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Determination of boldine in drug formulations by first-derivative synchronous spectrofluorimetry

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Abstract: A simple and sensitive method is proposed for the determination of boldine by first-derivative synchronous spectrofluorimetry based on its native fluorescence in 0.1 N sulphuric acid. The constant wavelength difference ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) chosen to optimize the determination is 90 nm. The linear concentration range of application is between 2.0 and 50.0 $\mu g\ l^{-1}$ of boldine, the detection limit 0.4 $\mu g\ l^{-1}$ and the relative standard deviation at a concentration of 25 $\mu g\ l^{-1}$ was 2.1%. The method has been satisfactorily applied to the determination of boldine in pharmaceutical formulations.

Keywords: *Boldine; derivative spectroscopy; synchronous spectrofluorimetry; drug formulations.*

Introduction

Boldine, 5,6,6a,-7-tetrahydro-1,10-dimethoxy-6-methyl-4H-dibenzo[de,g]quinoline-2,9-diol, is an alkaloid derived from apomorphine and is used as a diuretic and laxative.

Several chromatographic methods have been proposed for its determination in Boldo extracts from *Peumus boldus Molina* leaves (a herb from Chile used for urinary and liver treatment) [1, 2] and in tablets and syrup [3]. These methods involve the use of extracting solvents and other separation steps and therefore they are slow and tedious for routine analysis.

Gürkan [4] has reported data for the native fluorescence of boldine in different solvents and its dependence on pH.

Derivative synchronous spectrofluorimetry has been shown to be a satisfactory technique for the analysis of organic compounds with advantages in both selectivity and sensitivity over conventional spectrofluorimetry. For example, *iso*-propyl phenylcarbamate after reaction with fluorescamine [5] and dichlone [6], gibberellic acid [7] and berberine [8] have been assayed by derivative synchronous spectrofluorimetry.

Selective analysis of inorganic ions by derivative synchronous spectrofluorimetry has been reported: aluminium with 8-hydroxyquinoline-5-sulphonic acid [9], beryllium with 4-hexyloxysalicylaldehyde-4-ethoxysalicylhydrazone [10], boron with chromotropic acid [11], cadmium with benzyl-2-pyridylketone-2-quinolylhydrazone [12], magnesium with salicylaldehyde-2-pyridylhydrazone [13] and 2-quinizarin sulphonate [14], scandium with 1,2,7-trihydroxyanthraquinone [15] and zinc with 2-furaldehyde-2-pyridylhydrazone [16].

In this paper a simple, quick and sensitive first-derivative synchronous spectrofluorimetric method is proposed for the determination of boldine, based on its native fluorescence. The procedure does not require a separation step and has satisfactorily been applied to the determination of boldine in different pharmaceutical products.

Experimental

Apparatus

All spectrofluorimetric measurements were performed using a Perkin–Elmer LS 5 luminescence spectrometer, equipped with a xenon discharge lamp (9.9 W) pulsed at line frequency, Monk-Gillieson F/3 monochromators, a Rhodamine 101 counter to correct the excitation spectra, a Hamamatsu R298 photomultiplier and a Braun Melsungen Thermomix 1441 thermostat. In order to compare all the spectrofluorimetric measurements and ensure reproducible experimental conditions, the LS 5 spectrometer was checked daily with a fluorescent polymer standard of *p*-

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terphenyl (10^{-7} mol l^{-1}) having a relative fluorescence intensity of 90% when measured at the wavelength of maximum emission (λ_{em}) of 340 nm and wavelength of excitation (λ_{ex}) of 295 nm, excitation and emission slit-widths of 2.5 nm and a sensitivity factor of 0.594. For synchronous fluorescence measurements, both excitation and emission monochromators were locked together and scanned simultaneously.

