Spectrophotometric Determination of Ephedrine with Ninhydrin.

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Summary: A new convenient and highly sensitive method for micro-amounts determination of ephedrine with ninhydrin is reported. Details of the working conditions for the method are discussed. The Beer-Lamberts' law is effectively obeyed in ephedrine concentration range of 4-36 $\mu g/ml$. The method has also been used for determination of ephedrine in ephedrine hydrochloride tablets and ephedrine sulphate injections and the results are found consistent.

Ephedrine (1) is an important alkaloid which is used in the treatment of bronichial asthama, in allergic conditions like utrecaria and also in nocturnal anuresis. It is also used to raise the blood pressure in cases of shock and spinal anaesthesia effects.

Along with different other types e.g. volumetric[1-3], gravimetric[4-5] and titrimetric[6-8], several colorimetric[9-12] and spectropnotometric[13-20] methods for determination of ephedrine are available in the literature. Out of these, the colorimetric and the spectrophotometric methods are the most sensitive. However, some of these later methods require rather difficult and time-consuming procedures e.g. the colorimetric method reported by Rojahn and Seifert[10] requires the precipitation of the alkaloidal picrate, washing the precipitate, cleaning it with ammonia and extracting the base with chloroform before determining the amount of picric acid, which had stoichiometrically combined with the precipitated base, colorimetrically as the ammonium salt in aqueous solution. Another method[9] requires extraction of the coloured compound produced in benzene before measuring the color intensity. Some other spectrophotometric methods[15,19] are not applicable in aqueous medium.

We, in this paper, report a new, simple, convenient and more sensitive spectrophotometric method for determination of ephedrine. The procedure of this method is much easier than some of the earlier reported methods. Also, our method does not require extraction of the coloured compound produced at any stage. Furthermore, the method is applicable in aqueous medium and does not require highly acidic or basic conditions. In this method we have used ninhydrin (II), also known as indane- 1,2,3-trione monohydrate, as a color producing agent, Earlier this reagent has been effectively used for spectrophotometric determination of some organic compounds e.g. p-phenylenediamine and p-amino-phenol[21], pyridine-4-carboxyhydrazide[22] and barbituric acid[23]. For spectrophotometric determination of ephedrine our method consists of mixing the aqueous solution of ephedrine and ninhydrin, adjusting the pH to 8.0 using boric acid-sodium hydroxide buffer solution, heating the mixture on a water-bath (60°) for fifteen minutes and recording the absorbance of the solution at 402 mm. Both ephedrine and ninhydrin as well as boric acid-sodium hydroxide buffer solution do not

CH(OH)–CH(CH$_3$)–NH–CH$_3$

I

II
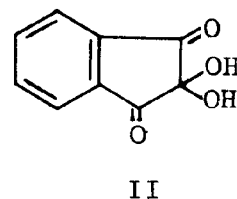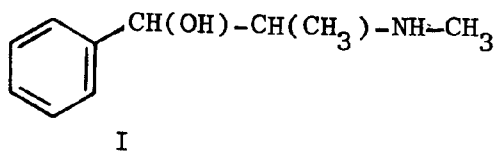
absorb at this particular wavelength. Also, the blue color produced is stable for not less than six hours and the Beer-Lamberts' law is obeyed in the concentration range of 4-36 μ g/ml of ephedrine. Thus, the sensitivity of the method is much higher than some of the earlier methods reported[13-14] for determination of ephedrine.

Experimental

Reagents: Ephedrine hydrochloride (pure) Marker Alkalaid Co., ninhydrin (Puriss p.a) Fluka, boric acid (b.p.) E. Merck, potassium chloride (reagent grade) and sodium hydroxide (reagent grade) B.D.H.

Apparatus and Instruments: The UV and Visible spectra were recorded on a Hitachi model 323 recording spectrophotometer. A matched set of two quartz cells of 1-cm path-length was used for these recordings. Fischer model Accumet 230 pH-meter with a set of glass and calomel electrodes was used for pH adjustments and measurements.

