

Spectrophotometric determination of ajmaline and brucine by Folin Ciocalteu's reagent

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Abstract: A rapid and simple spectrophotometric procedure is described for the determination of ajmaline and brucine. The method is based on the development of blue coloured product due to reduction of tungstate and/or molybdate in Folin Ciocalteu's reagent (FCR) by ajmaline and brucine in alkaline medium. The colour is stable for more than 48 h. The chromogenic reaction has λ_{\max} at 540 nm with molar absorptivity 1.64×10^4 and 2.37×10^3 $\text{l mol}^{-1} \text{cm}^{-1}$ in the Beer's law range 1–8 $\mu\text{g ml}^{-1}$ and 10–100 $\mu\text{g ml}^{-1}$ for ajmaline and brucine, respectively.

Keywords: spectrophotometry, ajmaline, brucine, chemical kinetics, Folin Ciocalteu's reagent.

INTRODUCTION

The determination of ajmaline (rauwolfia alkaloid) and brucine (strychnos alkaloid) is of importance to pharmaceutical chemists and toxicologists. Ajmaline not only lowers blood pressure but also has a potent antiarrhythmic effect. Studies have shown that ajmaline specifically depresses intraventricular conduction, suggesting this would be particularly effective in the treatment of re-entrant ventricular arrhythmias.^{1,2} Spectrophotometric methods have been reported for the determination of some rauwolfia³ and strychnos^{4,5} alkaloids but no reference is available for ajmaline. The use of Folin Ciocalteu's reagent is reported in the spectrophotometric determination of phenols⁶ and salbutamol sulphate.⁷

The present work describes the use of FCR for the spectrophotometric determination of ajmaline and brucine. The method is based on the reduction of tungstate and/or molybdate in FCR by ajmaline and brucine in alkaline medium resulting in a blue coloured product (λ_{\max} 540 nm).

EXPERIMENTAL

Reagents

Ajmaline (Extra pure, SRL, India) and brucine (AR, CDH, India) were used. Folin Ciocalteu's reagent and sodium carbonate were of analytical reagent grade (Qualigens, India).

Stock solutions of ajmaline and brucine were prepared by dissolving exactly 0.5 g of the alkaloid in about 10 ml of ethanol, stirring for 10 min and diluting to volume with double distilled water in a 50 ml volumetric flask. A working solution of ajmaline ($100 \mu\text{g ml}^{-1}$) was prepared by diluting 1 ml of stock solution with water and brucine ($1000 \mu\text{g ml}^{-1}$) was prepared by diluting 10 ml of its stock solution to 100 ml with water.

Apparatus

A thermostatically controlled water bath was used and absorbance measurements were made using a Bausch and Lomb Spectronic-20 spectrophotometer.

General procedure

Appropriate volumes of working solutions of ajmaline or brucine were transferred into a series of 50 ml volumetric flasks. Sodium carbonate solution (10 %, 15 ml) was added to each followed by Folin Ciocalteu's reagent (20 %, 15 ml). The flask and its contents were swirled and diluted to the mark with double distilled water. The flask was placed in a water bath maintained at $50 \pm 2^\circ\text{C}$ for 30 min. After cooling under tap water, the absorbance at 540 nm was measured against the reagent blank.

RESULTS AND DISCUSSION

In the alkaline medium, ajmaline and brucine react instantaneously with the FCR resulting in blue coloured products with similar spectra (Fig. 1). The similarity of λ_{max} for both the alkaloids seems to be due to a common reaction mechanism *i.e.*, the oxidation of the alkaloids and the reduction of FCR (blue colour). A strict sequence of addition of reagents is necessary as it plays an important role. FCR is a mixture of acids and involves the following chemical species:



The blue colour formation by FCR with alkaloids seems analogous to Folin phenol protein reaction.⁸ Alkaloids probably reduce tungstate and/or molybdate in FCR producing reduced species which have a characteristic intense blue colour with λ_{max} at 540 nm.

