3.Results and Discussion

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 (1.0×10^{-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 (1.0×10^{-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 $$RE = 100\,(\Delta X^{\backslash}/X^{\backslash}).$$

$$\Delta X^{\backslash} = S.t / (n)^{1/2}$$

Where:

t = the tabulated value.
$\sum$ = Summation.
X= independent variable (concentration).
Y= dependent variable (percentage of bending).
n = number of observation
$X^{\backslash} = \sum (X/n)$

The regression coefficient (r) of each parameter was calculated and compared with each other. The highest one is the optimum conditions when regression coefficient (r) was calculated and it's result by minus sign denoting that the curve is inverted, if the independent variable X was increased, the dependant Y decreased and vice versa.

3. 1. 1. 10. Validity to beer's law

Under optimum conditions of pH, time, solvent and reagent concentration the Drugs under investigation react with anionic dyes to form ion - pair complexes, which are often color development and can be subsequently measured colorimetrically. This character is applied, for determination of Fexo., Fluox and Azithr. through the measuring the absorbance of the formed color ion – pair at the corresponding optimum wavelength λ_{Max}, using BPB. Various parameters affecting the reaction development were studied. A calibration graph was constructed using standard solution of Fexo., Fluox and Azithr. with BIB. Under the optimum conditions, a linear relationship was obtained between the absorbance and concentration of the drug within the range listed in Table (1). The correlation coefficient slopes and intercepts, standard deviation, relative standard deviation, and relative error of the calibration data for Fexo., Fluox and Azithr are calculated using the equations on page

(50and 51). The reproducibility of the method was determined by running six replicate samples, each containing 6.0 µg/ml of the drug in case of Fexo., 5.0 µg/ml in case of Fluox and 5.0 µg/ml in case of Azithr. at these concentration, the relative standard deviation was found to be 0.4439, 0.7462 and 0.8842 % respectively as shown in Table (1). For more accurate results, Ringbom optimum concentration range was determined by plotting log [C] in µg/ml against percent transmittance T % and the linear portion of the S – shape curve gave accurate range of the analysis Fig (10) and the results are recorded in Table (1). The molar Absorptivity, Sandell sensitivity, detection and quantification limits are calculated from the standard deviation of the absorbance measurements obtained from Beer's law and recorded in Table (1). Representative curves on the validity to beer's law for BPB are shown in Fig (9).

3. 1. 1. 11. Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of each of Fexo., Fluox and Azithr were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table (2). The percent standard deviation and the percentage rang of error at 95% confidence level were calculated. The results can be considered as very satisfactory, at least for the level of concentrations examined.

3. 1. 1. 13. Analytical applications

The validity of the proposed procedures is tested for determining Fexo., Fluox and Azithr in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graph using standard addition technique. There was no shift in the

absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t-value and variance ratio F-test with those obtained using official method on the sample of the same batch. The student's t-values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that their is no significant difference between accuracy of the proposed and the official method Tables (3-5). The accuracy of the proposed method when applied to pharmaceutical preparations is evaluated by applying standard addition technique. In which variable amounts of the drugs (Fexo., Fluox and Azithr.) were added to the previously analyzed portion of pharmaceutical preparations. The results shown in Table (6-8), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystalline cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl p-hdroxy benzoate, propyl p-hydroxy benzoate, hydroxyl ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (1) Analytical data and characteristics of colored product ,precision and accuracy of the studied drugs using BPB.

Parameters	Bromophenol blue		
	Fexofenadine	Fluoxetine	azithromycine
pH	2.4	2.2	2.2
Wavelength λ_{max} (nm)	411	410	413
Stability constant	6.5847	6.7340	6.8120
Beer's low limits(µg/ml)	1 .0- 6.0	1.0 – 7.0	1.0 – 6.0
Ringbom limits (µg/ml)	1.2 – 5.4	1.33 – 6.85	1.4 – 5.6
Regression equation* slope (b)	0.1900	0.2476	0.0885
Intercept (a)	0.0149	0.0478	0.0335
Standard deviation (SD)	0.0052	0.0087	0.0041
Correlation coefficient (r)	0.9997	0.9999	0.9996
Detection limit (µg/ml)	0.0156	0.0261	0.0123
Quantitation limit (µg/ml)	0.052	0.087	0.0041
Molar absorptivity $X10^4$ ($1mol^{-1} cm^{-1}$)	10.29	8.564	6.629
Sandell sensitivity(($\mu g\ cm^{-2}$)	0.0052	0.0042	0.0011
Error**%	0.2123	0.3551	0.1673
RSD %	0.4439	0.7462	0.8842

*with respect to A=a+ b C where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drugs in (µg/ml)

** Average of six determinations.

Table (2): Evaluation of the accuracy and precision of the proposed method Using BPB.

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[a] (%)	RE (%)	Confidence[b] Limits
Fexofenadine Hydrochloride	3.0	3.01	100.33	1.6208	1.169	3.01 ± 0.0352
	5.0	5.02	100.04	0.6939	0.940	5.02 ± 0.0472
	7.0	6.97	99.57	0.3191	0.4246	6.97 ± 0.0296
Fluoxetine Hydrochloride	2.0	2.01	100.50	1.5085	0.9552	2.01 ± 0.0192
	4.0	3.98	99.50	0.9569	0.9120	3.98 ± 0.0363
	6.0	6.01	100.16	0.3352	0.4808	6.01 ± 0.0289
Azithromycine	2.0	1.99	99.50	1.9138	1.065	1.99 ± 0.0212
	4.0	4.02	100.50	1.2318	0.8300	4.02 ± 0.0332
	6.0	5.99	99.83	0.5185	0.7762	5.99 ± 0.0465

[a] Relative standard deviation for six determinations.
[b] 95% confidence limits and five degrees of freedom.

Table (3): Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fexo. In it's pharmaceutical forms using PBP.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Allerfen tab. 60 mg per tab	60	60.97	101.62	60	59.99	99.98	0.1088	1.323
Telfast tab. 120 mg per tab.	120	119.98	99.98	120	120.01	100.01	0.7418	2.324
Fexon tab 180 mg per tab.	180	179.99	99.99	180	180.01	100.01	0.2516	1.735
Fastofen tab. 120 mg per tab.	120	119.95	99.95	120	120.4	100.33	0.1734	2.183
Histafree tab. 120 mg per tab.	120	119.97	99.97	120	120.05	100.04	0.4826	1.935
Fastel tab. 120 mg per tab.	120	120.03	100.03	120	120.02	100.02	0.2649	2.816

* : Average of six determinations.

** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (4):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fluox. In it's pharmaceutical forms using PBP.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Flutine cap. 20 mg per cap	20	19.99	99.95	20	20.00	100.00	0.7643	2.133
Prozac cap. 20 mg per cap.	20	20.01	100.05	20	20.03	100.15	0.5815	2.542
Depreban cap. 20 mg per cap.	20	19.99	99.95	20	20.04	100.20	0.7953	1.834
Fluozac cap. 10 mg per cap.	10	9.98	99.80	10	10.02	100.20	0.9528	1.965
Florosin cap. 20 mg per cap.	20	20.02	100.10	20	20.03	100.15	1.4382	1.536
Octazac cap. 20 mg per cap.	20	20.01	100.05	20	20.02	100.10	1.1142	2.946

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (7) : Determination of Fluox. in its pharmaceutical dosage forms applying standard addition technique using BPB.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Flutine cap. 20 mg per cap.	2.0	0.0	1.99	99.50
		2.0	3.98	99.50
		3.5	5.51	100.18
		4.5	6.49	99.85
Prozac cap. 20 mg per cap.	2.0	0.0	2.02	101.00
		2.5	4.52	100.44
		3.5	5.52	100.36
		4.5	6.49	99.85
Depreban cap. 20 mg per cap.	2.0	0.0	2.01	100.50
		2.0	3.98	99.50
		3.0	4.97	99.40
		4.0	5.97	99.50
Fluozac cap. 10 mg per cap.	2.0	0.0	1.98	99.00
		2.0	3.99	99.75
		3.5	5.52	100.36
		4.5	6.51	100.15
Florosin cap. 20 mg per cap.	2.0	0.0	2.01	100.50
		2.0	3.98	99.50
		3.5	5.53	100.55
		4.5	6.51	100.15
Octazac cap. 20 mg per cap.	2.0	0.0	2.02	101.00
		2.5	4.49	99.78
		3.5	5.53	100.54
		4.5	6.53	100.46

* : Average of six determinations.

Table (8) : Determination of Azithr. in its pharmaceutical dosage forms applying standard addition technique using BPB.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Azalide cap. 200 mg per cap.	2.0	0.0	1.97	98.50
		2.0	3.97	99.25
		3.0	4.99	99.80
		4.0	5.96	99.33
Aziwok susp. 250 mg .	1.5	0.0	1.52	101.33
		2.5	4.02	100.50
		3.5	4.96	99.20
		4.5	5.95	99.17
Xithrone cap. 250 mg per cap.	1.5	0.0	1.49	99.33
		2.5	3.99	99.75
		3.5	5.01	100.20
		4.5	5.97	99.50
Zithromax cap. 250 mg per cap.	2.0	0.0	2.02	101.00
		2.0	4.01	100.25
		3.0	5.3	106.00
		4.0	5.98	99.67
Zisrocin cap. 500 mg per cap.	2.0	0.0	1.99	99.50
		2.5	4.54	100.89
		3.5	5.55	100.91
		4.0	6.01	100.17
Zithrokan cap. 500 mg per cap.	1.5	0.0	1.47	98.00
		2.5	4.03	100.75
		3.5	5.02	100.40
		4.5	5.96	99.33

* : Average of six determinations.

