of the two isomers could not be determined by UV measurement. This determination was possible using NMR. As observed for the testosterone oxime (2), the vinyl proton had a different chemical shift for the two isomers. The spectra (over the 4–8-ppm range) for I crystallized from acetone and from aqueous ethanol are shown in Fig. 5. Crystallized I from acetone had a single resonance attributable to the vinyl proton at 5.95 ppm; I crystallized from aqueous ethanol ($A^{1\%}_{1cm}$ 457 at 282 nm) had resonances at 5.95 and 5.60 ppm. That the resonance at 5.60 ppm was due to *Ib* was confirmed by monitoring changes in the two resonances with time. After storage for 144 hr with exposure to daily diffuse sunlight, the acetone-crystallized I had developed a resonance at 5.60 ppm (Fig. 6A). In the sample crystallized from aqueous ethanol, the resonance at 5.60 ppm had become smaller and the one at 5.95 ppm had become larger than in the initial spectrum (Fig. 6B).

The vinyl resonance integration values for the aqueous ethanol-crystallized sample showed the mixture to be 50% *Ia* and 50% *Ib*. After 144 hr in solution, both crystal types showed 67% *Ia* and 33% *Ib*. Using these ratios along with an apparent $A^{1\%}_{1cm}$ of 457 for I crystallized from ethanol and an apparent $A^{1\%}_{1cm}$ of 492 for the equilibrium mixture, the $A^{1\%}_{1cm}$ of *Ia* was calculated as 554 and that for *Ib* was 365 at 282 nm.

For the oximes of testosterone derivatives, Mazur (2) observed that the vinyl proton in the *anti*-isomer always resonated about 40 Hz upfield from the *syn*-isomer. For I, the 22-Hz upfield shift of *Ib* relative to *Ia* suggests that *Ia* is the *syn*-isomer and *Ib* is *anti*. This assignment is consistent with an ~20-Hz shift to lower field predicted when the vinyl proton is *cis* to the azo group(*syn*) compared to *trans* (*anti*) (1).

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Spectrophotometric Determination of Tolbutamide, Thiamine Hydrochloride, and Pyridoxine Hydrochloride in Combination Products

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Abstract □ The first derivative curve is used for tolbutamide determination in unit-dose tablets and in combination products. The absorbance contribution from tablet excipient and coexisting components, thiamine and pyridoxine, is thereby nullified. The interference from tolbutamide during thiamine and pyridoxine determination is eliminated by solvent extraction and pH-induced differential spectrophotometry. Thiamine is measured at the isosbestic point of pyridoxine. The latter is determined by the differential absorbance measurement at two wavelengths with the consequent computation of the delta absorbance value.

Keyphrases □ Tolbutamide—analysis, combination tablets with thiamine and pyroxidine, spectrophotometry, first derivative curve □ Thiamine—analysis, combination tablets with tolbutamide and pyridoxine, spectrophotometry, first derivative curve □ Pyridoxine—analysis, combination tablets with thiamine and tolbutamide, spectrophotometry, first derivative curve □ Spectrophotometry—analysis, tolbutamide in combination tablets □ Antidiabetic agents—tolbutamide, spectrophotometric analysis, in combination tablets

Spectrophotometric determination of a weakly absorbing compound like tolbutamide in tablets without any interference from the tablet excipients is challenging. The problem is made more difficult when such a compound is combined with thiamine hydrochloride and pyridoxine hydrochloride.

Quantitation methods for multicomponent mixtures often employ multiple separation steps using chromatography or solvent extraction (1, 2). UV spectrophotometric methods that demand solution of simultaneous equations have also been used (3). Mixtures of two known absorbing substances have been determined spectrophotometrically (4). This method was modified (5) in terms of the extinction ratio. The application of the absorbance ratio to binary mixture analysis was recommended (6, 7).

The orthogonal function method was proposed in two-component spectrophotometric analysis (8). Recently, dual wavelength spectrophotometry (9) was applied to the simultaneous determination of mixtures (10) and to masking of unwanted components (11). The first derivative curve was useful in distinguishing substances with overlapping spectra (12) and in the quantitative analysis of two-component mixtures (13).

