CHAPTER III

Results and Discussion

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3. 1. Determination of the studied drugs by complex formation with acid dye

3. 1. 1. Absorption spectra of the studied drugs with BPB

In order to investigate the optimum reaction conditions for complete color development of the ion-pair complex formed between the studied drugs and acid dye BPB (1.0 x 10^{-4} M), the effect of different experimental variables were studied and recorded below.

3. 1. 1. 1. Effect of pH

In order to establish the optimum pH value for each ion-pairs formed between the studied drugs and BPB the investigated, Fexo., Fluox. or Azithr. was allowed to react with the BPB in aqueous buffered solution of pH's (2.0 – 12.0). The absorbance intensity was measured at its $\lambda_{max.}$ The highest absorbance values were obtained at pH 2.4 in case of Fexo., 2.2 in case of Fluox. and 2.2 in case of Azithr. which are selected for ion-pairs formation. These results are shown in Fig. (1). Furthermore, the amount of buffer was examined and found to be 2.0 ml in case of Fexo. and Fluox. and 3ml in case of Azithr. As shown in Fig. (2). The optimum wavelength corresponding to each ion–pair complex of the drugs with BPB is at 411 nm in case of Fexo., 410 nm in case of Fluox and at 413 nm in case of Azithr. As shown in Fig. (3).

3. 1. 1. 2. Effect of time

The time required for complete color development of the ion - pair formed between each of Fexo., Fluox. and Azithr. with BPB was investigated. Allowing the reactants to stand for different time intervals, it was observed that the time has no effect on the maximum color intensity. Consequently 2.0 min was enough for standing. The formed ion – pairs were found to be stable for more than 24 hours in case of Fexo., Fluox and Azithr. The shaking time required for complete color development of ion-pair complex formed between the drugs and BPB was investigated. Allowing the reactants to stand and shaking for different time intervals, it was observed that 2.0 min are quite sufficient to obtain maximum color intensity, before extraction by chloroform in the three cases (Fexo., Fluox and Azithr. complexes), as shown in Fig (4) .

3. 1. 1. 3. Effect of the extracting solvent

The polarity of the solvent affects both extraction efficiency and absorbance intensity. The results obtained using different extracting solvents (benzene, chloroform, carbon tetrachloride. Hexane, ethylene chloride), applying the BPB reagent on the drugs under consideration indicated that chloroform is the best solvent for extraction in the three cases (Fexo, Fluox and Azithr Ion-pairs). This solvent is selected due to its slightly higher sensitivity and the considerably lower extraction of the reagent itself. Complete extraction was attained by extraction with 3.0 ml of the solvent in one batch.

3. 1. 1. 4. Effect of reagent concentration

When various concentrations of BPB were added to fixed concentrations of Fexo., Fluox and Azithr drugs, 2.0 ml of BPB (1.0 x 10^{-4} M) in the three cases (Fexo., Fluox and Azithr.) solution as shown in Fig. (5) were found to be sufficient for the production of the maximum and reproducible color intensity. Higher concentration of the reagent decreased the absorbance and color intensity of the formed ion – pair.

3. 1. 1. 5. Composition of the ion-pair complexes and the stability constant of it.

In order to investigate the molecular ratio of the complexes formed between the drugs under investigation and BPB at the selected conditions, the molar ratio and continuous variation methods were carried out. The results indicated that the molar ratio of the drugs to dye was found to be (1: 1) in all ion – pair formed. The shape of the curves indicated that the complexes were labile, as shown in Figs. (6&7). Hence, a large excess of reagent must always be used to enhance the formation of the complex.

3. 1. 1. 6. Suggested mechanism

The mechanism which exhibit the acid dye technique is an ion – pair mechanism in which ion – pair is formed between negative ion produced form ionization of BPB, which is converted into BPB sodium salt in the buffer solution and positive ion e.g. Fexofenadine Hydrochloride as shown in Fig (8) the ion – pair formed exhibits maximum absorbance at 410 nm in case of Fexofenadine hydrochloride, 411 nm in case of Fluoxetine Hydrochloride and at 413 nm in case of Azithromycine.

3. 1. 1. 7. Interference

No interference (less than ± 3.0% in absorbance is considered non – interference) was observed in the determination of Fexo., Fluox and Azithr with BPB from the presence of additives and exceptions that are usually present in pharmaceutical formulations. Also there were no interference from common degradation products which resulted from oxidation of the studied drugs, which are likely to occur at normal storage conditions.

3. 1. 1. 8. Evaluation of the stability constants of the ion - pair complexes

Spectrophotometric methods can be applied for the determination of the stability constant of the ion – pair complexes. Generally, the spectrophotometric methods that are usually applied to establish the stoichiometry of the complexes can also be used for the determination of their stability constants in the solution. The overall formation constants of the concerned ion – pair complexes were calculated using the spectrophotometric data of the mole ratio and continuous variation methods applying the following equation (78)

$$K_n = (A/A_m) / (1 - A/A_m)^{n+1} C_R n^2$$

Where

A, is the absorbance at reagent concentration C_R.
$A_{m,}$ is the absorbance at full color development.
n, is the stoichiometric ratio of the complex.
K_{n}, is the stability constant.

3. 1. 1. 9. Statistical analysis

The statistical analysis of each variable was made showing the sample mean ($X^{\backslash}$) and the sample standard error are calculated according the mean (S.E.). The mean value and the standard error are calculated according to the following equation.

Mean value $(X^{\backslash}) = \sum_i (Xi/n)$
Standard Deviation $(S.D.) = \sum_i (X_i - X^{\backslash})^2 / (n-1)$

$$(S.E.) = (S.D.)/(n)$$

Where:

n = Number of observations.
$\sum$ = Summation.
X_i = individual observations.

The regression equation $A = a + b\,C$

Where:

A = absorbance
C = the concentration in µg/ml.

The slope (b) and regression coefficient (r) were calculated using the following formula.

Slope $(b) = \sum_i [(X_i - X^{\backslash})(Y_i - Y^{\backslash})] / \sum_i (X_i - X^{\backslash})^2$

Regression coefficient

$$(r) = \sum_i [(X_i - X^{\backslash})(Y_i - Y^{\backslash})] / \{[\sum_i (X_i - X^{\backslash})^2][(Y_i - Y^{\backslash})]^2]\}^{1/2}$$

Standard deviation for the slope

$$(S_b) = [\sum_i (Y_i - Y^{\backslash})]^2 / n-2]^{1/2} / [\sum_i (X_i - X^{\backslash})^2]^{1/2}$$

Where:

The fitted Y- values ($Y_i^{\backslash}$) are the points on the calculated regression line corresponding to the individual X- values.

Standard deviation of the intercept (S_d).

$$S_d = \{\sum_i (Y_i - Y^{\backslash})^2 / n-2\}^{1/2} \{\sum_i X_i^2 / n \sum_i (X_i - X^{\backslash})^2\}$$

Relative standard deviation
$$RSD = 100(S/X^{\backslash}).$$
Relative error $RE = 100\,(\Delta X^{\backslash}/X^{\backslash})$.