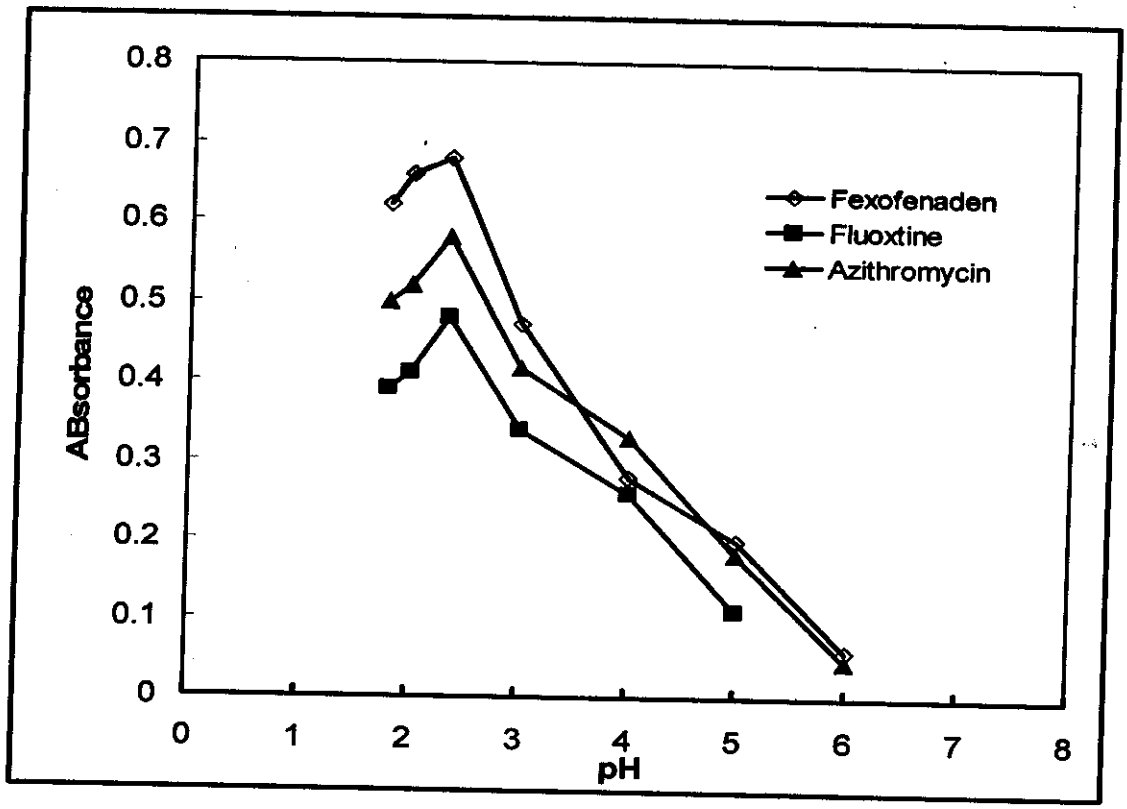


Fig (1): Effect of pH on the absorbance of the studied drugs (1.0×10^{-4} M) BPB.

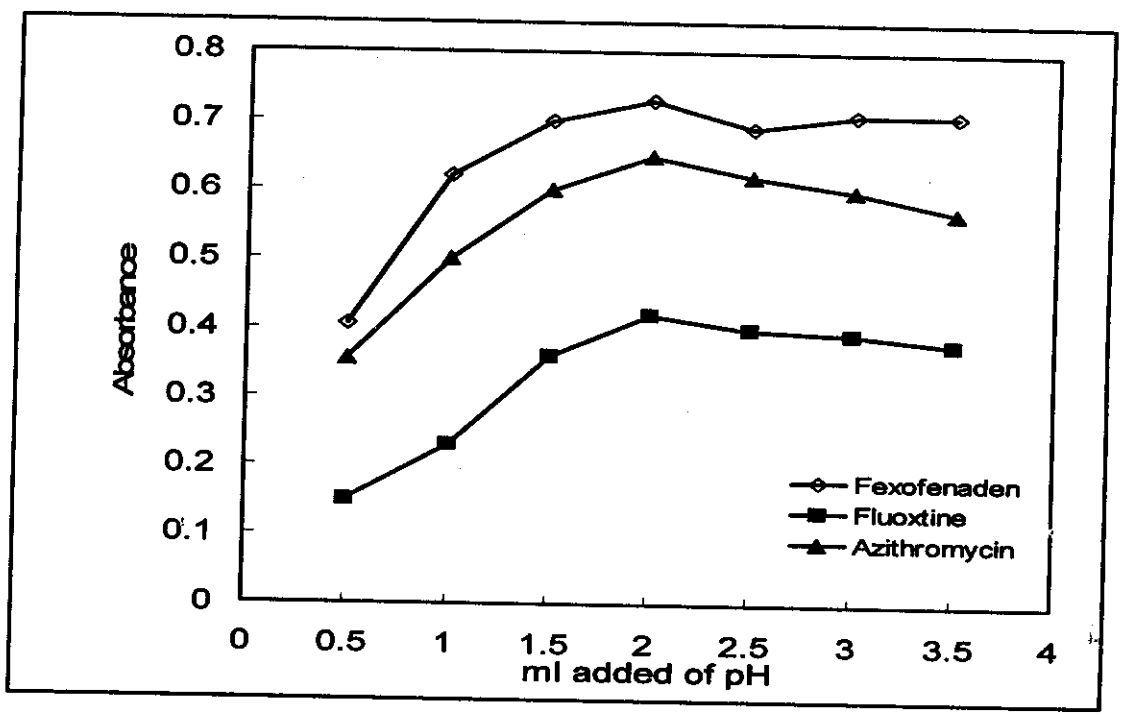


Fig (2): Effect of ml added of pH on the absorbance of the studied drugs (1.0×10^{-4} M) BPB.

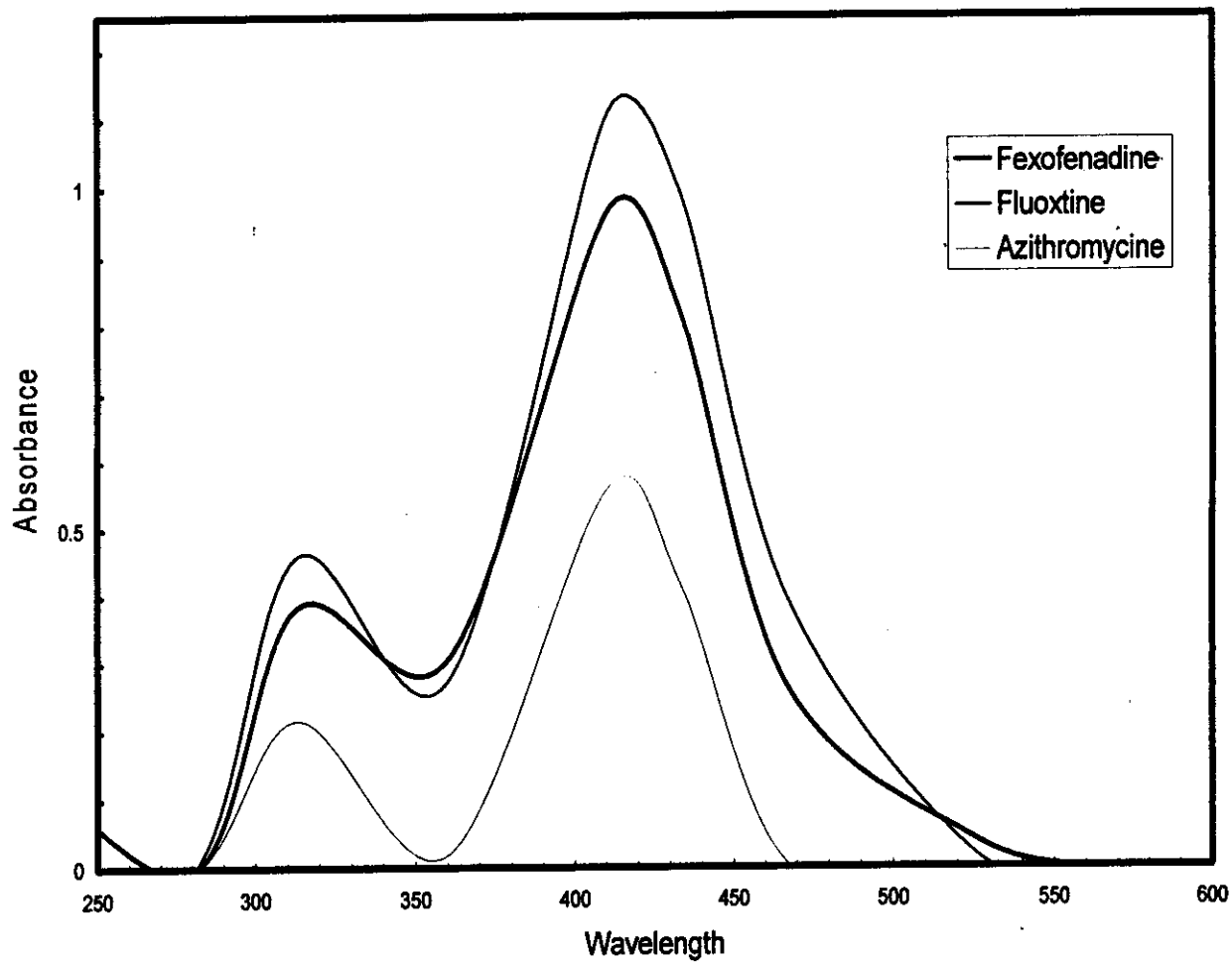


Fig (3): Absorption spectra of ion-pair complex of 5 µg/ml of the studied drugs with acid dye BPB (1.0×10^{-4} M).

