has been implied in explaining the mode of action (Manzar & Alam, 1992; Manzar & Kost, 1980; Hager *et al*., 1986; Borazan & Ajeena, 1988; Manzar, 1981 and 1982).

The immediate appearance of colour is a characteristic feature of the charge transfer complexes. The colour formed with bromothymol blue is undoubtedly due to complex formation (Gyorgyi, 1960; Hutzinger, 1969; Manzar & Alam, 1992; Manzar & Kost, 1980; Hager *et al*., 1986; Borazan & Ajeena, 1988, Manzar, 1981; 1982 and Gyorgyii, 1961).

The colours obtained from various indole compounds with bromothymol blue have been shown in table 1. All colours were produced immediately on mixing the solution of each indole compound with bromothymol blue solution separately. All colours were stable over the period of measurement.

These colours were ascribed to charge transfer transitions between the acceptor and the donor complexes (Hammond & Burkardf, 1970). Structurally similar indole gave similar colour with the complexing agent like bromothymol blue. The colours formed with the substituted indoles show a clear and consistent correlation with the electron donor-acceptor properties of the substituents (Hutzinger, 1952). The indole compounds gave a new colour (greenish-yellow) at λ_{max} with bromothymole blue which is accordaning to the Mulliken Theory most the often produces complexation 1:1 (Mulliken, 1952). Results showed that the present method is suitable for all those substituted indoles which form coloured complexes with good complexing agent such as iodine and bromothymol blue (Manzar *et al*., 2000). In case of indole-bromothymol blue complexes the stability of colour is not less than 35 minutes which is good enough for analytical work.

The absorption characterization of indole complexes provide a useful information from the qualitative and quantitative aspects and also with regard to the basic understanding of parameters influencing complexation a shown in table 2.

According to literature, the absorption band of bromothymol blue with various vitamin B lies at 410 nm and coloured complexes with thiamine showed maximum absorption at 420 nm in chloroform (Gupta & Cadwallader, 1968). Experimentally the absorption spectra of yohimbine with bromothymol blue was found to absorb at wavelength 415 nm. While rescinnamine and reserpine showed λ_{max} at 416 nm. The Beer's & Lambert's law relationship of the bromothymole blue complex is effectively obeyed in the concentration range studies. Iso molecular series method was used to determine the complex composition. The complexes were formed into the ratio of 1:1 as shown in table 3. The ratio 1:1 is the most suitable for analytical work. For the first time, this ratio was allowed a complete quantification of mentioned indole compounds with bromothymol blue form the charge transfer complex.

Result of the analysis and the precision data of each complex of indole with bromothymol blue is shown in table 4. Results in table 4 exhibit that the statistical values are quite satisfactory for analytical purpose.

Results show that the present method is suitable for all those substituted indoles which form stable coloured complexes with good complexing agent like bromothymol blue. The stability of coloured complex is achieved upto 35 minutes which is good enough for analytical purpose.

Choice of solvent

Selection of solvent for the study of charge transfer complexes of indole-bromothymol blue is of considerable importance. The use of bromothymol blue as an acceptor makes the choice of solvent very limited. The stability of bromothymol blue complexes with indole is the best in chloroform as compared to other solvents like carbon-tetrachloride and n-heptane etc.

Molecular ratio

It has been established that indole forms complexes usually in 1:1 ratio (Manzar *et al.,* 2000). In the present study, isomoleculer series method used to determine the molecular ratio of bromothymol blue complexes with indole compounds. This method has already been used to determine the molecular ratio of indole compounds with different complexing agents (Manzar & Alam, 1992; Manzar & Kost, 1981; Hager *et al*., 1986; Borazan & Ajeena, 1988; Manzar, 1982; Manzar, 1982; Manzar, 1981;

Table 1

Characteristic properties of coloured indole complexes with bromothymol blue ($1x10^{-4}$ M)

S. No.	Indoles	Colour*	λ_{max}	Stability of colour (minutes)	Concentration in $Mx10^{-5}$ or $Mx10^{5}$
1.	Rescinnamine	Greenish yellow	Immediate	5-40	2.0-12.0
2.	Reserpine	Greenish yellow	Immediate	5-40	2.0-12.0
3.	Yohimbine	Greenish yellow	Immediate	5-45	2.0-12.0

*Colour appeared immediately.

Hutzinger, 1952; Mulliken, 1952; Manzar *et al.,* 2000). It has been found that the molecular composition of the coloured complexes of indolic compounds with bromothymol blue was in the ratio of 1:1. The formation of 1:1 molecular ratio is also a strong evidence that forms indole complexes with bromothymol blue (Mulliken, 1952).