The LS 5 spectrometer was interfaced to a IBM PS/2 30-286 microcomputer using RS 232C connections for spectral acquisition and subsequent manipulation of spectra as described previously [17]. The contour plots in the excitation and emission plane were obtained by linking points of equal fluorescent intensity. Smoothed and derivative spectra were calculated by the Savitzky–Golay method [18, 19] using the Beckman Data Leader Software [20]. A Canon BJ-300 printer was used for graphical representation.

Reagents

All the experiments were performed with analytical-reagent grade chemicals. Double-distilled demineralized water was used throughout.

Boldine stock solution, 1.0 mg ml^{-1} was prepared by accurately weighing about 25 mg of the reagent (Sigma, St Louis, MO) and diluting to 25 ml with 0.1 N sulphuric acid. This solution, stored in a dark glass bottle at 4°C, is stable for several weeks. Working solutions were obtained by appropriate dilution with 0.1 N sulphuric acid.

Basic procedure

An aliquot of the sample solution containing between 0.1 and 2.5 μg of boldine was transferred to a 50-ml calibrated flask and diluted to the mark with 0.1 N sulphuric acid. A blank solution of 0.1 N sulphuric acid was also prepared.

The synchronous spectra were recorded at 20.0 ± 0.5°C, using the following fixed instrumental parameters: $\Delta\lambda = \lambda_{em} - \lambda_{ex} = 90$ nm, a scan speed of 240 nm min^{-1}, and a spectrometer response time of 1 s. The spectra were then stored on a disk file, corrected for the blank signal and smoothed by the use of 15 experimental points. The first-derivative spectra were calculated by the Savitzky–Golay method [18, 19] with an interval of 8 nm. First-derivative analytical signals were measured as the vertical distance from the first-derivative synchronous spectrum at $\lambda_{ex}/\lambda_{em}$ = 269/359 nm to $\lambda_{ex}/\lambda_{em}$ = 318/408 nm.

A calibration graph was constructed in the same way by using boldine solutions of known concentration.

Procedure for pharmaceuticals

Sambil tablets (Lacer S.A.) and Menabil Complex tablets (Menarini S.A.): 10 tablets were powdered and mixed, then 0.5 g of the mixture were extracted with 100 ml of 0.1 N sulphuric acid sonication and filtered through Whatman No. 1 filter paper.

Boldosal (Ern S.A.): 0.5 g were dissolved in 100 ml of 0.1 N sulphuric acid.

Aliquots of the sample solutions were treated as described under *Basic procedure* except that for the Menabil Complex preparation the first-derivative analytical signal was measured as the vertical distance in the first-derivative synchronous spectrum at $\lambda_{ex}/\lambda_{em}$ = 318/408 nm to the base line, owing to the interference from the matrix at $\lambda_{ex}/\lambda_{em}$ = 269/359 nm.

Results and Discussion

Spectral characteristics and effect of experimental variables

Boldine in 0.1 N sulphuric acid has two excitation maxima at 280 and 300 nm and an emission maximum at 370 nm. Figure 1 shows the three-dimensional spectra of boldine after correction for the contribution of the blank solution. These spectra are represented as an isometric projection in which the emission spectra have been plotted at 4-nm increments of the excitation wavelength. For optimum excitation and emission, slit-widths of 5 nm were selected in both instances.

The effect of pH on the fluorescence of boldine has been studied by Gürkan [4], who showed that although the maximum fluorescence intensity occurred at pH 9.0–10.0, the optimum pH in the range was rather critical and the solution had to be adequately buffered. However, boldine also fluoresces at strongly acidic pH and in order to make the method simple, 0.1 N sulphuric acid was selected, thus avoiding the use of a complex buffer solution.