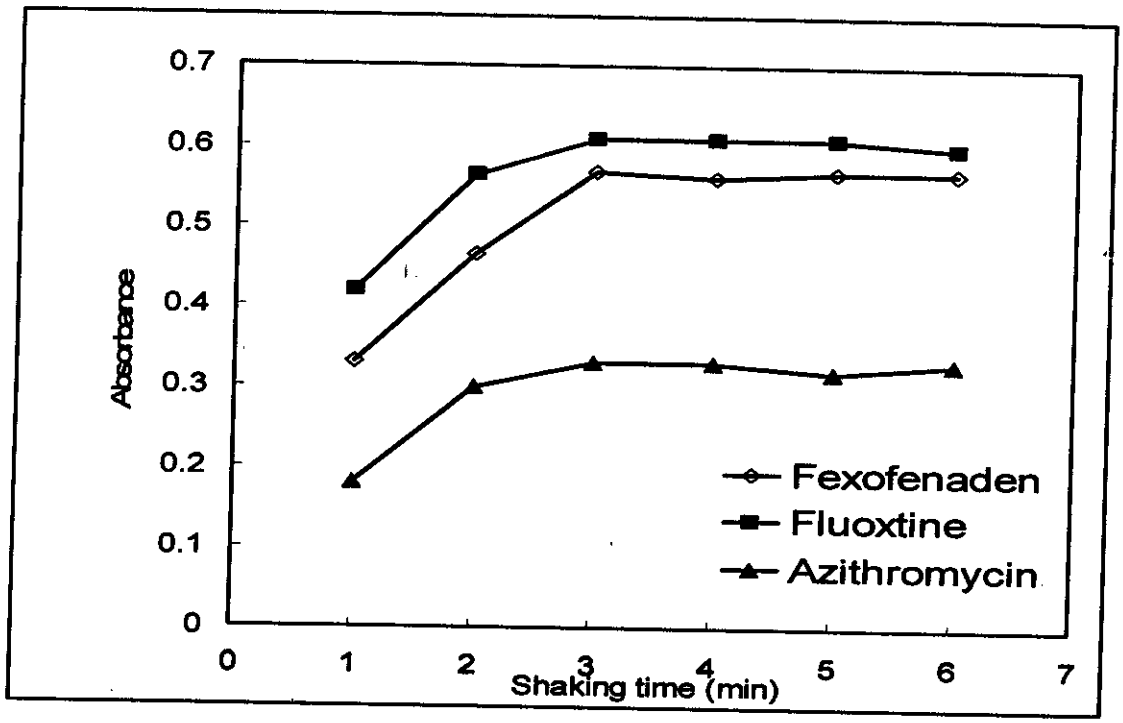


Fig. (4) : Effect of shaking time on the absorbnce of the studied drugs solution using (1.0×10^{-4} M) BPB.

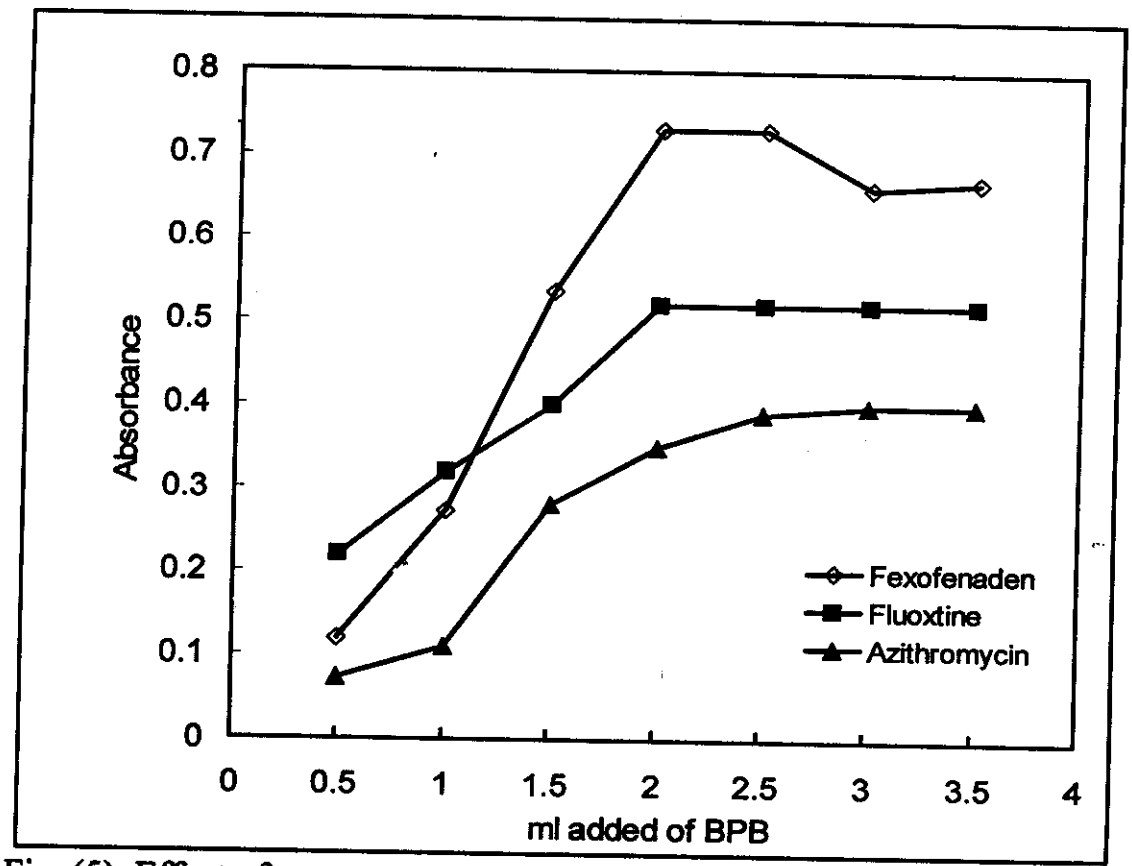


Fig. (5): Effect of reagent concentration on the absorbance of the studied drugs solution using (1.0×10^{-4} M) BPB.

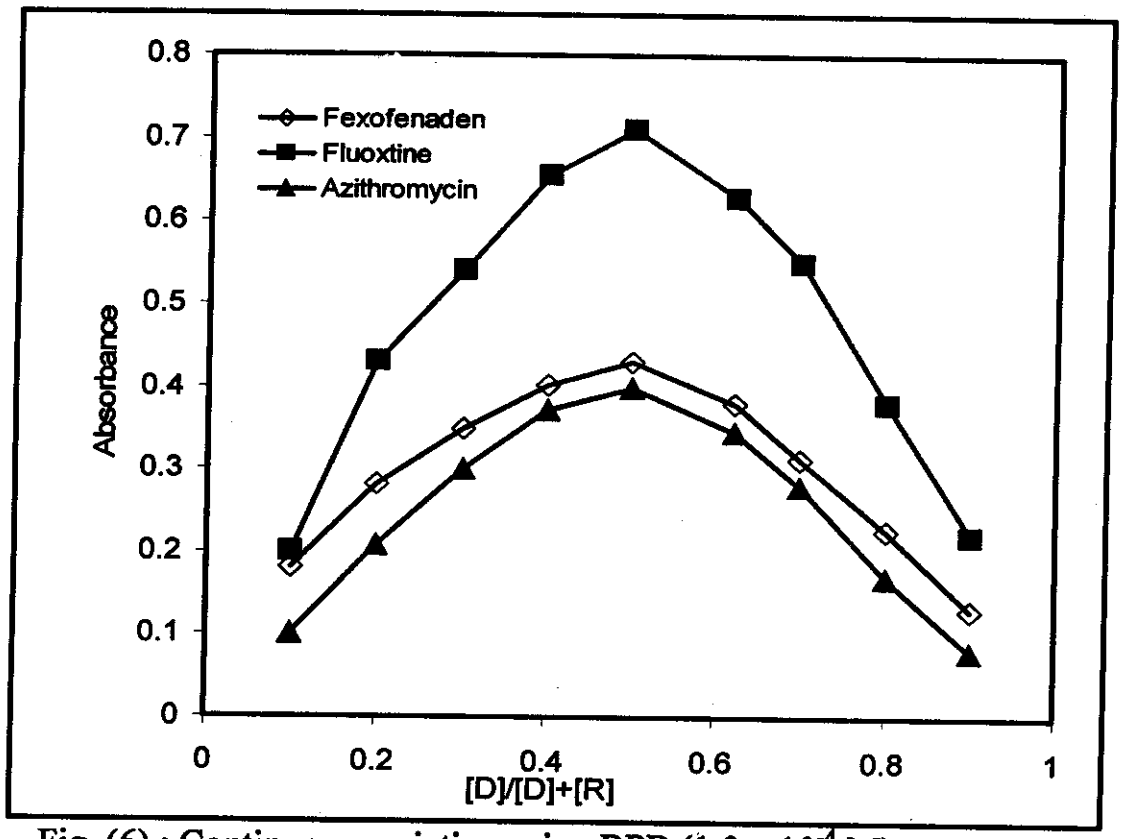


Fig. (6) : Continuous variation using BPB (1.0×10^{-4} M) reagent with (1.0×10^{-4} M) of the Drugs under consideration.

