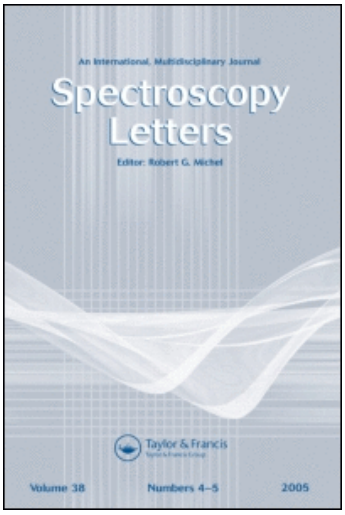

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Simultaneous Determination of Barbital and Thiobarbituric Acid by Derivative Spectrophotometry

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SIMULTANEOUS DETERMINATION OF BARBITAL AND THIOBARBITURIC ACID BY DERIVATIVE SPECTROPHOTOMETRY

Key words: Barbital, thiobarbituric acid, derivative spectrophotometry, simultaneous determination.

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ABSTRACT

A new method for the simultaneous determination of barbital and thiobarbituric acid by derivative spectrophotometry is proposed. The method allows the resolution of mixtures of the two components over the concentration ranges 0.37–4.70 μg/ml (barbital) and 0.40–4.50 μg/ml (thiobarbituric acid) provided the ratio between their concentrations does not exceed 5:1. It was applied to the determination of the two compounds in synthetic samples and blood serum.

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INTRODUCTION

Derivative UV-visible spectrophotometry is a relatively recently developed instrumental technique which has so far been successfully applied to the determination of compounds of biochemical[1] pharmaceutical[2] environmental[3] clinical[4] and miscellaneous interest[5]. The derivative mode has also been applied in other techniques such as IR[6] atomic absorption[7] and plasma spectrophotometry[8] as well as in conventional and synchronous spectrofluorimetry[9] and has been of great aid in solving problems arising from spectral overlap[10] and matrix effects[11].

In this work we developed a straightforward, fast method for the simultaneous determination of barbital and thiobarbituric acid by first- and second-derivative spectrophotometry. The method was applied to the determination of the two compounds in synthetic samples and blood serum.

EXPERIMENTAL

Apparatus

The instrumental set-up used consisted of a Perkin-Elmer 550S UV-visible spectrophotometer furnished with quartz cells of 1 cm pathlength, a Radiometer PHM84 potentiometer fitter with a combined glass–saturated calomel electrode, an Afora centrifuge working at 1200 rpm and featuring automatic braking, and a Selecta 512 ultrasound generator.

Reagents

The reagents used included 0.1 M aqueous standards of barbital and thiobarbituric acid, prepared by direct weighing from the commercially available products(Merck) and stored at room temperature in the dark, and a $Na_2B_4O_7$/NaOH buffer of pH 9.20 and $C_{tot} = 2 \times 10^{-2}$ M. The ionic strength was adjusted to 0.25 M with $NaClO_4$.

All reagents used were analytical grade chemicals and de-ionized water was employed throughout.

Simultaneous determination of barbital and barbituric acid in synthetic samples

In 25-ml volumetric flasks were placed 2.5 ml of 2.5 M $NaClO_4$, 5 ml of the $Na_2B_4O_7$/NaOH buffer of pH 9.20 and appropriate volumes of the standards of the two compounds providing concentrations over the ranges 0.37–4.70 μg/ml (barbital) and 0.40–4.50 μg/ml (thiobarbituric acid) on final dilution to the mark with de-ionized water. After 5 min, the first- and second-derivative spectra of the sample were recorded between 350 and 190 nm against a blank containing neither analyte. The instrumental conditions used were as follows: chart speed, 30 nm/min; wavelength scanning rate, 120 nm/min; slit width, 2 nm; response time, 10 s (first-derivative) and 7 s (second-derivative).

From first-derivative spectra were measured the height from the baseline of the peak at 296 nm and the minimum at 246 nm, while from second derivative spectra were measured the peak heights at 294 nm and 244 nm (zero-crossing for thiobarbituric acid). By com-

paring the measured signals with the corresponding calibration graphs (Table I) the two components were readily determined.

Determination of barbital and thiobarbituric acid in blood serum

A volume of 2.0 ml of blood serum containing the two analytes at concentrations in the ranges 3.50–80.00 μg (barbital) and 3.50–75 μg (thiobarbituric acid) was added 0.30 ml of 40% trichloroacetic acid, and the mixture was stirred for 1 min and centrifuged at room temperature for 10 min. An aliquot of the supernatant not larger than 1.5 ml was then analysed by the procedure described above for synthetic samples using normal Keytrol serum vials supplied by ITC-Diagnostic that were also previously added trichloroacetic as blanks.

