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Simultaneous determination of melatonin–pyridoxine combination in tablets by zero-crossing derivative spectrophotometry and spectrofluorimetry

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Abstract

Two methods have been developed for the analysis of melatonin (M) and pyridoxine hydrochloride (PH) in combination. The first method depends on first- and second-derivative ultraviolet spectrophotometry, with the zero crossing technique of measurement. First-derivative amplitudes at 296 nm and second-derivative amplitudes at 294 and 322 nm are selected for the determination of M and PH, respectively. The second method is based on the native fluorescence of both M and PH, in methanol and 0.1 M hydrochloric acid, respectively, after a preliminary solvent extraction procedure. The relative standard deviation of both methods was less than 2.0%. The two methods have been successfully applied to the determination of both drugs in laboratory-prepared mixtures and in tablets © 1998 Elsevier Science B.V.

Keywords: Melatonin; Pyridoxine hydrochloride; Simultaneous determination; Derivative spectrophtometry; Spectrofluorimetry; Dosage form

1. Introduction:

Melatonin (M), the chief hormone of the pineal gland in vertebrates is the natural way to combat aging, stimulate immune function, reduce risk of cancer and heart disease and offer a better night's sleep and was recently shown to be a potent hydroxyl radical scavenger and antioxidant [1]. Certain vitamins and mineral supplements, including pyridoxine hydrochloride (PH), and cyanocobalamin and the minerals calcium and magnesium are known to have sedative effects on the body. Brands of M containing PH have been produced [2]. It was claimed that PH produces an increase in the natural production of M (Amoun Pharmaceutical Industries, Cairo, Egypt, personal communication).

A survey of the literature revealed that the analysis of PH either in single or multicomponent mixtures has been reported through HPLC [3], TLC-spectrophotometry [4], and spectrophotometry [5,6]. An extended bibliography can be found in the analytical profile [7]. M in edible plants, aqueous solutions, rat pineals and serum has been

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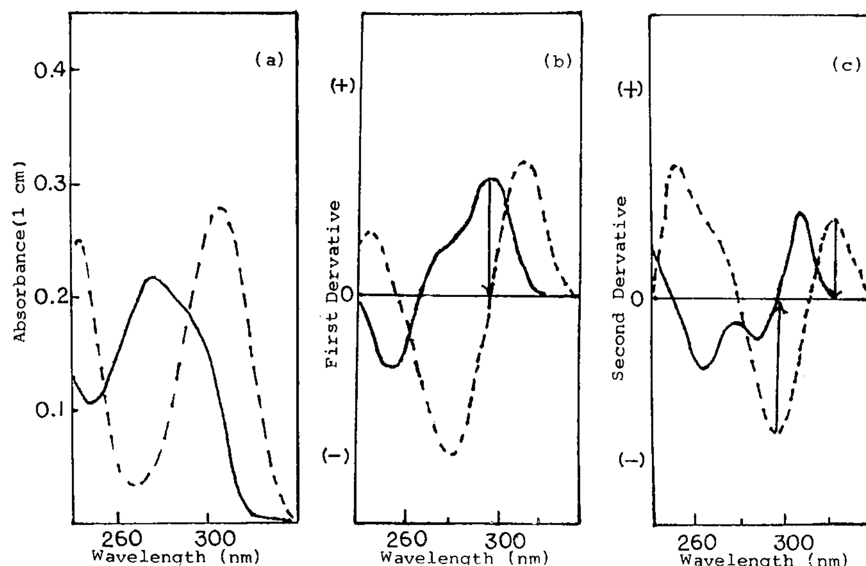

Fig. 1. (a) Zero-order; (b) first derivative; and (c) second derivative spectra of 4 μg ml^{-1} melatonin (—) and 4 μg ml^{-1} pyridoxine hydrochloride (- - -) in 0.1 M sodium hydroxide.

identified and estimated using several chromatographic techniques either HPLC [8–11] or GC [12] and radioimmuno assay procedure [11].

To our knowledge no analytical method could be traced for the analysis of M–PH combination in pharmaceutical dosage form. In this connection a simple and reliable method for simultaneous assay of both drugs in mixture seemed to be necessary.

In this paper, two methods based on UV-derivative spectrophotometry and spectrofluorimetry are proposed for the quantitation of both drugs. The developed methods were extended to determine the content of both drugs in commercial tablets.