EXPERIMENTAL

Materials

1. Rescinnamine [Methyl-18-0-{3,4,5-trimethoxy cinnamoyl} reserpate], $C_{35}H_{40}N_2O_9$. Mol. Wt. = 632.7
2. Reserpine [Methyl - 18-0-{3,4,5-trimethoxy benzyl] reserpate}, $C_{33}H_{40}N_2O_9$ Mol.wt. = 608.7
3. Yohimbine [Methyl -16, 17 - didehydro -19 - α-methly-18-oxayohimbine -16- carboxylate], $C_{21}H_{24}N_2O_3$ Mol. Wt. = 325.4

The above mentioned indolic compounds were synthesized in the laboratory.

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

Methods

Preparation of solutions (A)

Preparation of indole drug solution.

A x 10^{-4} M solution of :
Rescinnamine (3.20 mg/50 ml)
Reserpine (3.10 mg/50 ml)
Yohimbine (1.76 mg/50 ml)
Each indole stock solution having 10^{-4}M was prepared separately in chloroform in a 50ml volumetric flask. After preparation it was wrapped in aluminum foil to protect from light.

The above solutions (A x 10^{-4} M) of rescinnamine, resperpine and yohimbine were further diluted to 2 x 10^{-5} M separately with chloroform in a 50 ml volumetric flask. The light protected diluted solutions were then used for spectrophotometric determination.

Preparation of complexing agent solution (B)

Bromothymol blue B x 10^{-4} M solution of (3.12 mg/50 ml) was prepared in chloroform in a 50 ml volumetric flask and wrapped in aluminum foil to protect from light.

Confirmation of completion of indoles

Indole drug solutions (A) were treated with the solution of the complexing agent bromothymol blue (B) for the development of coloured complexes. The colour appeared immediately at room temperature. The formation of coloured complexes started after five (5) minutes and remained for 40 minutes as shown in table 1. The time period for the stability of coloured complexes was quite sufficient for analytical purpose. Structurally similar indoles gave similar colour with the complexing agent bromothymol blue. The colour formed with substituted

Table 2
Optical characteristics of indole complex with bromothymol blue

S. No.	Indole compound	Concentration range M x 10^{-5} at 416 nm	ε mole^{-1} cm^{-1}
1.	Rescinnamine	2.0 - 12.0	2944.4
2.	Reserpine	2.0 - 12.0	2732.0
3.	Yohimbine	2.0 - 12.0	2069.5

Table 3
Molecular ratio of Rescinnamine and yohimbine - bromothymol blue complex determination by iso molecular series method

Complex	Ratio / Absorbance						
	1:9	2:8	3:7	5:5	7:3	8:2	9:1
Rescinnamine – bromothymol blue complex	0.421	0.551	0.664	0.895	0.582	0.477	0.392
Yohimbine – bromothymol blue complex	0.228	0.410	0.511	0.717	0.491	0.331	0.210

Table 4
The precision data of indole compounds by complexing with bromothymol blue

S. No.	Indole Compound	Weight(g)	Added concentration	Observed concentration 10^5	S.D.	RSD%
1.	Rescinnamine	0.00240	7.50 x 10^{-5}	7.47	0.0020	0.91
2.	Reserpine	0.00171	5.50 x 10^{-5}	5.45	0.0018	1.21
3.	Yohimbine	0.00123	7.00 x 10^{-5}	6.86	0.0025	1.76

indoles showed a clear and consistent correlation with the electron donor acceptor properties of the substituents (Hutzinger, 1969).

Method of analysis

1.00 ml to 6.00 ml of individual indoles (sol. A) was pipetted out and placed into 10ml volumetric flask separately. Flask were wrapped in aluminum foil to protect from light. A 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°C to 28°C) 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 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 using Beer's & Lambert's law. Standard Deviation of proposed method was calculated by using the equation

$$S.D = \sqrt{\frac{\sum (xi - \bar{x})^2}{n-1}} \qquad (1)$$

where x_i = each variable
x = mean variable
n = number of variable
Σ = Summation of all variables

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

$$\%RSD = \frac{S.Dx100}{\bar{x}} \qquad (2)$$

Iso molecular series method was used to determine the composition of complexes (Manzar, 1982 and 1982).

CONCLUSION

On the basis of the results obtained during the study, the proposed method of analysis may be recommended for quantitative determination of indole alkaloids and their derivatives by complexing with bromothymol blue. The method is simple convenient and economical. The time period required for each analysis is not more than 20 minutes. The relative standard deviation (RSD) of each sample is within 2%.

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