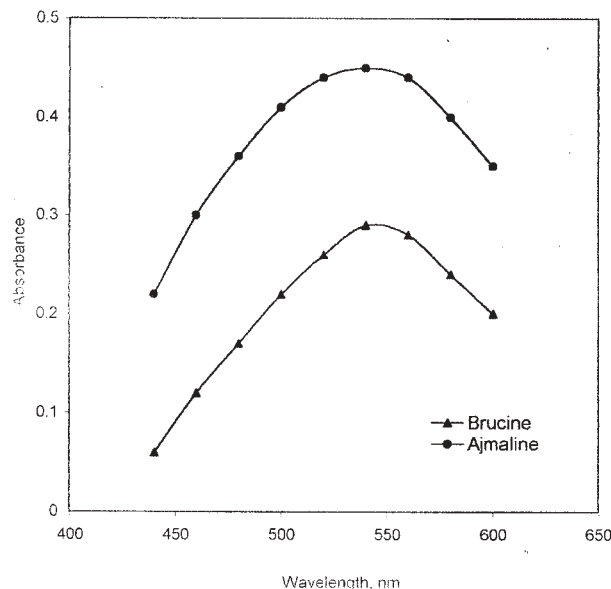


Fig. 1. Absorption spectra of the reaction products of Folin Ciocalteu's reagent with ajmaline ($10 \mu\text{g/ml}$) and brucine ($50 \mu\text{g/ml}$).

Under the conditions described, the calibration graphs for the measurement at 540 nm are linear over the concentration ranges 1–18 $\mu\text{g ml}^{-1}$ (ajmaline) and 10–100 $\mu\text{g ml}^{-1}$ (brucine). The regression equations derived for the calibration systems by the least-squares method⁹ were:

$$A = 0.0009 + 0.044 c \quad \text{Ajmaline}$$

$$A = 0.024 + 0.006 c \quad \text{Brucine}$$

with correlation coefficients of 0.923 and 0.996, respectively; c is the concentration of the alkaloid in $\mu\text{g ml}^{-1}$. The slope of the calibration curves reflects the degree of formation of the alkaloid–FCR product.

The optical characteristics, such as Beer's law limits, molar extinction coefficients and per cent relative standard deviations (for five measurements spanning three fourth of the Beer's law limits) were calculated. The results are summarized in Table I.

TABLE I. Optical characteristics and precision

Parameters	Ajmaline	Brucine
Beer's law limit ($\mu\text{g/ml}$)	1–18	10–100
Molar extinction coefficient ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	1.64×10^4	2.37×10^3
% Relative standard deviation	0.860	0.590

The effect of various experimental conditions on the absorbance was studied. The maximum absorbance was obtained with a 3 % concentration of sodium carbonate (Fig. 2) and a 6 % one of FCR (Fig. 3). Three concentrations were, therefore, selected as optimal. The effect of temperature (30–50 °C) and reaction time (0–30 min) on the absorbance revealed that the maximum and constant absorbance is obtained after 30 min at 50 °C (Fig. 4). The colour of the product was stable for more than 48 h.

The effect of the FCR concentration on the reaction rate of ajmaline was studied at 50 °C. Its concentration was varied from 4–8 % keeping the ajmaline concentration constant

