Spectrophotometric Determination of Some Indole Drugs

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Summary: A spectrophotometric method has been developed for the quantitative determination of indole drugs, i.e., yohimbine, reserpine and rescinnamine on complexation with iodine. The coloured complexes exhibit absorption maxima in the region 360-369 nm. The RSD (Relative Standard Deviation) of the method is 2.0%. The method is simple, rapid and convenient for routine analysis of the drugs.

Introduction

A large number of natural biologically important compounds are based on indole nucleus. Many of these are alkaloids and are used as antihypertensive and tranquillizing agents [1]. Some important examples are yohimbine, reserpine, rescinnamine, vinblastine and vincristine. The indole alkaloids have been the subject of considerable study as described in a comprehensive monograph [2]. Indole nucleus has been shown to act as a good electron donor in charge transfer complexes [3-5].

Intense colours are usually associated with charge transfer complexes in the solid state as well as in solution [6-12]. This property has been used as a basis of the quantitative determination of natural indoles [13].

A spectrophotometric methods for determining indole and its derivatives as charge-transfer complexes have been reported [14-16].

The object of this study is to develop a spectrophotometric method for indole compounds based on the above principle complexation with iodine.

Results and Discussion

Indole nucleus acts as a good electron donor in charge transfer complexes. A considerable amount of work involving charge transfer properties of indoles are available because several biologically important compounds possess the indole ring system and charge transfer phenomenon has been implied in explaining the mode of action [13,16-18].

The immediate appearance of colour is characteristic feature of the charge transfer complexes. The colour formed with iodine is undoubtedly due to complex formation [11,19].

The colour obtained for various indole compound with iodine has been shown in Table I. All colours were produced immediately on mixing the solution and were stable over the period of measurement (15 minutes).

Table I: Characteristic properties of coloured Indole complexes with iodine

S.No:	Indole compounds	Colour	Appearance of colour	Stability of colour (Minutes)	Concentration $M - X\ 10^{-4}$
1	Reserpine	Light yellow	Immediate	15-30	1.0 – 5.0
2.	Rescinnamine	Golden yellow	Immediate	10-30	1.0 – 5.0
3.	Yohimbine	Bright yellow	Immediate	5 – 35	10 – 6.0

These colours are ascribed to charge transfer transitions between the acceptor and the donor complexes [7]. Structurally similar indole gave similar colour with the complexing agents. The colours formed with the substituted indoles show a clear and consistent correlation with the electron donor-acceptor properties of the substituents [20]. The indole compounds gave a new colour (yellow) with iodine which is accordance to the Mullikens theory and most often 1:1 ratio [21].

Results show that, the present method is suitable for all those substituted indoles which form

stable coloured complexes with good complexing agent such as iodine. In case of indole-iodine complexes the stability of colour is not less than 15 minutes which is good enough for analytical work.

The absorption characterization of indole complexes provide a useful information from the qualitative and quantitative analytical aspects and also with regard to the basic understanding of parameters influencing complexation as shown in Table II.

Table II: Optical characteristics of indole complexes with iodine

S.No.	Indole compounds	Concentration range M X 10^{-4}	λ max	Molar absorbtivity
1	Yohimbine	1.0-5.0	360	3327.5
2.	Reserpine	1.0-5.0	369	2246.73
3.	Rescinnamine	1.0-6.0	369	2529.50

According to literature, the absorption band of indole-iodine complex lies at 367nm while theoretically it is expected to be in the region of about 370 nm [22]. Experimentally the absorption spectra of yohimbine with iodine was found to absorb at wavelength of 360 nm while reserpine and rescinnamine complexes with iodine showed λmax at 369 nm. The precision data of the complexes of indole compounds with iodine shown in Table III, IV. The Beer's lamberts law relationship of the iodine complexes is effectively obeyed in the concentration range studies.

Table III: Molecular ratio of yohimbine, reserpine and rescinnamine-iodine complex determination by isomolecular series method

Ratio/Absorbance	1:9	2:8	4:6	5:5	6:4	8:2	9:1
Yohimbine (S)	0.316	0.549	0.881	1.015	0.816	0.365	0.172
Rescinnamine (R)	0.015	0.031	0.058	0.075	0.064	0.044	0.032
Reserpine (Res)	0.725	0.343	0.528	0.651	0.558	0.378	0.265

S = Yohimbine (1×10^{-4} M)
R = Rescinnamine (2×10^{-5} M)
Res. = Reserpine (1×10^{-4} M)
Iodine (1×10^{-4} M)

Solvent Effect

Selection of solvent for the study for charge transfer complexes of indole-iodine is of considerable importance. The use of iodine as an acceptor makes the choice of solvent very limited. The stability of iodine complex is the best in chloroform as compared to carbon tetrachloride, cyclohexane and n-heptane [23]. The maximum λ max of iodine-indole complex is achieved in chloroform [24].