Stock sloutions: (i) A stock solution of ninhydrin was prepared by dissolving 200 mg of the reagent in 100 ml distilled water. This solution, thus, contained 2.00 mg/ml of ninhydrin.

(ii) A stock solution of ephedrine was made after extraction of ephedrine from ephedrine hydrochloride as follows. The free base was liberated with equivalent moles of sodium hydroxide, extracted with ether solvent and recrystallised from ethanol. 100 mg of ephedrine was then dissolved in 100 ml distilled water. This solution contained 1.00 mg/ml of ephedrine.

(iii) Boric acid-sodium hydroxide buffer solution was prepared by mixing 4.0 ml of 0.50 M aqueous hydroxide solution with 50.0 ml of an aqueous solution containing 1.5460 g (0.50 moles) of boric acid and 1.8637 g (0.50 moles) of potassium chloride and raising the total volume to 100 ml with distilled water. This solution when diluted five times gave a solution with pH ranging from 7.9 - 8.0.

Preparation of the Calibration Curve: 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and 2.2 ml of stock solution of ephedrine was taken in different 50-ml volumetric flasks. 2.0 ml of ninhydrin stock solution and 10.0 ml of boric acid-sodium hydroxide buffer solution was pipetted out in each of these flasks. After making up the volume with distilled water, the flasks were thoroughly shaken and pH of each solution was checked. It was observed that pH of all these solutions were fairly constant ranging from 7.9 to 8.0. All these solutions were then subjected to heat using a water-bath (60°) for fifteen minutes. The solutions were then allowed to cool to room temperature (15°) and absorbance of each solution was measured at 402 nm using aqueous ninhydrin solution (80 μ g/ml) as reference. However, it was pre-established that addition of boric acid-socium hydroxide buffer solution did not effect either the absorbance or the wavelength of the maximum absorbance (λ_{max}). The Calibration curve was then made by plotting the absorbance as a function of ephedrine concentration. Except for the first sample (containing 2.0 μ g/ml of ephedrine) which had no absorbance at 402 nm all other points resulted in a linear plot (Fig. 1) with negative deviation starting at concentration of ephedrine higher than 36 μ g/ml. This calibration plot was subsequently used for determination of ephedrine as such, in ephedrine hydrochloride tablets and in ephedrine sulphate injections.

Results and Discussions

When an aqueous solution of ephedrine (10^{-3}M) is mixed with an equimolar aqueous solution of ninhydrin, at room temperature (15°) a yellow coloured solution is immediately obtained. This color slowly darkens and in about two hours it turns blue. This blue color remains stable for more than six hours. However, when the mixture of an aqueous solution of ninhydrin and ephedrine is heated at 60° the blue color appears within fifteen minutes. The absorption spectrum of this blue coloured solution showed two bands in the visible region; a sharp band with λ_{max} at 402 nm and the other, relatively broad band with λ_{max} at 570 nm. However,
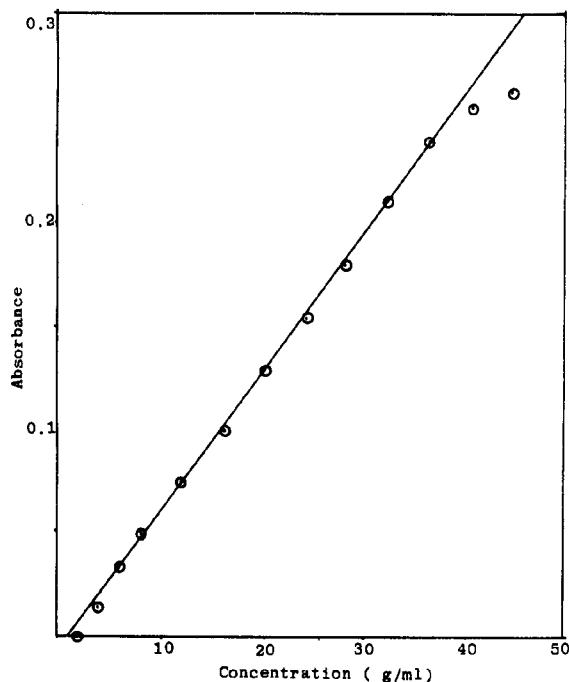
Fig. 1: Calibration polt for ephedrine