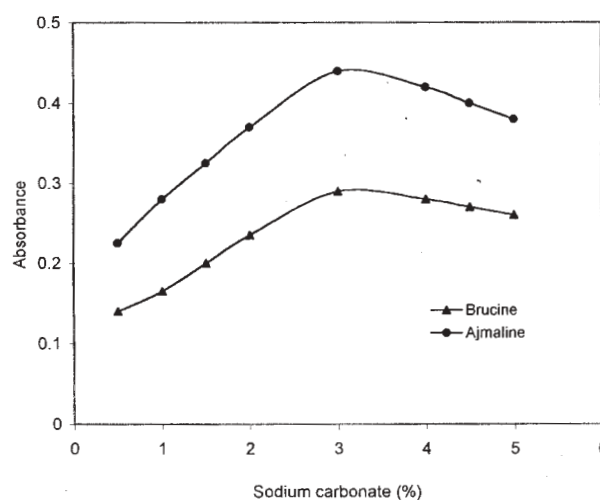


Fig. 2. Effect of sodium carbonate concentration on the absorbance of ajmaline (10 $\mu\text{g/ml}$) and brucine (50 $\mu\text{g/ml}$).

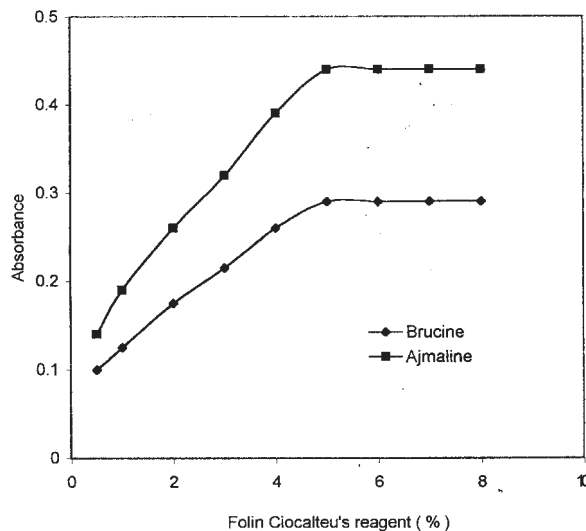


Fig. 3. Effect of the concentration of Folin Ciocalteu's reagent on the absorbance of ajmaline ($10 \mu\text{g/ml}$) and brucine ($50 \mu\text{g/ml}$).

($3.063 \times 10^{-4} \text{ mol dm}^{-3}$). The results (Table II) show that an increase in the FCR concentration causes an increase in the rate constant. The values of $k_{1\text{obs}}$ are greater than the values of $k_{2\text{obs}}$ by a factor of about 7–12. No significant changes in the values of $k_{1\text{obs}}$ and $k_{2\text{obs}}$ occurred when the ajmaline concentration was varied from 1.53×10^{-4} to $4.59 \times 10^{-4} \text{ mol dm}^{-3}$, keeping the FCR at a constant excess concentration, which confirms the first order dependence on the alkaloid concentration.

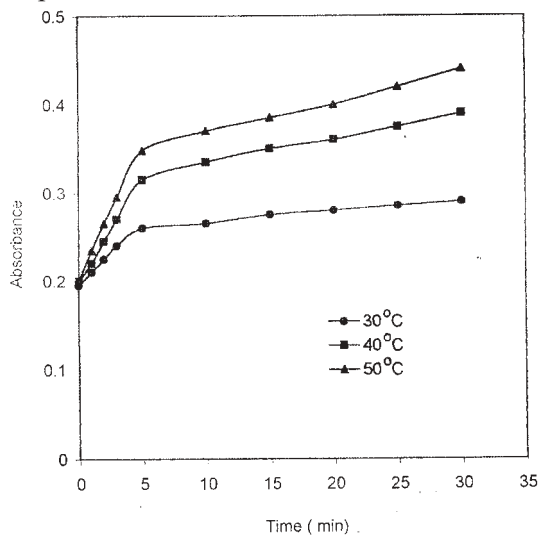


Fig. 4. Effect of reaction time and temperature on the absorbance of ajmaline ($10 \mu\text{g/ml}$).