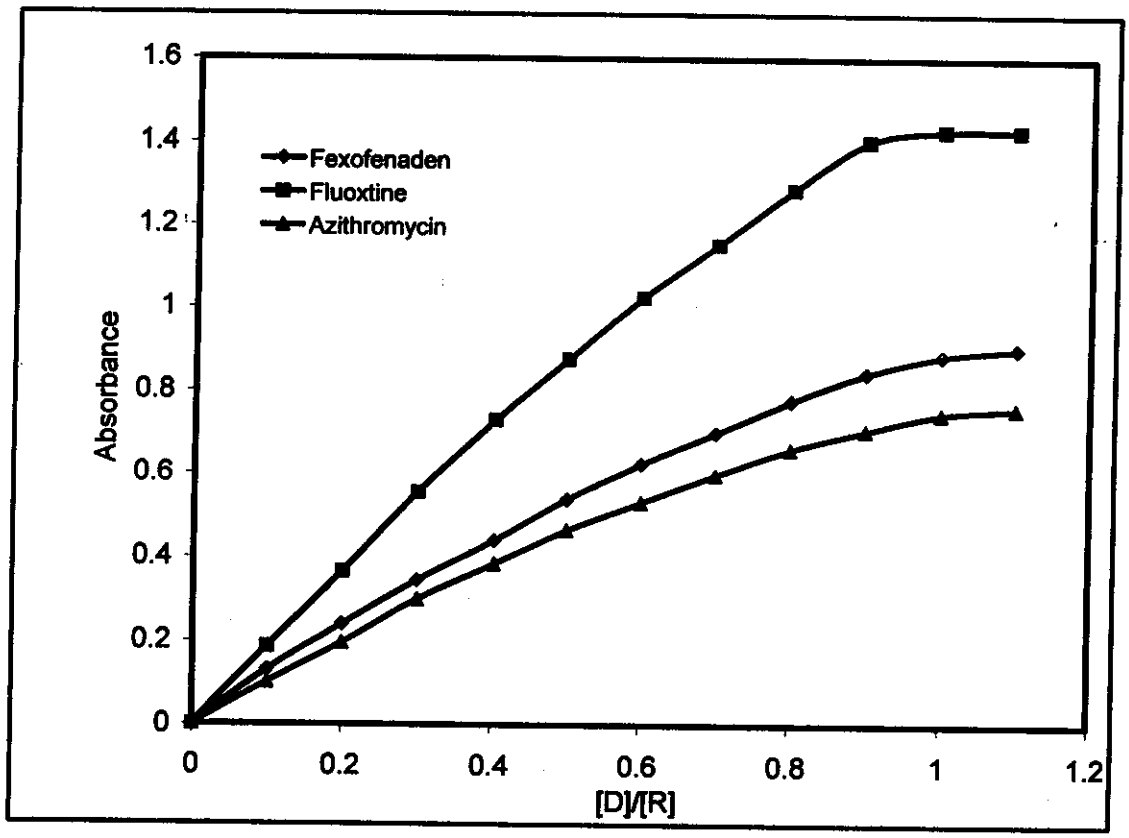
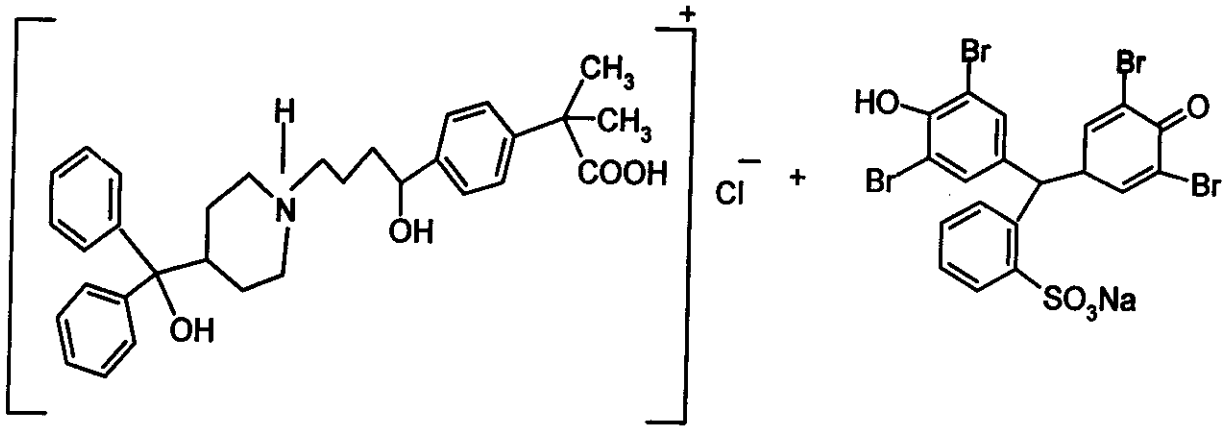


Fig. (7): Mole ratio for BPB- Drugs (1.0×10^{-4} M) under consideration.

Fig (8): proposed mechanism of the reaction between Fexofenadine hydrochloride and bromophenol blue sodium salt.

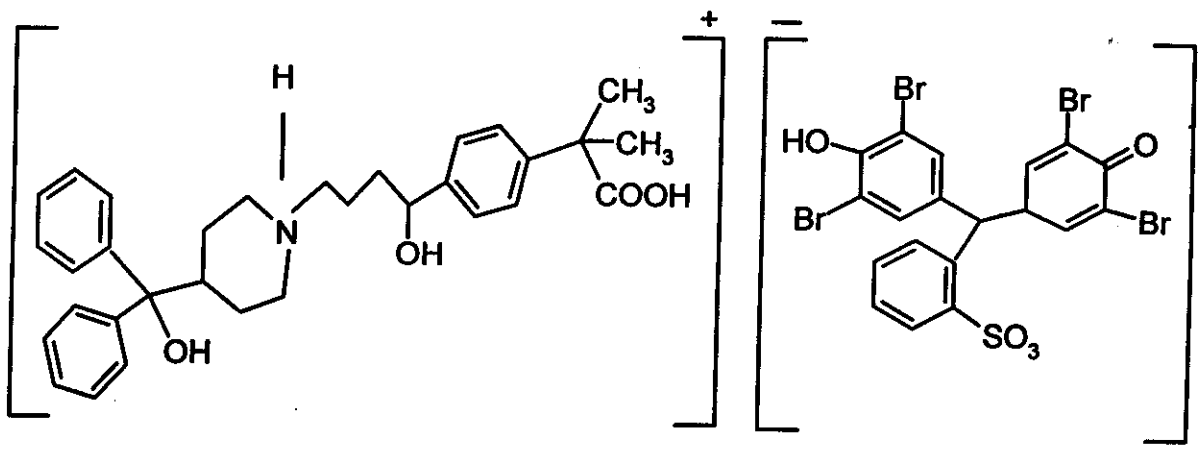


Fexofenadine Hydrochloride

Bromophenol blue Sodium salt

buffer (pH 2.4)

- NaCl



Fexofenadine hydrochloride -BPB complex

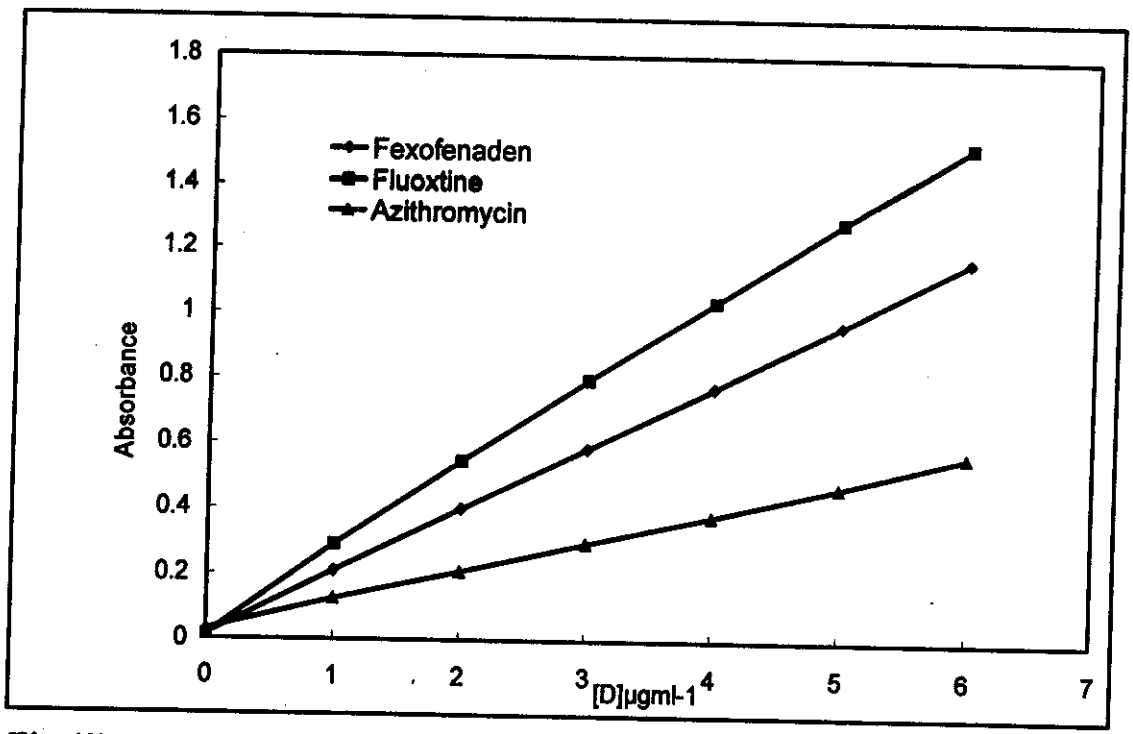


Fig (9): Application of Beer's law for the studied drugs using the optimum volume of (1.0×10^{-4} M) BPB.

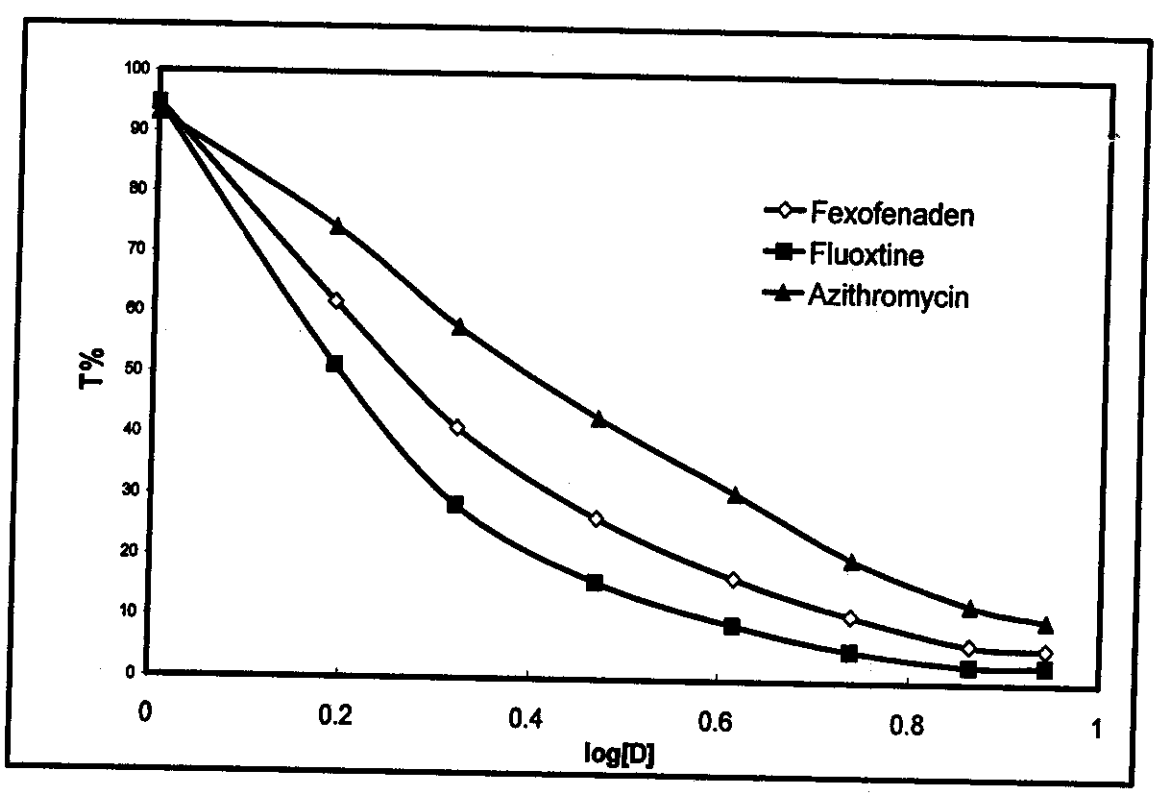


Fig (10): Ringbom plots for the studied drugs solution using the optimum volume of (1.0×10^{-4} M) BPB.