Fig. 2: Effect of heat and time on absorbance of ephedrine-ninhydrin system, [ephedrine] = 20µg/ml, [ninhydrin] = 80 µg/ml. 'A' without heating, 'B' with heating at 60°.

the absorbance at 402 nm was much higher than that at 570 nm. Therefore, the λ_{max} at 402 nm was selected for the spectrophotometric determination of ephedrine. Ninhydrin, ephedrine and boric acid do not absorb at this wavelenght. The extinction co-officient of absorbance of the blue coloured solution, at 402 nm, as calculated from the slope of the calibration plot is 1083.

Effect of heat and time on absorbance: Fig. 2 shows the effect of heat as well as time on absorbance at 402 nm. This is evident from the figure that when the aqueous mixture of ninhydrin and ephedrine are kept at room temperature the absorbance gradually increases and becomes constant after 100 minutes. However, when the mixture is heated at 60° same absorbance in observed within fifteen minutes. In order to make the determination method quicker it was decided to heat the aqueous mixture of ninhydrin and ephedrine using a water-bath at 60° for fiteen minutes.
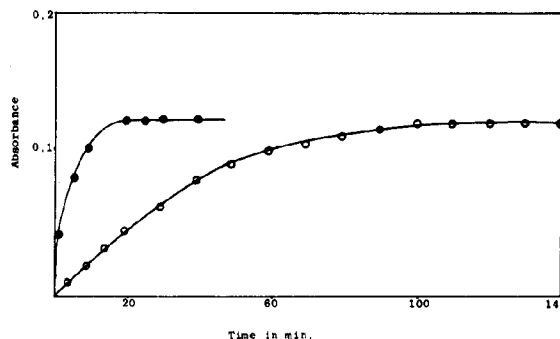
Effect of pH on absorbance: The effect of variation of pH on the absorbance of ninhydrin-ephedrine system at 402 nm was studied in the pH range of 4.0 - 12.0. The effect is illustrated by Fig. 3. This figure reveals that the absorbance initially increases with the increase in pH; becomes constant in the pH range of 7.5 - 8.5 and then starts decreasing. It is important to mention that no change in the λ_{max} was observed with the variation in the pH. Also, the intensity of the color of solutions at pH higher than 10.0 decreased rapidly and the solutions became almost colorless after about fifty minutes. However, at pH range of 7.5 - 8.5, the color remained stable for more than six hours. Therefore, it was found feasible to use this pH range and stability of color for spectrophotometric determination of ephedrine.
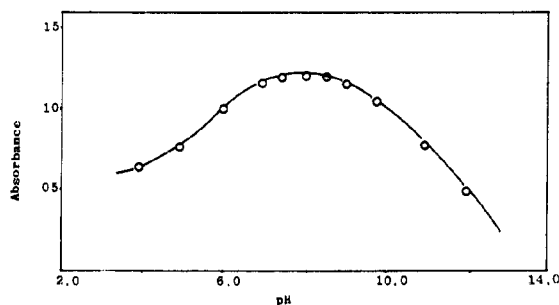

Fig. 3. Effect of pH on absorbance of ephedrien-ninhydrin system, [ephedrine] = 20 µg/ml, [ninhydrin] = 80 µ/ml.

Minimum molar ratio of ninhydrin reagent: The minimum molar ratio of ninhydrin to ephedrine for producing maximum absorbance, at the specified conditions, was determined by Jobs' method[24]. This ratio was established to be 1:1. Excess of ninhydrin had no effect either on absorbance or on the λ_{max} or ninhydrin-ephedrine system.

Sensitivity of the method: The spectrophotometric method for determination of ephedrine was found to be highly sensitive and using this method micro-amounts of ephedrine can be determined accurately. It appears from the calibration plot (Fig.1) that the Beer-Lamberts' law is obeyed within the concentration range of 4-36 μg/ml of ephedrine. However, the calibration plot does not start from the origin and negative deviation from the Beer-Lamberts' law starts appearing at concentration of ephedrine higher than 36 μg/ml. The method was applied to determine the amount of ephedrine in several standard aqueous solutions and the results of these determinations are reproduced in Table 1. It is evident from these results that the % difference in the amount of ephedrine taken and found by this method is relatively small and never exceeds ± 2.0%. Also, the sensitivity of the method is much higher than some of the other spectrophotometric methods[13-14].