The present investigation was concerned with tolbutamide determination in unit-dose tablets and combination products by use of the first derivative curve. pH-Induced difference spectrophotometry (14–16) was utilized for thiamine and pyridoxine determination by independent absorbance measurements.

EXPERIMENTAL

Materials—Tolbutamide[1], thiamine hydrochloride[2], and pyridoxine hydrochloride[1] unit-dose tablets[3] contained 500 mg of tolbutamide/tablet. The combination product[4] contained 500 mg of tolbutamide, 5 mg of thiamine hydrochloride, and 3 mg of pyridoxine hydrochloride.

Reagents—All reagents were analytical grade, and solvents were spectroscopic grade.

[1] El-Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt.
[2] Alexandria Co. for Pharmaceutical and Chemical Industries, Egypt.
[3] Batch 039, Hoechst Orient Saa, Cairo, Egypt.
[4] Batch 14364, El-Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt.

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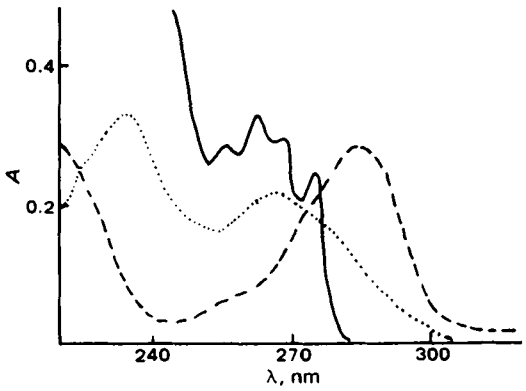

Figure 1—*UV absorption spectra of 0.15 mg of tolbutamide/ml (—), 0.01 mg of thiamine hydrochloride/ml (···), and 0.01 mg of pyridoxine hydrochloride/ml (- -). The solvent was 95% ethanol.*

Instruments—A photoelectric spectrophotometer[5] with 1-cm silica cells was used for all measurements.

Assay of Tolbutamide in Unit-Dose Tablets—Twenty tablets were powdered and mixed, and an accurately weighed quantity (~0.6 g) was extracted with 95% ethanol. The solution was diluted with ethanol to give ~0.25 mg of tolbutamide/ml. The absorbance was measured at 274 and 276 nm using 95% ethanol in the reference cell. The absorbance difference between 274 and 276 nm, $\Delta A(274 - 276)$, was computed.

Assay for Combination Product—For the tolbutamide component, the above-mentioned method was used. For the thiamine and pyridoxine components, another ~0.6-g powdered tablet portion was weighed accurately, extracted, and diluted with water. Two equal volumes were suitably diluted, with 0.1 *N* H_2SO_4 or 0.01 *N* NaOH, to give ~0.016 mg of thiamine/ml and 0.01 mg of pyridoxine/ml. The acid solution absorbance was measured at 265 nm using an alkaline solution in the reference cell (thiamine component). The alkaline solution absorbance was measured at 300 and 310 nm using an acid solution in the reference cell (pyridoxine component).

RESULTS AND DISCUSSION

The tolbutamide absorption spectrum (Fig. 1) showed three absorption maxima at 256, 262, and 274 nm and a shoulder at 268 nm. The first derivative curve, obtained by plotting $\Delta A(\lambda_1 - \lambda_2)$ *versus* λ_m [where $\lambda_m = (\lambda_1 + \lambda_2)/2$], is presented in Fig. 2. The working wavelengths chosen for the tolbutamide assay were 274 and 276 nm rather than the peaks located at shorter wavelengths.

The choice of these wavelengths was based on the high $\Delta A(274 - 276)$ with a consequent increase in sensitivity. The absorbance contribution from tablet excipients (mostly nonbenzenoid) at longer wavelengths was expected to be nil since the diverse components were characterized by constant absorption spectra (*i.e.*, with zero slope). More important, the absorption spectrum slopes of the coexisting components (thiamine and pyridoxine) in the compound tablets were opposite to each other and distinctly lower (Fig. 1) than the tolbutamide spectrum slope. Consequently, the first derivative curves of thiamine and pyridoxine contribute equally but with opposite signs (Fig. 2). Therefore, they canceled each other with the net result of no interference from these compounds. The first derivative curve in the tolbutamide determination in unit-dose tablets and in combination products gave highly accurate results (Tables I and II).