3. 1. 2. Absorption spectra of the studied drugs with BCG

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 BCG (1.0 x 10^{-4} M), the effect of different experimental variables were studied and recorded below.

3. 1. 2. 1. Effect of pH

In order to establish the optimum pH value for each ion-pairs formed, between the studied drugs and BCG the investigated. Fexo., Fluox. and Azithr. Was allowed to react with the BCG in aqueous type of buffered solution of various pH ranges (2.0 – 12.0). The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr). to measure The absorbance value at $\lambda_{max.}$ The highest absorbance values were obtained at pH 2.2 in case of Fexo., 2.4 in case of Fluox. and 2.2 in case of Azithr. which are selected for ion-pairs formation. These results are shown in Fig. (11) Furthermore, the amount of buffer was examined and found to be 2.0 ml in each case of Fexo., Fluox and 3 ml in case of Azithr. As shown in Fig. (12). The maximum wavelength corresponding to each ion–pair complex of the drugs with BCG is at 409 nm in case of Fexo., 408 nm in case of Fluox and at 411 nm in case of Azithr. as shown in Fig (13).

3. 1. 2. 2. Effect of time

The effect of time required for complete color development of the ion - pair formed between the studied drugs and BCG 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 in case of Fexo and Fluox., which is measured directly against

blank prepared by the same way without the drug, whereas in case of Azithr 3.0 min are quite sufficient to obtain maximum color intensity, before extraction. The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr), the optimum shaking time is 2.0 min in each case Fexo. and Fluox, and 3.0 min in case of Azithr as shown in Fig (14). The formed ion-pairs were found to be stable for more than 12 hours.

3. 1. 2. 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 BCG reagent on the drugs under consideration indicated that chloroform is the best solvent for extraction in the three cases (Fexo., Fluox and Azithr.) ion-pair, 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 time.

3. 1. 2. 4. Effect of reagent concentration

When various concentrations of BCG were added to fixed concentrations of each of Fexo., Fluox and Azithr. it was found that 2.0 ml of BCG (1.0×10^{-4} M) in the three cases (Fexo., Fluox and Azithr). were found to be sufficient for the production of the maximum and reproducible color intensity. Higher concentration of the reagent was affects in color intensity. The absorbance decreased gradually with increasing reagent concentration as shown in Fig (15).

3. 1. 2. 5. composition of the ion-pair complexes and the stability constant of it.

The stoichiometeric of the ion-pair complex was established by the mole ratio and continuous variation methods using both variable reagent BCG (1.0×10^{-4} M) and the Fexo., Fluox and Azithr,. The results showed that the stoichiometeric ratio of the complexes 1:1 (reagent: drugs) and the shape of resulting curves indicated that the complex is labile, as shown in Figs. (16&17). Consequently, a large excess of reagent must be always used to enhance the formation of the complex. The stability constant of the complex was calculated by using the data of the mole ratio and job's continuous variation methods applying the equation given in page 51. The results of the stability constants are recorded in Table (9).

3. 1. 2. 6. Suggested mechanism

The acid dye-drug technique is an ion – pair mechanism in which ion – pair is formed between negative ion produced from ionization of BCG, which is converted into BCG sodium salt in the buffer solution and positive ion of Fexofenadine Hydrochloride as shown in Fig (18). the ion – pair formed exhibits maximum absorbance at $\lambda_{max.}$ 409 nm in case of complex with Fexofenadine hydrochloride, 408 nm in case of complex with Fluoxetine Hydrochloride and at 411 nm in case of complex with Azithromycine.

3. 1. 2. 7. Validity to beer's law

A calibration graph was constructed using standard solution of the studied drugs. Under the optimum conditions, a linear relationship is obtained between the absorbance and concentration of the studied drugs

in the concentration ranges listed in Table (9). The correlation coefficient, slopes and intercepts of the calibration data for Fexo., Fluox or Azithr are calculated using the equations given in (pages 51, 52). The reproducibility of the method was determined by running six replicate samples, each containing 5.0 μg/ml of Fexo., Fluox and Azithr in the final assay solution. At this concentration, the relative standard deviation was found to be ≤ 0.8512 for more accurate results, Ringbom optimum concentration ranges are calculated and the mean molar absroptivity, Sandell sensitivity, detection and quantification limit are calculated from Beer's law. All this results are recorded in Table (9), while representation curves of the validity to Beer's law and Ringbom plot for BCG are shown in Figs. (19&20)

3. 1. 2. 8. Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Fexo., Fluox and Azithr were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table (10). The percent standard devotions and the percentage rang of error at 95% confidence level were calculated. The results can be considered as very satisfactory, at least for the level of concentrations examined.

3. 1. 2. 9. Analytical applications

The validity of the proposed procedures is tested for determining Fexo., Fluox and Azithr in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum due to the

presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t-value and variance ratio F-test with those obtained using official method on the sample of the same batch. The student's t-values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that their is no significant difference between precision of the proposed and the official method Table (11-13). The accuracy of the proposed method when applied to pharmaceutical preparations is evaluated by applying standard addition technique. In which variable amounts of the drugs (Fexo., Fluox and Azithr.) were added to the previously analyzed portion of pharmaceutical preparations. The results shown in Table (14-16), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystalline cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl p-hdroxy benzoate, propyl p-hydroxy benzoate, hydroxyl ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (9) Analytical data and characteristics of colored product ,precision and accuracy of the studied drugs using BCG.

Parameters	*Bromo Cresol Green*		
	Fexofenadine	Fluoxetine	Azithromycine
pH	2.2	2.4	2.2
Wavelength λ_{max} (nm)	409	408	411
Stability constant	5.9856	5.8562	5.983
Beer's law limits(µg/ml)	1.0 – 8.0	0.5- 7.0	2.0 – 11
Ringbom limits (µg/ml)	1.1 – 7.4	0.8 – 6.6	2.3 – 10.3
Regression equation* slope (b)	0.13	0.26	0.056
Intercept (a)	0.0945	0.0848	0.0931
Standered deviation (SD)	0.0085	0.0045	0.0054
Correlation coefficient (r)	0.9998	0.9998	0.9994
Detection limit (µg/ml)	0.025	0.0134	0.0162
Quantitation limits (µg/ml)	0.085	0.0045	0.054
Molar absorptivity (10^4) ($1mol^{-1}$ cm^{-1})	7.30	9.13	4.25
Sandell sensitivity(($ng\ cm^{-2}$)	0.0076	0.0038	0.0017
Error**%	0.347	0.1837	0.2205
RSD %	0.7800	0.7276	0.8512

*with respect to A=a+ b C where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drugs in (µg/ml).
** Average of six determination.

Table (10): Evaluation of the accuracy and precision of the proposed method Using BCG.

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD [a] (%)	RE (%)	Confidence [b] Limits
Fexofenadine Hydrochloride	2.0	2.017	100.85	1.1339	1.0808	2.017 ± 0.0218
	3.5	3.51	100.29	1.2883	1.2279	3.51 ± 0.0431
	5.0	5.02	100.40	0.7482	0.7131	5.02 ± 0.0358
Fluoxetine Hydrochloride	2.0	1.97	98.50	0.6604	0.6294	1.97 ± 0.0124
	4.0	3.98	99.50	0.8594	0.8191	3.98 ± 0.0326
	6.0	6.01	100.10	1.2429	1.1846	6.01 ± 0.0712
Azithromycine	2.0	2.02	101.00	0.6284	0.2020	2.02 ± 0.0121
	4.5	4.51	99.78	1.4214	1.3547	4.51 ± 0.0611
	6.0	5.99	99.80	0.7461	0.7112	5.99 ± 0.0426

[a] Relative standard deviation for six determinations.
[b] 95% confidence limits and five degrees of freedom.