The effect of temperature on the relative fluorescence intensity (RFI) of the compound was studied in the range 5–70°C. The results obtained show that RFI decreases when the

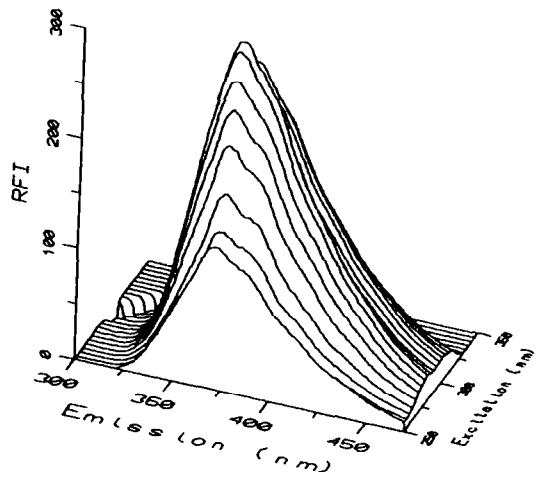

Figure 1
Projected three-dimensional spectrum of the boldine in 0.1 N sulphuric acid. Increments in excitation wavelengths were 4 nm for each emission scan and the scan speed was 240 nm min^{-1}.

temperature increases, but this effect is completely reversible. The decreases in RFI were 15% at 20°C, 39% at 40°C and 56% at 60°C compared to that at 5°C. All fluorescence measurements reported here were made at 20.0 ± 0.5°C.

Fluorescence develops immediately and remains constant for several hours. No photodecomposition was observed.

Selection of the optimum $\Delta\lambda$ *for synchronous scanning*

In Fig. 2 the three-dimensional spectrum has been transformed into a contour plot in the excitation–emission plane. The contour representation of the fluorescence profile of the boldine solution provides the most suitable trajectory in the excitation–emission matrix, in order to obtain synchronous fluorescence spectra. The parallel diagonal line superimposed on the contour plot represents the scan path through the excitation–emission matrix that would be obtained with synchronous scans at the wavelength interval chosen. The optimum path for determining boldine was $\Delta\lambda$ = 90 nm because it passes through the maximum, allowing the determination of boldine without loss of sensitivity [21].

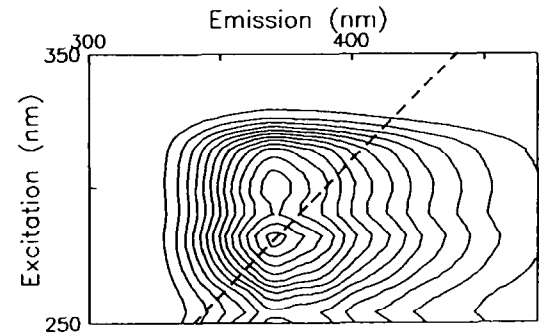

Figure 2
Contour plot of the excitation–emission matrix of the boldine in 0.1 N sulphuric acid. The synchronous fluorescence path (---) slices the data matrix at $\Delta\lambda$ = 90 nm.

Figure 3 shows the synchronous fluorescence spectrum of boldine, corrected for the blank signal, obtained with a constant interval between the emission and excitation wavelengths of 90 nm.

Instrumental parameters

A scan speed of 240 nm min^{-1} and a luminescence spectrometer response time of 1 s were selected after verifying that these parameters hardly affect the derivative signal obtained, because the differentiation is obtained numerically and not electronically.

To reduce the noise levels on the synchronous derivative spectra, a smoothing function based on the Savitzky–Golay method and 15 experimental points [18, 19] were used. For the calculation of the derivative spectra by the Savitzky–Golay method, an interval of 8 nm was selected to give the best signal-to-noise ratio.

Figure 4 shows the first-derivative spectrum of boldine obtained with these optimized variables after the contribution of the blank solution has been subtracted.