Determination of ephedrine in tablets: Seven ephedrine hydrochloride tablets (manufactured by Marker Alkaloid Co., Quetta) labelled to contain 30 mg of ephe-

drine hydrochloride per tablet were subjected to determination of ephedrine by this method. For this purpose each tablet was weighed, powdered and dissolved in about 60 ml distilled water. Then, equivalent moles of sodium hydroxide were added to liberate the free base. After filtration of the binders, the volume of the solution was raised to 100 ml with distilled water in a volumetric flask. From each tablet solution two independent determinations of ephedrine were made as follows. 5.0 ml of the tablet solution was taken in a 50-ml volumetric flask containing 2.0 ml of aqueous ninhydrin stock solution (2.0 mg/ml). This solution was then diluted with about 30 ml distilled water and then 10 ml of boric acid-sodium hydroxide buffer solution was added

Table 1. Determination of Ephedrine

Amount taken (μg/ml)	Amount found (μg/ml)	% difference
5.00	5.10	+2.00
7.50	7.45	−0.67
10.00	10.15	+1.50
15.00	14.80	−1.33
20.00	19.80	−1.00
25.00	25.10	+0.40
30.00	30.30	+1.00

Table 2. Determination of Ephedrine in Ephedrine Hydrochloride Tablets

Tablet No.	Weight of tablet (mg)	Amount of ephedrine Calculated	Amount of ephedrine found	% difference
1	44.20	24.57	24.17	− 1.63
			24.27	− 1.63
2	44.28	24.57	24.22	− 1.43
			24.29	− 1.14
3	44.28	24.57	24.45	− 0.54
			24.29	− 1.14
4	44.35	24.57	24.85	+ 1.14
			24.45	− 0.54
5	44.35	24.57	25.06	+ 2.00
			24.97	+ 1.63
6	44.40	24.57	24.97	+ 1.63
			24.69	+ 0.54
7	44.45	24.57	24.57	0.00
			24.97	+ 1.63

*Calculated from the labelled amount of ephedrine hydrochloride (30 mg)

Table 3. Determination of Ephedrine in Ephedrine Sulphate Injections

Injection No.	Amount of ephedrine		
	*Calculated (mg)	found (mg)	% difference
1	23.13	23.46	+ 1.43
2	23.13	23.40	+ 1.21
3	23.13	22.93	−0.43
4	23.13	22.76	− 1.55
5	23.13	23.33	+ 0.86
6	23.13	22.68	−1.99
7	23.13	23.50	+ 1.64

*Calculated from the labelled amount of ephedrine sulphate (30 mg).

to it. After making up the volume with distilled water, the flasks were thoroughly shaken and the pH of the solution was checked. In each case it was found to be 7.9 - 8.0. The solution was then heated on a water-bath (60°) for fifteen minutes, cooled to room temperature (15°) and the absorbance recorded at 402 nm using aqueous ninhydrin solution (80 μ g/ml) as reference. From this absorbance the amount of ephedrine in the tablet was calculated using the calibration plot (Fig. 1). The results of these determination are reported in Table 2. It is evident that these results are consistent among themselves but differ slightly from the labelled amount of ephedrine. This discrepency is explained by the fact that the weights of the tablets were not uniform.

Determination of ephedrine in ephedrine sulphate injections: Seven ephedrine sulphate injections (manufactured by Marker Alkaloid Co., Quetta) each labelled to contain 30 mg ephedrine sulphate were analysed for

the amount of ephedrine by the same procedure as described for the tablets above. The results of these determinations are listed in Table 3. These results are also consistent and agree well with the labelled amount. Here also, the % difference in the amount of ephedrine labelled and determined by our method does not exceed ± 2.0 %.

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