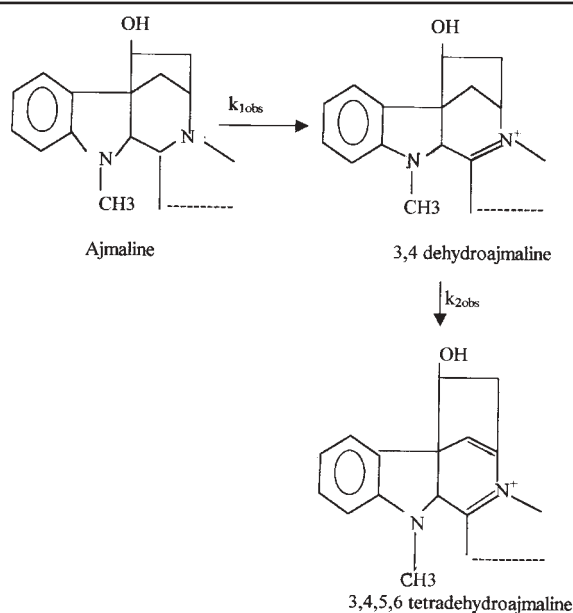
Figure 4 shows that the initial rate of the reaction is very fast, during the first 5 min, then the rate of reaction decreases remaining virtually constant after 30 min. The oxidation of ajmaline seems to proceed as two consecutive, pseudo-first-order reactions with rate constants $k_{1\text{obs}}$ and $k_{2\text{obs}}$. A similar oxidation mechanism has been proposed for reserpine.^{10–12}

TABLE II. Observed rate constants for the reaction of ajmaline with Folin Ciocalteu's reagent at 50 °C in 3 % sodium carbonate with a ajmaline concentration of $3.063 \times 10^{-4} \text{ mol dm}^{-3}$

Folin Ciocalteu's reagent/%	$k_{1 \text{ obs}}/\text{min}^{-1}$	$k_{2 \text{ obs}}/\text{min}^{-1}$	$k_{1 \text{ obs}}/k_{2 \text{ obs}}$
4.0	0.024	0.0020	12.0
6.0	0.028	0.0040	7.0
8.0	0.032	0.0045	7.1

TABLE III. Recovery results of ajmaline

Amount added/ $\mu\text{g mol}^{-1}$	Recovery*/%		t_{cal}
	Proposed method	Official method	
3.5	100.59 \pm 0.97	100.12 \pm 0.80	0.84
5.0	100.75 \pm 0.56	100.23 \pm 0.49	1.56
6.5	99.83 \pm 0.77	100.37 \pm 8.0	1.07
8.0	101.21 \pm 0.89	100.44 \pm 0.53	1.66
9.5	100.23 \pm 0.82	99.51 \pm 0.79	1.41



Statistical analysis of the result (Table III) obtained by applying the newly proposed and official procedure¹³ indicated that the difference between the mean recoveries obtained for ajmaline was statistically insignificant. The performance of the method was also assessed by the *t*-test. At 95 % confidence level, the calculated student *t*-values did not exceed the tabulated values, indicating that the proposed and official methods are equally accurate. The investigated method is simpler, faster and more sensitive than the official one. These advantages favor its application in the analysis and quality control of pharmaceutical formulations containing ajmaline.

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ИЗВОД

СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ АЈМАЛИНА И БРУЦИНА
КОРИШЋЕЊЕМ FOLIN CIUCALTEU-ОВОГ РЕАГЕНСА

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Описана је брза и једноставна процедура за одређивање ајмалина и бруцина. Метода је заснована на стварању плаво обојених производа који настају редукцијом волфрамата и/или молибдата у Folin Ciocalteu-овом реагенсу (FCR) ајмалином или бруцином у алкалном раствору. Боја је стабилна дуже од 48 часова. Реакција која ствара обојење даје λ_{\max} на 540 nm са моларном апсорбанцијом 1.64×10^4 , односно $2.37 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ са применљивошћу Беровог закона у области концентрација $1\text{--}8 \text{ }\mu\text{g ml}^{-1}$, односно $10\text{--}100 \text{ }\mu\text{g ml}^{-1}$, за ајмалин, односно бруцин.

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