2. Experimental:

2.1. Apparatus

Spectrophotometric determinations were performed using a Perkin-Elmer Model 550S UV-visible spectrophotometer and a Hitachi Model 561 recorder. All derivative spectra were obtained using the following parameters: scan speed, 60 nm min^{-1}; chart speed, 120 mm min^{-1}; mode, ^1D (first derivative) and ^2D (second derivative); response time, 10 s; wavelength range, 240–400 nm and ordinate maximum and minimum settings $\pm$ 0.1 (^1D) and ± 0.01 (^2D).

All fluorimetric measurements were performed on a Perkin-Elmer Model 650-10S spectrofluorimeter equipped with 1-cm quartz cuvettes, a 150-W xenon lamp, excitation and emission grating monochromators, and a Perkin-Elmer Model 56 recorder.

2.2. Reagents and samples

All experiments were performed with analytical grade chemicals and solvents. Authentic samples of M and PH were kindly donated by Pharco Pharmaceuticals, Alexandria, Egypt, and were used without further purification. Viva-Max® 3 tablets (Amoun Pharm., Cairo, Egypt) labelled to contain 3 mg M and 10 mg PH (Vitamin B_6) were obtained from the market.

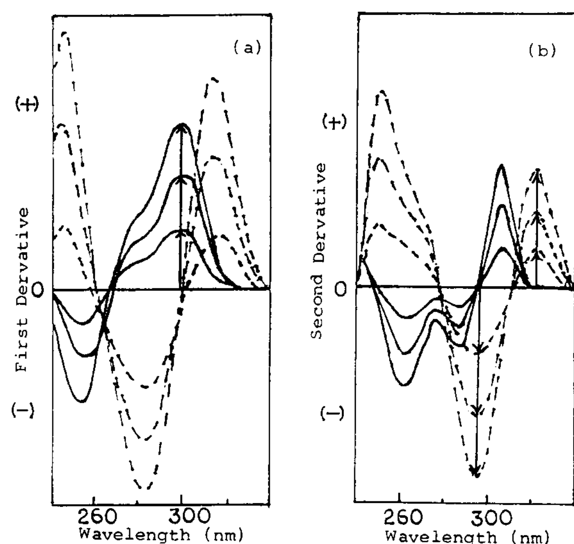

Fig. 2. (a) First derivative; (b) second derivative spectra of melatonin (—) and pyridoxine hydrochloride (- - -) at several different concentrations in 0.1 M sodium hydroxide solution: melatonin, 2, 4, and 6 μg ml^{-1} and pyridoxine hydrochloride, 2, 4 and 6 μg ml^{-1}.

2.3. Standard solutions and calibration graphs for spectrophotometric procedure

Stock solutions of either M (0.4 mg ml^{-1}) or PH (1.6 mg ml^{-1}) were prepared in hot water and stored refrigerated at 4°C in brown glass flasks. The working standard solutions were prepared by dilution of the stock solutions with 0.1 M sodium hydroxide solution to reach a concentration range of 2.0–8.0 μg ml^{-1} for both M and PH.

The ^1D and ^2D spectra were recorded against 0.1 M sodium hydroxide solution. The observed values of the ^1D amplitudes at 296 nm (for M) and the ^2D amplitudes at 294 and 322 nm (for PH) were plotted against the corresponding concentrations.

2.4. Standard solutions and calibration graphs for spectrofluorimetric procedure

Aliquots of the previously prepared stock solutions containing 0.40 mg M and 1.6 mg PH, were transferred into a separator containing 0.5 ml of 5 M sodium hydroxide solution. The contents of the separator were mixed and then extracted with two 10-ml portions of chloroform.

2.4.1. For M determination

The combined chloroformic extracts were collected into 25-ml volumetric flask and diluted to volume with chloroform. Aliquots ranging from 0.1–0.4 ml of chloroformic solutions corresponding to 1.6–6.4 μg of M were transferred to 10-ml volumetric flasks and evaporated to dryness on a boiling water bath. The residue in each flask was dissolved and completed to volume using methanol. The fluorescence intensities at 338 nm emission with excitation at 300 nm were measured. The observed fluorescence was corrected by subtracting the fluorescence intensity measured using methanol as a blank.

2.4.2. For PH determination

The aqueous layer remaining after extraction with chloroform, was transferred to a 10-ml volumetric flask, neutralized with 0.5 M hydrochloric acid and completed to volume with water.