Table (11):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fexo. In it's pharmaceutical forms using BCG.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Allerfen tab. 60 mg per tab	60	59.95	99.92	60	60.03	100.05	0.974	2.472
Telfast tab. 120 mg per tab.	120	119.96	99.97	120	119.98	99.98	0.733	2.183
Fexon tab 180 mg per tab.	180	179.94	99.97	180	179.97	99.98	0.894	2.831
Fastofen tab. 120 mg per tab.	120	120.03	100.03	120	120.01	100.01	0.732	1.974
Histafree tab. 120 mg per tab.	120	120.01	100.01	120	119.98	99.98	0.994	1.923
Fastel tab. 120 mg per tab.	120	119.96	99.97	120	119.98	99.98	1.052	2.842

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (12):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fluox. In it's pharmaceutical forms using BCG.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Flutine cap. 20 mg per cap	20	20.03	100.15	20	20.04	100.20	0.875	2.872
Prozac cap. 20 mg per cap.	20	19.96	99.80	20	20.00	100.00	0.694	1.956
Depreban cap. 20 mg per cap.	20	19.98	99.90	20	20.02	100.10	0.689	1.967
Fluozac cap. 10 mg per cap.	10	9.98	99.80	10	10.01	100.10	1.023	0.945
Florosin cap. 20 mg per cap.	20	19.97	99.85	20	20.04	100.20	1.056	2.043
Octazac cap. 20 mg per cap.	20	20.02	100.10	20	20.01	100.05	0.738	0.894

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (13):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Azithr. In it's pharmaceutical forms using BCG.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Azalide cap. 200 mg per cap.	200	200.06	100.03	200	200.05	100.06	0.842	2.851
Aziwok susp. 250 mg .	250	249.95	99.98	250	250.03	100.01	0.917	2.518
Xithrone cap. 250 mg per cap.	250	249.98	99.99	250	249.98	99.99	1.052	2.942
Zithromax cap. 250 mg per cap.	250	250.04	100.016	250	250.05	100.02	1.173	1.957
Zisrocin cap. 500 mg per cap.	500	500.07	100.01	500	500.08	100.01	0.917	1.948
Zithrokan cap. 500 mg per cap.	500	500.06	100.01	500	500.07	100.01	0.958	1.683

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (14): Determination of Fexo. in its pharmaceutical dosage forms applying standard addition technique using BCG.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Allerfen tab. 60 mg per tab.	2.0	0.0	2.03	101.50
		2.0	4.05	101.25
		3.0	5.01	100.20
		5.0	6.96	99.43
Telfast tab. 120 mg per tab.	2.0	0.0	1.97	98.50
		2.0	4.02	100.50
		3.5	5.49	99.82
		4.0	6.05	100.83
Fexon tab 180 mg per tab.	2.5	0.0	1.97	78.80
		2.5	4.96	99.20
		3.5	6.04	100.67
		4.0	6.49	99.85
Fastofen tab. 120 mg per tab.	2.0	0.0	2.02	101.00
		3.0	4.97	99.40
		4.0	6.01	100.17
		5.0	6.97	99.57
Histafree tab. 120 mg per tab.	3.0	0.0	2.96	98.67
		2.0	4.95	99.00
		3.0	6.03	100.50
		3.5	6.49	99.85
Fastel tab. 120 mg per tab.	2.0	0.0	1.98	99.00
		2.0	3.97	99.25
		4.0	5.95	99.17
		5.0	6.97	99.57

* : Average of six determinations.

Table(15): Determination of Fluox. in its pharmaceutical dosage forms applying standard addition technique using BCG.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Flutine cap. 20 mg per cap.	2.0	0.0	2.01	100.50
		2.0	3.95	98.75
		3.5	5.54	100.73
		4.5	6.49	99.84
Prozac cap. 20 mg per cap.	2.0	0.0	2.02	101.00
		2.5	4.53	100.67
		3.5	5.52	100.36
		4.5	6.51	100.15
Depreban cap. 20 mg per cap.	2.0	0.0	1.99	99.50
		2.0	4.02	100.50
		3.0	5.01	100.20
		4.0	6.07	101.17
Fluozac cap. 10 mg per cap.	2.0	0.0	2.03	101.50
		2.0	4.03	100.75
		3.5	5.49	99.82
		4.5	6.49	99.85
Florosin cap. 20 mg per cap.	2.0	0.0	2.01	100.50
		2.0	4.04	101.00
		3.5	5.51	100.18
		4.5	6.51	100.15
Octazac cap. 20 mg per cap.	2.0	0.0	1.99	99.50
		2.5	4.52	100.44
		3.5	5.49	99.81
		4.5	6.53	100.46

* : Average of six determinations.

Table(16): Determination of Azithr. in its pharmaceutical dosage forms applying standard addition technique using BCG.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Azalide cap. 200 mg per cap.	2.0	0.0	1.97	98.50
		2.0	4.03	100.75
		3.0	5.05	101.00
		4.0	6.04	100.66
Aziwok susp. 250 mg .	1.5	0.0	1.52	101.33
		2.5	4.05	101.25
		3.5	5.01	100.20
		4.5	5.95	99.16
Xithrone cap. 250 mg per cap.	1.5	0.0	1.53	102.00
		2.5	4.05	101.25
		3.5	5.06	101.20
		4.5	6.07	101.16
Zithromax cap. 250 mg per cap.	2.0	0.0	2.03	101.50
		2.0	4.04	101.00
		3.0	4.95	99.00
		4.0	6.06	101.00
Zisrocin cap. 500 mg per cap.	2.0	0.0	2.03	101.50
		2.5	4.54	100.88
		3.5	5.56	101.09
		4.0	5.96	99.33
Zithrokan cap. 500 mg per cap.	1.5	0.0	1.52	101.33
		2.5	4.01	100.25
		3.5	4.96t	99.20
		4.5	6.05	100.83

* : Average of six determinations.

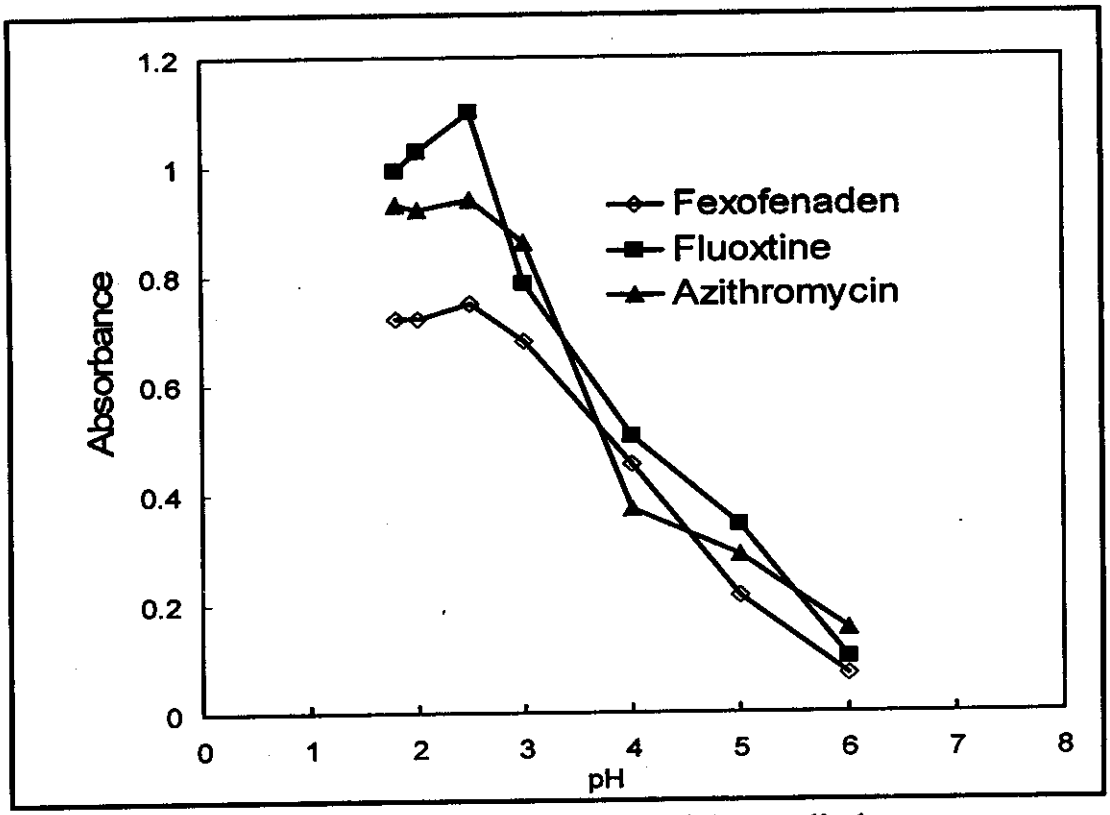


Fig. (11): Effect of pH on the absorbance of the studied drugs using (1.0×10^{-4} M) BCG.

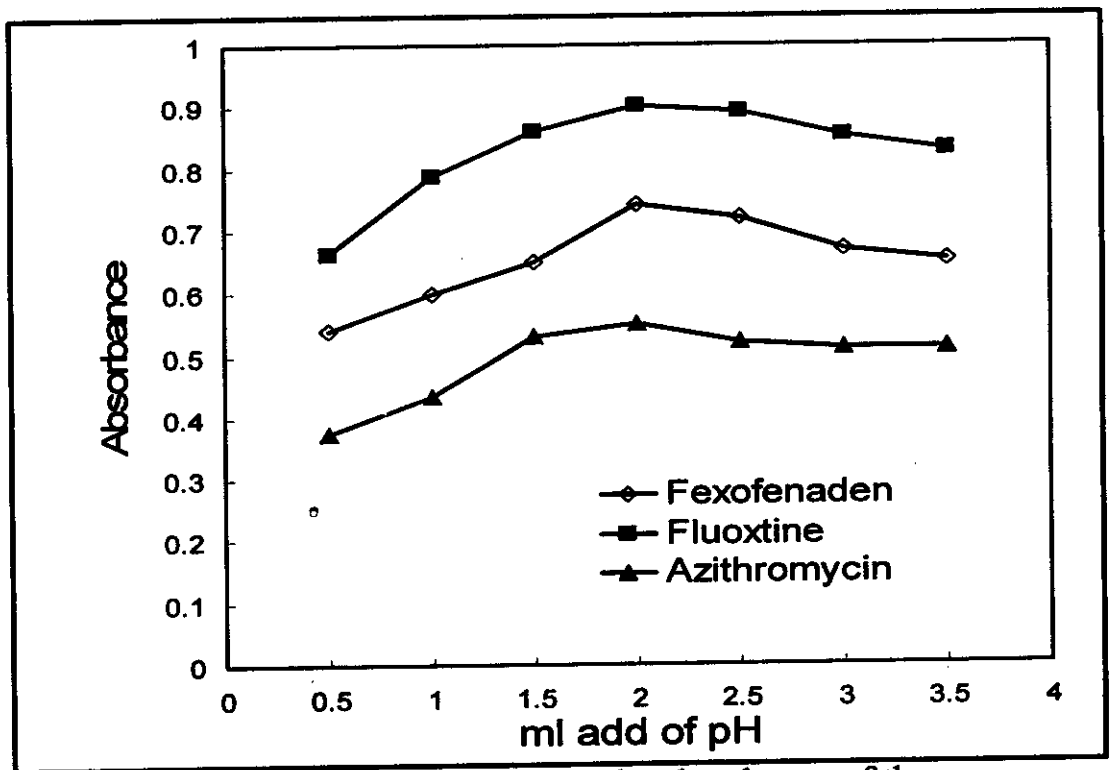


Fig (12): Effect of ml added of pH on the absorbance of the Studied drugs (1.0×10^{-4} M) BCG.