The tolbutamide concentration was calculated from the following regression equation[6], derived by using the least-squares method (17):

$$\Delta A(274 - 276) = 0.0053 + 0.915C \qquad \text{(Eq. 1)}$$

with[7] $r = 0.9998$, which was obtained by application of the procedure described for tolbutamide assay in tablets to known tolbutamide concentrations. Within a 0.1–0.7-mg/ml range, $\Delta A(274 - 276)$ *versus* C was linear.

Thiamine and pyridoxine in combination products could be determined using their pH-induced spectral changes. The interference from the major component, tolbutamide, was thereby eliminated since it is barely soluble in water, the solvent used for the tablet powder extraction. More important, tolbutamide exhibits virtually identical spectra in acid and alkaline media. Consequently, the pH-induced tolbutamide solution differential spectrum (prepared by extracting 500 mg of tolbutamide with water and subsequent acidification or alkalinization) displayed a negligible contribution (Fig. 3).

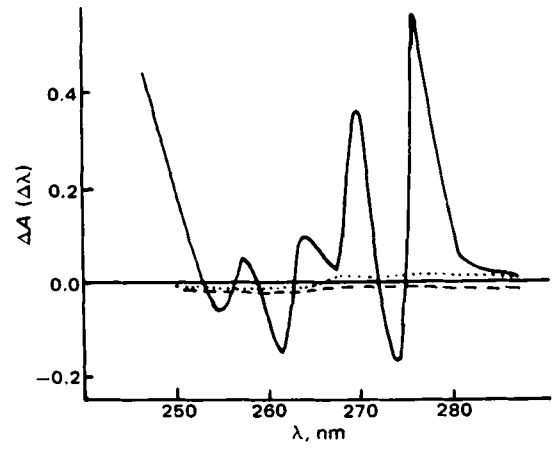

Figure 2—*First derivative curves from absorption spectra of tolbutamide (—), thiamine hydrochloride (···), and pyridoxine hydrochloride (- -).*

When the acid solution absorbance was measured against that of alkaline solution at 265 nm, thiamine could be determined without interference from pyridoxine since the latter showed an isosbestic point (Fig. 3). The delta absorbance measurement [A(acid) − A(alkaline)] at 265 nm was linearly related to the thiamine concentration over the 0.008–0.056-mg/ml range. The following regression equation, derived from ΔA_{265} measurements of known thiamine concentrations, describes the calibration curve:

$$\Delta A_{265} = -0.0108 + 3.03C \qquad \text{(Eq. 2)}$$

where $r = 0.9998$. For pyridoxine estimation, the delta absorbance at two wavelengths (300 and 310 nm) was measured with subsequent subtraction to obtain a value of $\Delta(\Delta A)(310 - 300)$. Such a choice for the two wavelengths satisfied the following fundamental conditions. The coexisting

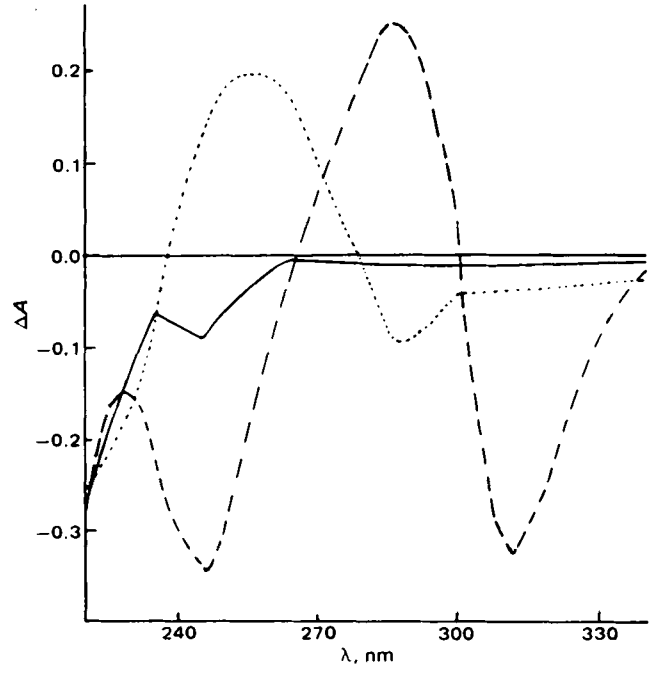

Figure 3—*Differential curves of 0.15 mg of tolbutamide/ml (—), 0.01 mg of thiamine hydrochloride/ml (···), and 0.01 mg of pyridoxine hydrochloride/ml (- -).*

[5] Prolabo, Paris.