Table 1
Analytical data of the calibration graphs for the determination of melatonin and pyridoxine hydrochloride by derivative spectrophotometry

Compound	Derivative mode, λ(nm)	Linearity range (μg ml^{-1})	Regression equation ($D=a+b\ C$)[a]	r^{b}	$S^{c}_{y/x}$	S^{d}_{a}	S^{e}_{b}
Melatonin	$^1D_{296}$	(2–8)	$D=0.04+6.24C$	0.9999	0.163	0.196	0.036
Pyridoxine	$^2D_{294}$	(2–8)	$D=0.41+7.25C$	0.9992	0.748	0.900	0.167
	$^2D_{322}$	(2–8)	$D=-0.035+4.48C$	0.9990	0.126	0.150	0.028

[a] Derivative value (^1D or ^2D) versus concentration (C) of each drug in μg ml^{-1}; standard specimens: $n=5$.
[b] Correlation coefficient; [c] $S_{y/x}$ = standard deviation of residuals; [d] S_a = standard deviation of intercept of regression line; [e] S_b = standard deviation of slope of regression line.

Table 2
Detection and quantification limits for the determination of melatonin and pyridoxine hydrochloride by the proposed methods

Compound	Proposed method	S_B^a	C_L (μg ml^{-1})[b]	C_Q (μg ml^{-1})[c]
Melatonin	$^1D_{296}$	0.011	5.3×10^{-3}	0.018
	Fluorimetry($\lambda_{ex}=305$ nm) ($\lambda_{em}=338$ nm)	0.016	2.87×10^{-4}	9.57×10^{-4}
Pyridoxine	$^2D_{294}$	0.160	0.066	0.220
	$^2D_{322}$	0.170	0.114	0.379
	Fluorimetry($\lambda_{ex}=315$ nm) ($\lambda_{em}=395$ nm)	0.140	0.042	0.141

[a] S_B = standard deviation of blank; [b] $C_L=3S_{B/b}$; C_L = detection limit; b = slope of calibration graph; [c] $C_Q=10S_{B/b}$; C_Q = quantification limit.

Aliquots of the neutralized solution (0.05–0.3 ml), corresponding to 8.0–48 μg of PH were transferred into 5-ml volumetric flasks and diluted to volume with 0.1 M hydrochloric acid solution.

The fluorescence intensities at 395 nm emission wavelength with excitation at 305 nm were measured. The observed fluorescence was corrected by subtracting the fluorescence intensity measured using 0.1 M hydrochloric acid solution as a blank.

2.5. Determination of M–PH in tablets

Twenty tablets were weighed and finely powdered. A portion of the mixed powder equivalent to about 20 mg of M was accurately weighed, transferred to a 50-ml volumetric flask and extracted with hot water and filtered. Aliquots of the filtrate were treated as described under the calibration procedure for spectrophotometric and spectrofluorimetric methods.

3. Results and discussion

3.1. Derivative spectrophotometric method

The absorption (zero order) UV spectra of M and PH over the range 220–340 nm in 0.1 M sodium hydroxide are shown in Fig. 1a. M exhibits a broad band with maximum absorption at 275 nm; PH, however, absorbs over this wavelength region, with two peaks at about 244 and 306 nm. Because of the extensive overlap of the spectral bands of the two compounds, conventional UV spectrophotometry cannot be used for their determination in mixtures.

However, when ^1D and ^2D UV spectra are recorded, sharp bands of large amplitudes (Fig. 1b and 1c) are produced which may permit more selective identification and determination of the two compounds. The ^1D spectra (Fig. 1b) permits the determination of M at 296 nm (zero crossing of PH). On the other hand, PH can be determined without any interference from M through ^2D measurement (Fig. 1c) at 294 or 322 nm (zero crossing of M).

Fig. 2a and b show the ^1D spectra of PH and ^2D spectra of (M) at several different concentrations, as can be seen, the position of the iso-differential point for each compound is as stated above.

The calibration graphs, obtained by the recommended procedure, are linear over the range 2–8 μg ml^{-1} of M or PH. The calibration graphs prepared by plotting ^1D or ^2D values vs M or PH concentrations, respectively, all gave significant linearity with negligible intercepts, confirming the mutual independence of the derivative signals of the two compounds. In Table 1, the statistical parameters are given: the regression equations calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation of residuals ($S_{y/x}$). The linearity of calibration graphs and conformity of the ^1D and ^2D measurements to Beer's law are proved by the high values of the correlation coefficients (r) of the regression equations.