Molecular Ratio

It has been established that indole forms complexes usually in 1:1 ratio [13]. In the present study, isomolecular series method used to determine the molecular ratio of iodine complexes of the indole compounds. This method has already been used to determine the molecular ratio of indole complexes with different reagents [13, 17, 18, 25,]. It has been found that the molecular composition of the coloured complexes of the indolic compunds with iodine was found in the ratio of 1:1. The formation of 1:1 molecular ratio is also a strong evidence that indole forms complexes with iodine [21]. The result of isomolecular series method is shown in Table III.

Statistical Determination

The values of molar absorbtivity (ε) of the complexes were calculated for each individual compound as shown in Table II. Using these molar absorbtivity values the unknown concentration of indolic compunds were determined as shown in Table IV.

Table IV: Determination of indole compounds with iodine

Indole compounds	Weight (g)	Added conc M X 10^{-4}	Observed conc. M X 10^{-4}	RSD %	SD
Yohimbine	0.00573	3.25	3.23	0.73	0.0078
Reserpine	0.01365	4.48	4.47	0.30	0.0030
Rescinnamine	0.01063	3.35	3.34	.034	0.0029

On the basis of the results obtained during the study, the proposed method of analysis may be recommended for the quantitative determination of indole alkaloid by complexing with iodine. The method is simple and economical. The time period required for each analysis is not more than 30 minutes. The relative standard deviation (RSD) in each is within 2%.

Experimental

Material

1. Yohimbine [Methyl–16, 17–didehydro–19 α-methyl-18-oxayohimbine-16-carboxylate] $C_{21}H_{24}N_2O$

2. Reserpine [Methyl-18-O{3,4,5-trimethoxy benzoyl} reserpate] $C_{33}H_{40}N_2O_9$
3. Rescinnamine [Methyl-18-O-{3,4,5-trimethoxy cinnamoyl } reserpate] $C_{35}H_{40}N_2O_9$

Above donors were prepared in the laboratory.

4. Iodine and chloroform and other solvents were obtained from BDH of AR quality.
5. (i) Shimadzu W-150-02 double beam spectrophotometer and wave length 190-900 nm were used. (ii) Shimadzu W-240 visible recording spectrophotometer were also used.

Methods

Preparation of Solutions

Preparation of Indole Drugs Solution (Sol. A)

A X 10^{-4} M solution of yohimbine (1.762 mg/50 ml), reserpine (3.100 mg/50 ml), rescinnamine (3.200 mg/50 ml) were prepared in chloroform in 50 ml volumetric flask wrapped in aluminum foil to protect from light.

The above solution (A x 10^{-4} M) of yohimbine, reserpine and rescinnamine were further diluted to 2 x 10^{-5} M with chloroform in 50 ml volumetric flask. Wrapped the flask in aluminum foil to protect from light. The diluted solutions were then used for analytical determination.

Preparation of Complexing Agent Solution (Sol. B)

1 x 10^{-3} M solution of iodine (12.7 mg/50 ml)in chloroform was prepared in 50 ml volumetric flask and wrapped in aluminum foil to protect from light.

Colour Development

Indole Drug Solution (Sol. A) were treated with the solution of the complexing agent iodine (Sol. B) for the development of coloured complexes. The colour appeared immediately at room temperature, but the stability of coloured complexes started after some time and last for 15 to 30 minutes as shown in Table I. The time period for the stability of coloured complexes was quite sufficient for analytical purpose.

Method of Analysis

1.00 ml to 6.00 ml of individual indoles (Sol. A) was pipette out and placed into 10 ml volumetric flask separately. Flasks were wrapped in aluminum foil to protect from light. 1.0 ml of freshly prepared solution of complexing agent (Sol. B) was added to each flask and kept for a few minutes at laboratory temperature (25° – 28°) until a stable colour was obtained. After achieving the stability of the coloured complex solution, the volume of 10 ml volumetric flask was made up to the mark with the solvent (chloroform) and the absorbance of each coloured complex was measured at the respective absorption maximum of Shimadzu, W-240 visible recording spectrophotometer in a 1.0 cm cuvette using the reagent solution as a blank. Calibration curve was made for each indole solution and the validity of Beer's law was confirmed and was calculated by using Lambert-Beer's law.

Standard deviation of proposed method was calculated by using the equation

$$S.D = \sqrt{\frac{\varepsilon(Xi - X)^2}{n - 1}}$$

where

xi = each variable

$\bar{x}$ = mean of variable

n = number of variable

ε = summation of all variables

Relative standard deviation (RSD) was calculated in the form of percentage by using the relationship

$$\% RSD = \frac{S.D \times 100}{\bar{x}}$$

Iso-molecular series method was used to determine the composition of complexes [18,25].

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