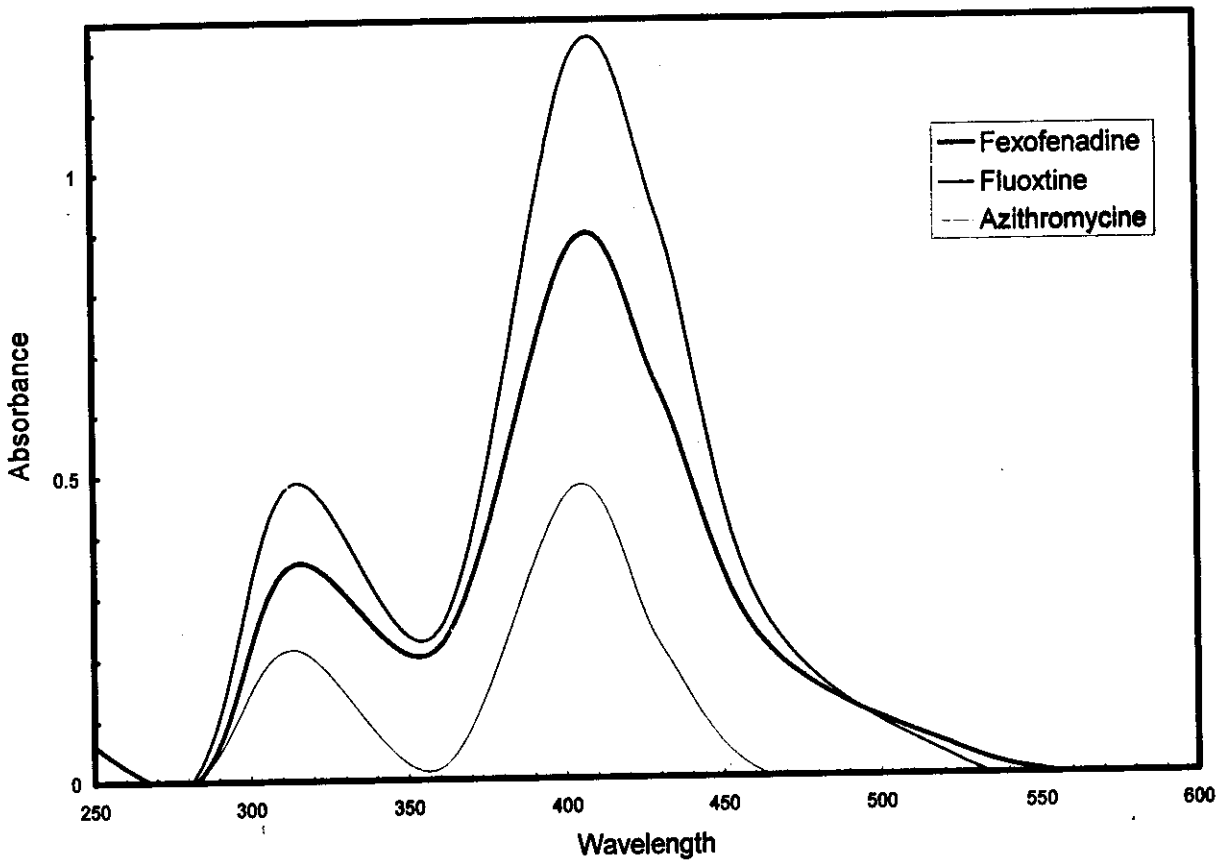


Fig (13): Absorption spectra of ion-pair complex of 10 µg/ml of the studied drugs with acid dye BCG (1.0×10^{-4} M).

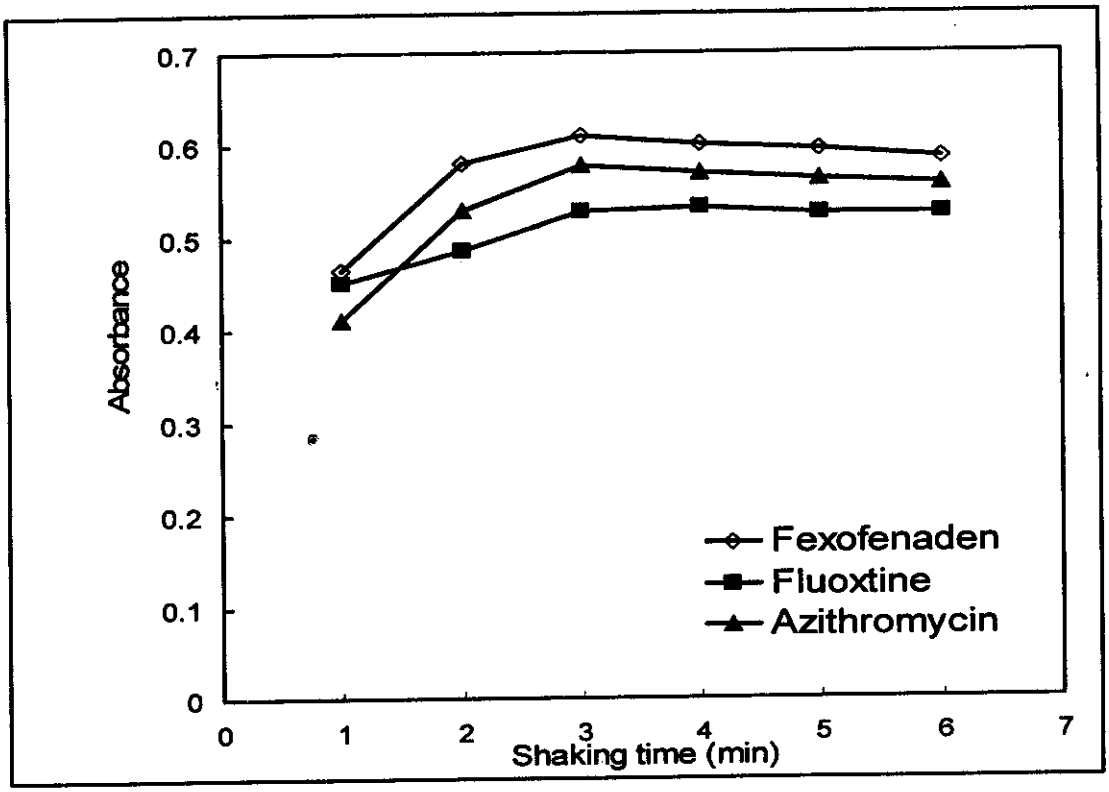


Fig (14): Effect of shaking time on the absorbance of the studied drugs solution using (1.0×10^{-4} M) BCG.

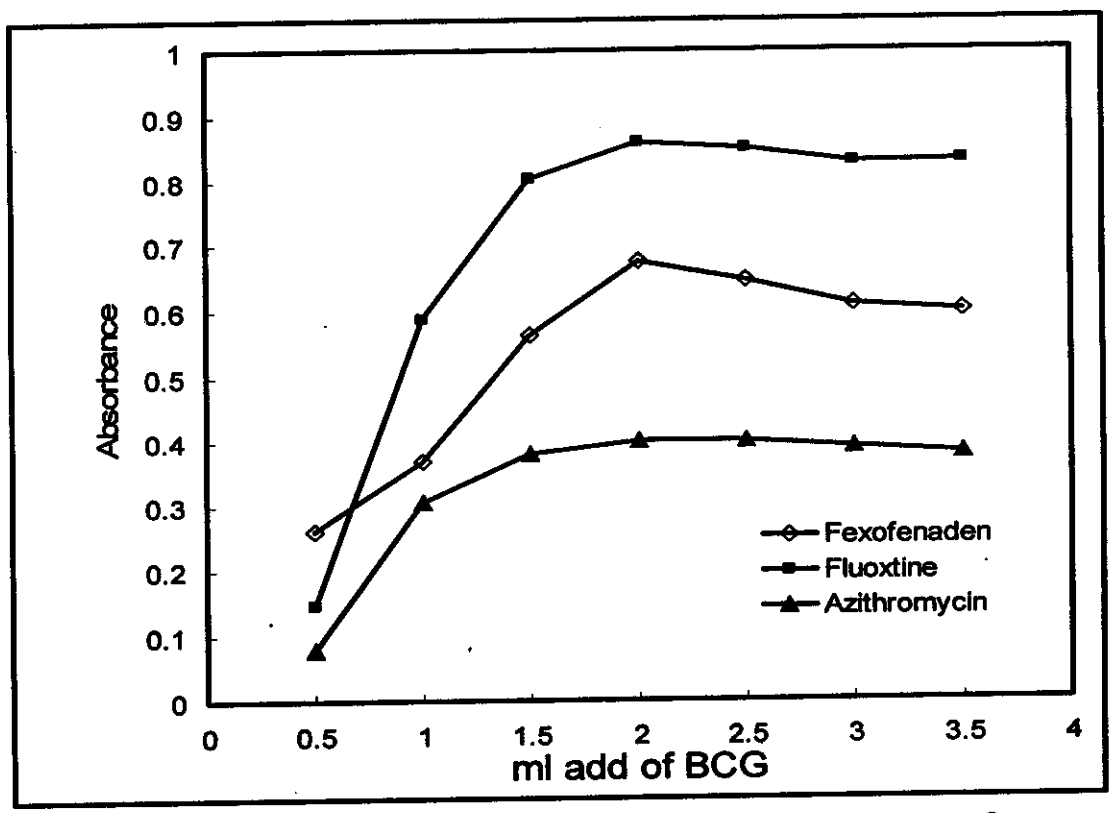


Fig. (15): Effect of reagent concentration on the absorbance of the studied drugs solution using (1.0×10^{-4} M) BCG.