RESULTS AND DISCUSSION

Before the resolution of barbital–thiobarbituric acid mixtures was tackled, the additivity of absorbances was checked Fig. 1. The next step in the process involved determining the influence of chemical and instrumental variables on the process; while the latter affected both the number and shape of the first-derivative peaks, the former only influenced the signal intensity. The instrumental variables studied included the wavelength scanning rate, slit width and response time. Preliminary assays showed a chart speed of 30 mm/min to provide optimal results. In fact:

(a) Increasing wavelength scanning speeds resulted in increased band broadening, which obviously hindered resolution of

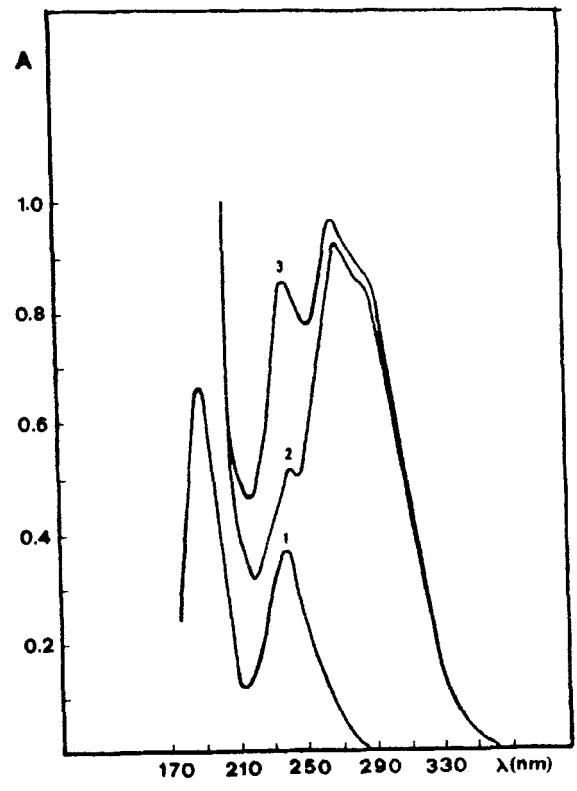

Figure 1.- Absorption spectra of system Barbital - Thiobarbituric acid. pH = 9.20; I = 0.25 M ($NaClO_4$). 1) $C_B = 2.10^{-5}$ M; 2) $C_{TBA} = 2.10^{-5}$ M; 3) $C_B = 2.10^{-5}$ M + $C_{TBA} = 2.10^{-5}$ M.

the mixture. A scanning speed of 120 nm/min was selected.

(b) The slit width had no significant effect on the spectral resolution, so an optimal value of 2 nm was chosen.

(c) The signal-to-noise ratio increased with decreasing response time. The optimal values chosen for the first- and second derivative were 10 and 7 s, respectively.

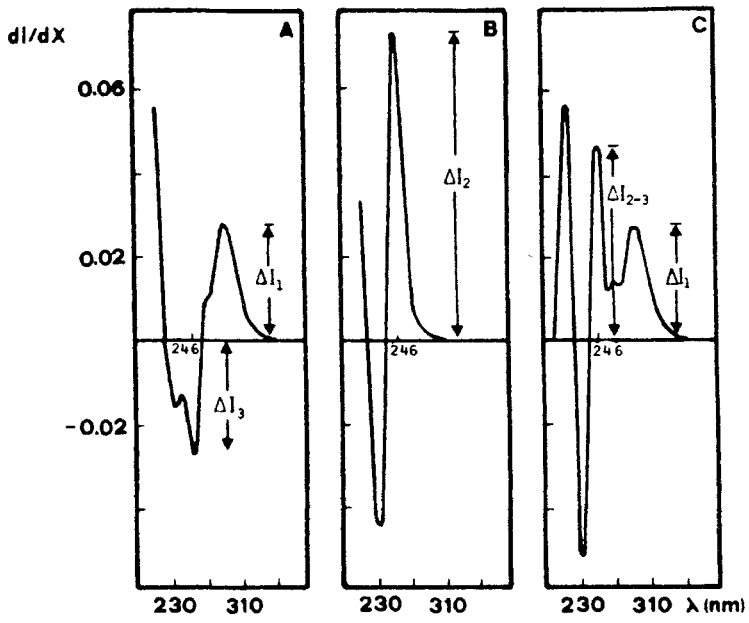

Figure 2.- First derivative spectra of system Barbital-Thiobarbituric acid, pH = 9.20; I = 0,25 M ($NaClO_4$) A) C_{TBA} = 1.60 μg/ml; B) C_B = 1.47 μg/ml and C) C_{TBA} = 1,60 μg/ml and C_B = 1.47 μg/ml