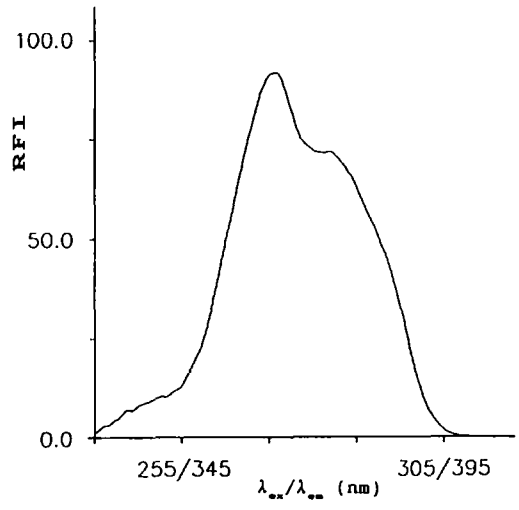

Figure 3
Synchronous fluorescence spectrum of boldine in 0.1 N sulphuric acid ($\Delta\lambda$ = 90 nm).

Table 1
Analysis of boldine in formulations

Proprietary name	Composition (%)*		Found†	Recovery (%)
Sambil tablets	Boldine	0.03	0.0271 ± 0.0005%	90.3
	N-(hydroxymethyl)nicotinamide	50.00		
	Wheat starch	4.70		
Boldosal	Boldine	0.02	0.0204 ± 0.0005%	102.0
	Inosine	0.25		
	$NaHCO_3$ an.	49.05		
	$NaHPO_4$ anh.	8.38		
	$MgSO_4$	1.05		
	Na_2SO_4 anh.	3.15		
Menabil Complex tablets	Boldine	0.07	0.068 ± 0.001%	97.3
	Methionine phenylbutyrate	7.14		
	Phenypentol succinate	3.57		
	Nicotinamide α-(hydroxy-1-cyclohexyl)-butyrate	3.57		
	Plattner's bile salts	3.57		
	Rhubarb dry extract	7.14		
	Artichoke extract	2.86		
	Belladonna dry extract	1.43		
	Cascara sagrade dry extract	3.57		

*Indicated by the suppliers.
†Mean values ± standard deviation of five determinations.

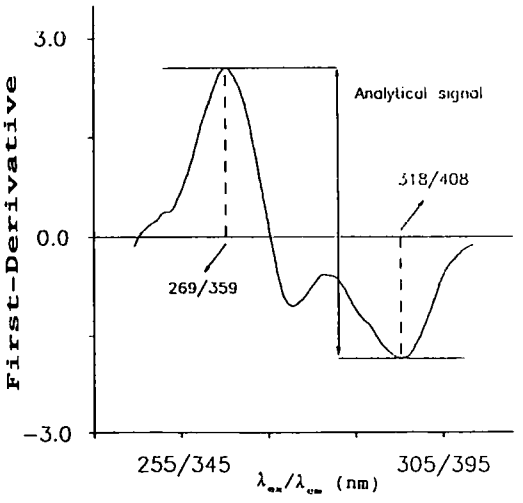

Figure 4
First-derivative synchronous spectrum of boldine in 0.1 N sulphuric acid (Δλ = 90 nm).

Analytical parameters

Under the recommended conditions, there is a linear relationship between the analytical signal (AS) and boldine concentration (C) over the range 2.0–50.0 μg l^{-1} [AS = 0.001 + 0.213C (r = 0.9997, n = 10)].

The reproducibility of the proposed method was checked with a series of 10 samples having boldine concentration of 25 μg l^{-1}; the relative standard deviation (RSD) was 2.1%.

The IUPAC detection limit (k = 3) [22] is 0.4 μg l^{-1} and the quantification limit (k = 10) [23] 1.4 μg l^{-1}.

Applications

The proposed method was applied to the determination of boldine in all the commercial drug formulations of The Spanish Pharmacopeia containing this alkaloid. The samples were prepared and analysed as described in the Experimental section.

It should be noted that the analytical signal chosen for the determination of boldine in Menabil Complex preparation was different to that described in *Basic procedure*. This is due to the serious interference from the matrix.

The results obtained, summarized in Table 1, show good agreement with the composition values indicated by the suppliers.

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