The detection limits [13] were 0.096 μg ml^{-1} for M and 0.069 μg ml^{-1} (at λ_{294}) or 0.109 μg ml^{-1} (at λ_{322} nm) for PH; while the quantification limits [14] were 0.32 μg ml^{-1} for M and 0.231 (at λ_{294}

Table 3
Precision and accuracy for the determination of melatonin and pyridoxine hydrochloride by the proposed methods

Method	Nominal value (μg ml^{-1})			Found ± SD (μg ml^{-1})[a]			RSD (%)[b]			E_r (%)[c]		
	M	PH		M	PH		M	PH		M	PH	
	$^1D_{296}$	$^2D_{294}$	$^2D_{322}$	$^1D_{296}$	$^2D_{294}$	$^2D_{322}$	$^1D_{296}$	$^2D_{294}$	$^2D_{322}$	$^1D_{296}$	$^2D_{294}$	$^2D_{322}$
Derivative spectrophotometry	2	2	2	1.97 ± 0.004	2.01 ± 0.005	2.02 ± 0.001	0.20	0.25	0.05	1.50	0.50	1.00
	2	4	4	1.98 ± 0.003	4.05 ± 0.001	3.98 ± 0.008	0.15	0.02	0.20	1.00	1.25	0.50
	2	5	5	2.00 ± 0.010	5.01 ± 0.005	5.03 ± 0.013	0.50	0.10	0.26	0.00	0.20	0.60
	2	6	6	1.99 ± 0.002	5.98 ± 0.013	6.03 ± 0.013	0.10	0.22	0.22	0.50	0.33	0.50
	2	7	7	1.98 ± 0.001	7.06 ± 0.001	7.00 ± 0.008	0.05	0.01	0.11	1.00	0.85	0.00
	2	8	8	1.99 ± 0.008	8.08 ± 0.001	7.98 ± 0.008	0.40	0.01	0.10	0.50	1.00	0.25
Fluorimetry	0.32	1.6	0.321 ± 0.001		1.6 ± 0.010		0.31	0.63		0.31		0.00
	0.32	3.2	0.323 ± 0.001		3.2 ± 0.001		0.31	0.03		0.93		0.00
	0.32	4.8	0.319 ± 0.001		4.8 ± 0.008		0.31	0.17		0.31		0.00
	0.32	5.6	0.320 ± 0.001		5.6 ± 0.008		0.31	0.14		0.00		0.00
	0.32	6.4	0.323 ± 0.001		6.4 ± 0.008		0.31	0.13		0.93		0.00

[a] Mean ± standard deviation for five determinations; [b] Percentage relative standard deviation; [c] Percentage relative error.

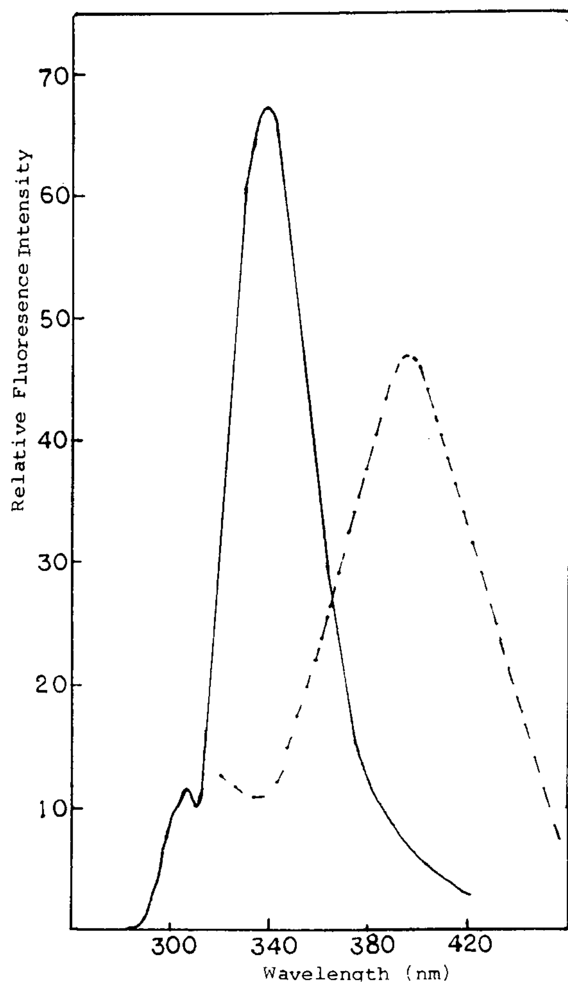

Fig. 3. Fluorescence emission spectra of 0.4 μg ml^{-1} melatonin (—) and 4.8 μg ml^{-1} pyridoxine hydrochloride (- - -) in methanol.

nm) or 0.367 μg ml^{-1} (at λ_{322} nm) for PH (Table 2).