The chemical variables studied were the pH and concentration of the buffer solution. The optimal pH for recording the absorption spectra was found to be between 9.0 and 10.0. Above pH 10.5, the two compounds decomposed[12] while below pH 8.5 the absorbance was markedly smaller. A pH of 9.20 was thus chosen as optimal. Of the different buffers assayed, only that consisting of $Na_2B_4O_7$/NaOH was found to result in a signal coinciding with that obtained in its absence. Since the buffer concentration did not seem to influence

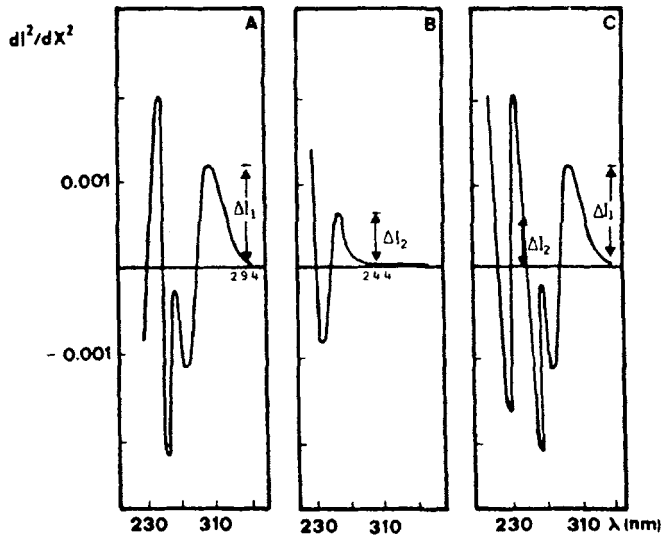

Figure 3.- Second derivative spectra of system Barbital-Thiobarbituric acid, pH = 9.20; I = 0,25 M ($NaClO_4$) A) C_{TBA} = 1.60 μg/ml; B) C_B = 1.47 μg/ml and C) C_{TBA} = 1,60 μg/ml and C_B = 1.47 μg/ml

analytical signal, a value of 2×10^{-2} M was chosen for subsequent experiments.

As can be seen from the first-derivative spectra (Fig. 2 A), the distances between the baseline and the peaks at 296 nm (ΔI_1) and 242 nm (ΔI_3) are related to the concentration of thiobarbituric acid and independent of that of barbital (Fig. 2 A). Also, the distance between the baseline and the maximum at 242 nm (ΔI_2) is related to the barbital concentration; and the distance ΔI_{2-3}, *i.e.* the difference between ΔI_2 and ΔI_3, is also related to the barbital con-

TABLE 1

Statistic Analysis of Methods for the Determination of Barbital and Thiobarbituric Acid.

Equation	r	S	R.S.D.	D.L.
First Derivative				
$I_1 = -3,6.10^{-2} + 2.72C_{TBA}$	0.999	$1.04.10^{-1}$	1.21	0.12
$I_2 = -5,7.10^{-2} + 3.36C_{TBA}$	0.998	$7.50.10^{-2}$	1.31	0.11
$I_3 = 5.2.10^{-2} + 2.50C_B$	0.997	$7.30.10^{-2}$	1.52	0.09
Second Derivative				
$I_4 = -1.9.10^{-1} + 1.97C_{TBA}$	0.999	$6.74.10^{-2}$	1.22	0.10
$I_2 = 4.5.10^{-2} + 2.64C_B$	0.997	$1.01.10^{-1}$	1.33	0.14

B = Barbital; TBA = Thiobarbituric Acid; C = μg/ml;

S = Standard Deviation; R.S.D. = Relative Standard Deviation;

D.L. = Detection Limit (μg/ml)

centration. The determination of the two compounds in mixtures was addressed by measuring ΔI_1 and ΔI_{2-3} on the first-derivative spectrum. By comparing ΔI_1 with the calibration curve was determined the amount of thiobarbituric acid present and hence the corresponding distance ΔI_3; also, by adding up ΔI_3 and ΔI_{2-3} was obtained ΔI_2, which allowed the amount of barbital in the mixture to be determined.

TABLE 2

Precision of the Method for the Determination of Barbital and Thiobarbituric Acid by First and Second Derivative.

Concentration (μg/ml)	First Derivative S.D.			
	Within assays[a]		Between assays[b]	
	B	TBA	B	TBA
0.80	5.09	5.03	0.10	1.21
2.20	2.45	1.79	0.14	0.14
3.50	1.18	1.16	0.09	0.05
	Second Derivative S.D.			
	Within assays[a]		Between assays[b]	
	B	TBA	B	TBA
0.80	6.44	4.10	0.98	0.97
2.20	1.97	1.55	0.54	0.42
3.50	1.10	0.70	0.08	0.03

B = Barbital; TBA = Thiobarbituric Acid; [a] = Eight individual determinations; [b] = The assays were run over a period of six days; S.D. = Standard Deviation

A similar study was performed on the second-derivative spectrum (Fig. 3 A). The distance between the baseline and the peak at 294 nm (ΔI_4) was related to the concentration of thiobarbituric acid and independent of that of barbital (Fig.3 B), which was determined directly by measuring ΔI_5 at 240 nm (zero-crossing for barbituric acid).

