

Method	Solution A, %	Solution B, %
Direct UV	162.8	160.8
Derivative UV	99.0	98.2
Three-point correction	71.4 68.4	63.9
$\frac{A_{\lambda_{max}}}{A_{\lambda_1}} = \frac{A_{\lambda_{max}}}{A_{\lambda_2}}$	1.174 1.076	1.130

The results demonstrate that the only points not seriously affected by the changing interference due to the background absorption were those of the derivative determinations. Also, as expected, when the relative amount of the active drug in the preparation increased, the relative error given by the three-point correction approached the value obtained by derivative analysis, fulfilling the requirement that the background absorbance must be weak relative to that of the substance being determined (1). The points represented for the three-point correction curve in Fig. 6 are the average values of two determinations on the same solution carried out at different absorbance ratios, as indicated in Table IV (Solution A). Solution B (Table IV), determined at a different $A_{\lambda_{max}}/A_{\lambda_1}$ ratio, gave a similar value. From a consideration of the absorption spectrum, slightly different wavelengths were selected on each side of the peak maximum at 241 nm to give different absorbance ratios (Table IV). Nevertheless, the results were comparable.

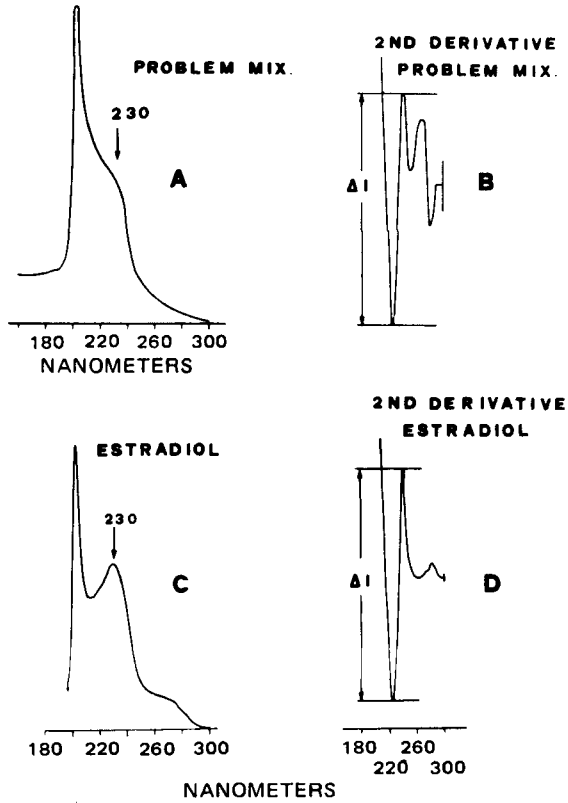

Figure 7—*Curve A, zero-order UV spectrum of an estradiol benzoate suspension in lecithin; curve B, second derivative UV spectrum of the same; curve C, UV spectrum of estradiol benzoate; and curve D, second derivative UV spectrum of estradiol benzoate.*

Table V—Estradiol Benzoate Determination in the Presence of Lecithin in Different Proportions

Method	Estradiol Benzoate–Lecithin Ratio		
	1:0.8	1:5	1:10
Direct UV reading	103	121.8	143.6
Derivative UV	101.2	105	108.8
Three-point correction[a]	96.8	111.3	115.0

[a] $A_{\lambda_{max}}/A_{\lambda_1}$ = 1152. Absorbances were read at 221.6, 231, and 237.6 nm for the three-point correction.

Estradiol Benzoate in Suspension—A final representative case where the derivative techniques demonstrated their superiority over other background-correction methods is illustrated by the assay of estradiol in a pharmaceutical suspension with lecithin. At the usual concentrations in the pharmaceutical preparation analyzed (1:0.8), the characteristic 230-nm spectrum of estradiol benzoate (Fig. 7C) was masked by the spectrum of the problem mixture (Fig. 7A). Under these conditions (*e.g.*, relatively high active drug to lecithin ratio), the three methods gave values that fit within a 3.5% margin of error, with the second derivative determination being the most accurate (Table V). However, when this ratio was diminished, the relative error increased proportionally, representing a case where the three-point correction cannot cope with the level of irrelevant background interference. Evidently, this method requires a cleanup procedure to remove as much of the interfering compound as possible, thus increasing the relative concentration ratio to more amenable values. The more simple approach is to cancel as much of this background effect as possible by the application of second derivative UV spectroscopic techniques.

Conclusions—An appraisal of the UV assay of active drugs in pharmaceutical preparations illustrates the kind of results that can be expected by application of graphical and derivative background correction procedures to samples whose background absorption characteristics preclude a direct reading of the UV spectra.

Depending on the ratio of the active drug to the rest of the components in a given formulation (excipients and degradation products), the direct quantitation of the uncorrected UV absorption spectra may lead to significant errors relative to the formulated values. These values, allowing for factors such as extraction efficiency whenever applicable, are well established by the formulation of the commercial product, except products with a limited shelflife.

By working with changeable matrixes (varying the active drug to excipient ratio), it also was shown that the derivative and three-point correction techniques provide comparable results in some cases. In general, both can compensate for the irrelevant UV absorption from the secondary sample ingredients, as long as it is within reasonable limits. However, the graphical Morton–Stubbs three-point correction procedure sometimes cannot cope with the level of interference, leaving the derivative spectroscopic procedure as the only method giving reliable results over a relatively wide span of drug to excipient ratios.

Likewise, the three-point or other related correction methods (3–7) are applicable qualitatively as long as there is an identifiable, though distorted, UV profile characteristic of the product assayed. On the other hand, there are situations where it becomes difficult, if not impossible, to infer the presence of the active product, because its characteristic UV pattern may be masked totally by the background. One such situation is the assay of estradiol.

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