In order to assess the precision, as percentage relative standard deviation (RSD%) and the accuracy, as percentage relative error (E_r%) of the proposed method, five replicate determinations were carried out on a M–PH synthetic mixtures of different proportions. The data shown in Table 3 indicate good accuracy and precision of the proposed procedure.

3.2. Spectrofluorimetric method

Fluorescence emission spectra of M and PH in methanol are shown in Fig. 3. The extensive overlap makes it difficult to distinguish between the two compounds in mixture. Trials involving the use of either acidic or basic media in order to resolve the fluorescence emission spectra of these compounds by either direct or synchronous fluorescence were not successful. Therefore M and PH in mixtures were determined after a preliminary solvent extraction procedure, which is based on the fact that PH, being a phenolic like compound, remains in solution upon alkalinization with alkali hydroxide and accordingly is not extracted with chloroform; while M, being a basic compound is readily extracted with chloroform from the alkaline solution.

Calibration graphs were constructed from six points over the concentration ranges shown in Table 4.This table also presents the results of the statistical analysis of the experimental data, the regression equations calculated from the calibration graphs, along with S_b, S_a and $S_{y/x}$. The values of the correlation coefficient (r) of the regression equations indicate good linearity. The detection and quantification limits (Table 2) shows the high sensitivity of the proposed spectrofluorimetric method.

The values of RSD% and E_r% (Table 3) as a measure for precision and accuracy, respectively for the spectrofluorimetric procedure can be considered very satisfactory.

Table 4
Analytical data from the calibration graphs for the determination of melatonin and pyridoxine hydrochloride by spectrofluorimetry

Compound	λ_{ex}	λ_{em}	Linearity range (μg ml^{-1})	Regression equation ($F=a+bC$)[a]	r	$S_{y/x}$	S_a	S_b
Melatonin	305	338	0.16–0.64	$F=0.336+167.14C$	0.9999	0.47	0.58	1.28
Pyridoxine	315	395	1.6–9.6	$F=0.259+9.91C$	0.9980	1.81	1.68	0.28

[a] Relative fluorescence intencity (F) versus concentration (C) of each drug in μg ml^{-1}; standard specimens: $n=6$.

Table 5
Determination of melatonin–pyridoxine combination in tablets by derivative spectrophotometry and spectrofluorimetry

Method	Melatonin (3 mg/tablet)		Pyridoxine (10 mg/tablet)		
	Derivative spectrophotometry	Fluorimetry	Derivative spectrophotometry		Fluorimetry
	$^{1}D_{296}$	λ_{ex} 305 λ_{em} 338	$^{2}D_{294}$	$^{2}D_{322}$	λ_{ex} 315 λ_{em} 395
Mean ± SD	100.29 ± 1.16	100.48 ± 0.79	100.63 ± 0.44	100.70 ± 0.71	100.87 ± 0.75
RSD (%)	1.16	0.79	0.44	0.71	0.74
E_r (%)	0.29	0.48	0.63	0.70	0.87
t^b	0.45	—	0.80	1.63	—
F^b	2.10	—	2.83	1.09	—

[a] Average of five determinations;
[b] Theoretical values of t- and F-tests at $P = 0.05$ are 2.31 and 6.93, respectively.

3.3. Analysis of tablets

The proposed methods were evaluated in the assay of commercial tablets. Five replicate determinations were carried out on an accurately weighed amount of pulverised tablets, giving excellent percentage recovery (100.29–100.87%) with RSD% less than 1.16 and E_r% less than 1.11 for either M or PH (Table 5). These results conform satisfactory with the lable claim and indicate the high precision and accuracy of the proposed methods when applied to tablets.

The performance of the spectrophotometric method was statistically compared with that of the spectrofluorimetric method by Student's t-test and variance ratio F-test (Table 5). The calculated (experimental) t- and F-values did not exceed the tabulated (theoretical) values in either test, indicating that there was no significant difference between the methods compared.

4. Conclusion

Derivative spectrophotometry and spectrofluorimetry are suitable techniques for the reliable analysis of combination of M and PH either in a pure form or in tablets.

The most striking features of the derivative method are its simplicity, selectivity and rapidity, which render it suitable for routine analysis in control laboratories. Although the spectrofluorimetric method requires an extraction procedure, it possesses the advantage of high sensitivity (expressed by the detection limits) which may be an incentive to other workers to apply to the biological fluids.

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