TABLE 3

Simultaneous Analysis of Synthetic Binary Mixtures of Barbital and Thiobarbituric Acid by the first and second derivative.

Amount added (μg/ml)		Ratio	Amount found (μg/ml)			
			1ª Deriv.		2ª Deriv.	
B	TBA	B:TBA	B	TBA	B	TBA
0.80	0.80	1:1	0.79	0.80	0.78	0.80
1.60	1.60	1:1	1.59	1.61	1.58	1.59
2.40	2.40	1:1	2.39	2.42	2.44	2.41
1.60	3.20	1:2	1.59	3.20	1.63	3.22
0.80	2.40	1:3	0.77	2.41	0.79	2.39
0.80	3.20	1:4	0.81	3.21	0.79	3.21
1.60	0.80	2:1	1.59	0.80	1.58	0.79
3.20	1.60	2:1	3.21	1.61	3.22	1.61
1.60	2.40	2:3	1.59	2.39	1.58	2.41
2.40	0.80	3:1	2.40	0.81	2.43	0.79
2.40	1.60	3:2	2.42	1.59	2.44	1.58
2.40	3.20	3:4	2.45	3.22	2.42	3.21
2.40	4.00	3:5	2.35	3.98	2.36	3.99
3.20	0.80	4:1	3.20	0.81	3.19	0.81
3.20	2.40	4:3	3.21	2.41	3.18	2.38

The equations of the calibration curves, applicability range, standard deviations and detection limits obtained are given in Table 1.

Statistical treatment of the results

The accuracy and precision of the proposed method were assessed at three different concentrations of each analyte over the applicability

TABLE 4

Effect of foreign species on the simultaneous determination of 1.47 μg/ml of Barbital and 1.60 μg/ml of Thiobarbituric Acid.

[Specie]/[B]	Specie added
100:1	K^+, Li^+, Ca^{2+}, Mg^{2+}, F^-, Cl^-, CO_3^{2-}
80:1	EDTA, DCTA, DCTA-Ca^{2+} (1:1)
25:1	starch, glucose, urea
5:1	$S_2O_3^{2-}$, Fe^{3+} *
1:1	Cu^{2+} ** Fe^{3+} *
≤ 1	ascorbic acid, uric acid, thiourea

* in excess of F^-
** in excess of DCTA-Ca^{2+}

range, *viz.* 0.80, 2.40 and 3.50 μg/ml. Five series of 8 samples each were prepared at each concentration assayed and the study was conducted over a period of 4 days [13]. The results obtained are listed in table 2.

The validity and applicability of the proposed method was checked on synthetic samples containing the two barbiturates in different proportions which were analysed as described above. The results obtained are listed in Table III. The simultaneous determination of the two components was feasible in barbital/thiobarbituric acid ratios within the applicability range of the method.

TABLE 5

Simultaneous Determination of Barbital and Thiobarbituric Acid in Blood serum by First and Second Derivative.

Amount Added (μg/ml)		1st Deriv. Amount Found* (μg/ml)		2nd Deriv. Amount Found* (μg/ml)	
B	TBA	B	TBA	B	TBA
0.92	0.99	0.93	0.99	0.90	0.99
1.84	0.50	1.82	0.51	1.80	0.49
1.84	0.99	1.82	0.98	1.83	1.01
1.84	1.99	1.83	2.00	1.81	1.97
2.08	0.76	2.09	0.77	1.98	0.77
2.76	1.49	2.77	1.47	2.70	1.51
2.76	2.98	2.76	3.00	2.71	2.97
2.76	3.50	2.71	3.51	2.69	3.51

All results are the mean od three determinations.

Effect of interferents

The effect of potential interfering ions on the simultaneous determination of 0.73 μg/ml barbital and 0.80 μg/ml thiobarbituric acid by using the first and second-derivative spectrum of the mixture was established by applying the above-described procedure to solutions containing each potential interferent at concentrations 100

times higher that the analytes. A given species was considered to interfere with the determination if it resulted in a deviation greater than ± 5% in the concentration of the two analyte concentrations. The results obtained in the interference study are summarized in Table 4.

Simultaneous determination of barbital and thiobarbituric acid in blood serum

The proposed method was applied to the determination of barbital and thiobarbituric acid in blood serum after deproteination. The results obtained are listed in Table 5.

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