[6] Concentrations in this equation and in subsequent equations are in milligrams per milliliter.

[7] r = correlation coefficient.

Table I—Tolbutamide in Unit-Dose Tablets

Tablet	Mean[a] Percentage[b] ± *SD*	
	A_{max} Method	$\Delta A(274 - 276)$ Method
Laboratory made	101.78 ± 0.42	100.58 ± 0.59
	9.476[c]	2.198[c]
Commercial product[d]	101.38 ± 0.47	100.07 ± 0.36
	6.565[c]	4.16[c]

[a] Mean of five determinations. [b] Percentage recovery in laboratory made and percentage found in commercial. [c] Calculated t-value for which theoretical t at 99% confidence level ($\alpha = 0.01$) = 4.604. [d] Rastinon.

components (thiamine and the soluble portion of tolbutamide) exhibited the same absorbance at these wavelengths [*i.e.*, $\Delta(\Delta A)(310 - 300)$ = zero], so variations in their concentrations had no influence on absorbance measurements. Furthermore, $\Delta(\Delta A)(310 - 300)$ was linearly related to concentration in the 0.004–0.028-mg/ml range of pyridoxine, thus permitting its determination. The regression equation derived from the data obtained is as follows:

$$\Delta(\Delta A)(310 - 300) = 0.0058 + 26.89C \qquad \text{(Eq. 3)}$$

where $r = 0.9999$.

The applicability of Eq. 1 in the tolbutamide assay and of Eqs. 2 and 3 in thiamine and pyridoxine determination was tested by analysis of laboratory-made mixtures. The results obtained are of good accuracy and reproducibility (Table II).

The first derivative curve method [$\Delta A(274 - 276)$] for tolbutamide determination in unit-dose tablets was compared with the traditional spectrophotometric method (A_{max} method). The latter was carried out by direct absorbance measurement at λ_{max} 262 nm, and the following equation was used for the concentration calculation:

$$A_{262} = 0.019 + 2.170C \qquad \text{(Eq. 4)}$$

where $r = 0.9999$.

The unit-dose tablets were analyzed using $\Delta A(274 - 276)$ and A_{max} methods (Table I). By applying the t test (17), the results were subjected to statistical analysis. At the 99% confidence level ($p = 0.01$), the ΔA method gave a value not significantly different from the true value whereas the A_{max} method gave a value significantly different from the true value. Therefore, the former method was more accurate than the latter method.

In combination tablets, tolbutamide–thiamine–pyridoxine were present in the ratio 166:1.66:1. Although tolbutamide represented the major component, the interference from strongly absorbing coexisting components was significant because of the weak absorptivity of tolbutamide (Fig. 1). In term of absorbance, the interference from thiamine and pyridoxine was about 20%. The first derivative curve method diminished such interference since the slope of the tolbutamide absorption curve was distinctly higher than the slopes of the coexisting component spectra (Figs. 1 and 2). The interference from the coexisting components was eliminated completely because of the opposite slopes of the thiamine and pyridoxine curves.

Table II—Three Components in Tolbutamide Combination Tablets

Preparation	Mean Percentage[a] ± *SD*		
	Tolbutamide[b]	Thiamine[c]	Pyridoxine[c]
Laboratory made	100.82 ± 0.56	100.04 ± 0.39	100.16 ± 0.49
Commercial[d]	101.63 ± 0.45	106.61 ± 0.59	106.99 ± 0.66

[a] Percentage recovery in laboratory-made mixture and percentage found in commercial tablets. [b] Mean of 15 determinations. [c] Mean of 10 determinations. [d] Tolvit.

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