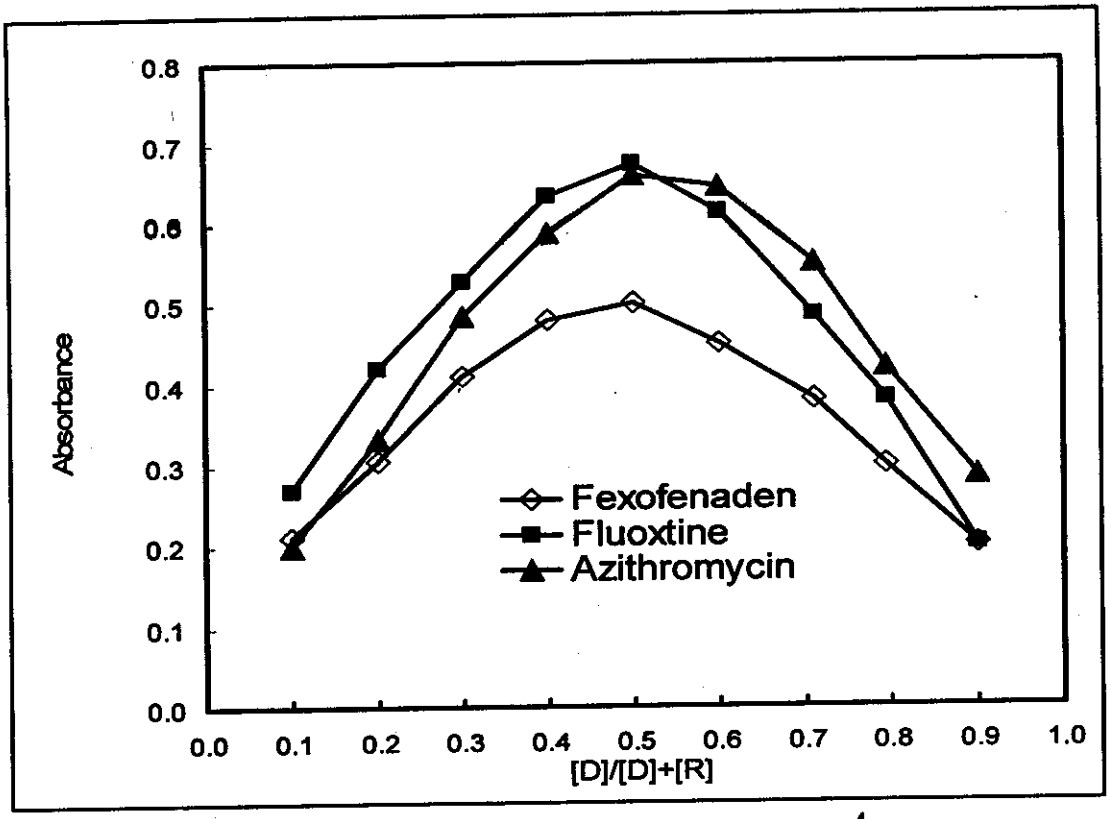


Fig. (16) : continuous variation using BCG (1.0×10^{-4} M) reagent with (1.0×10^{-4} M) of the Druges under consideration.

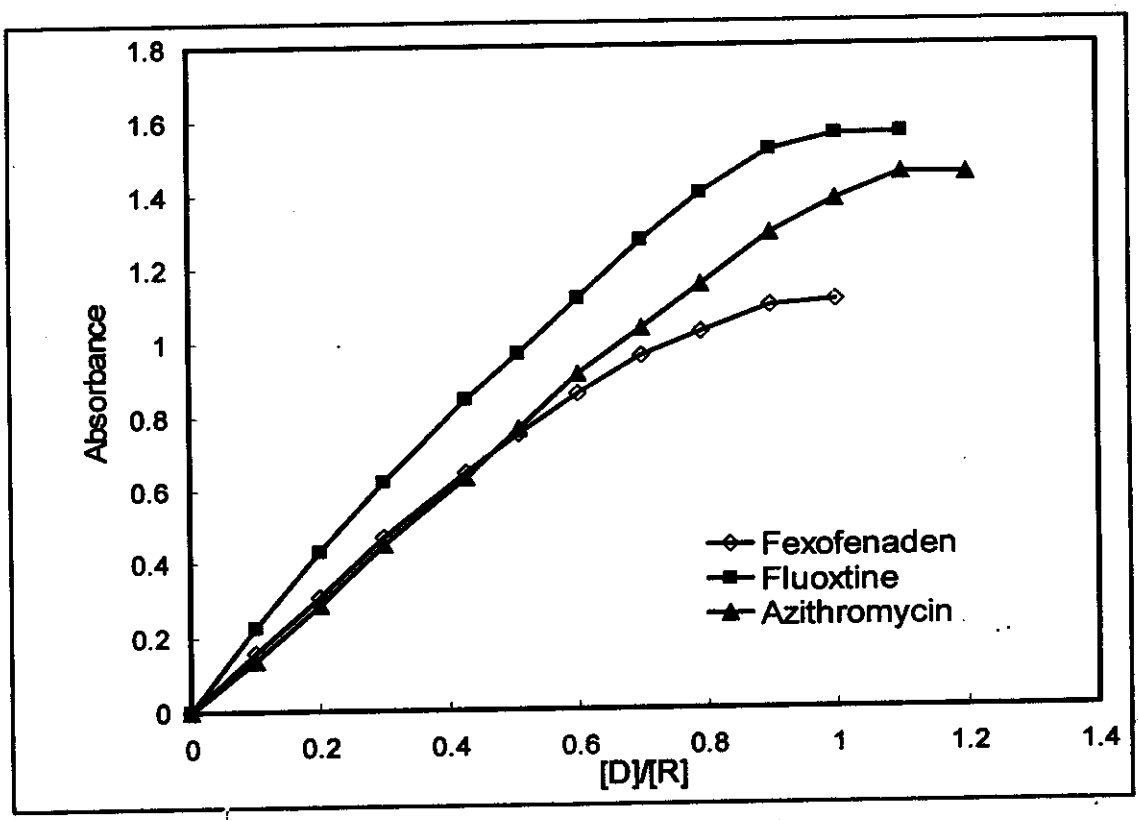
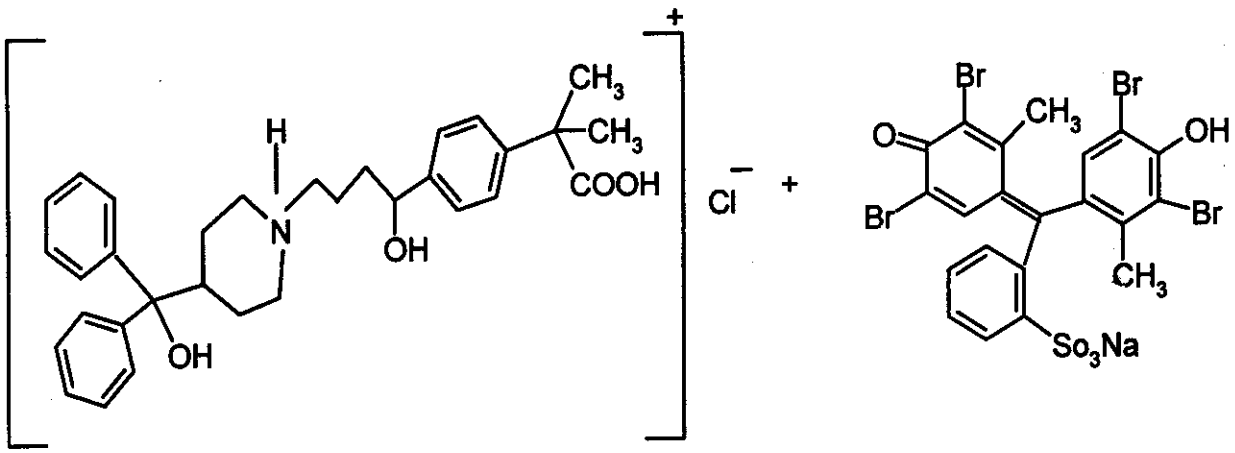


Fig. (17): Mole ratio for BCG- Drugs (1.0×10^{-4} M) under consideration.

Fig (18): proposed mechanism of the reaction between Fexofenadine hydrochloride and Bromocresol green sodium salt.

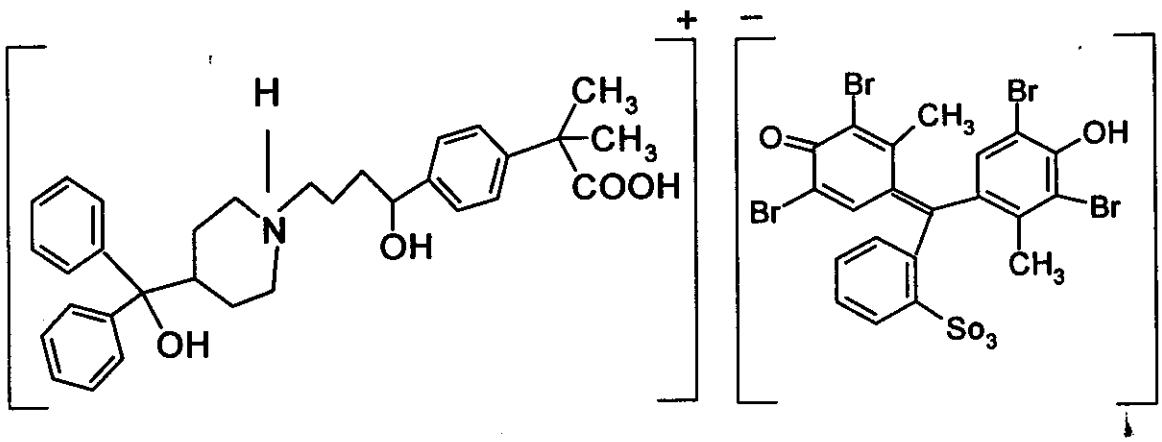


Fexofenadine hydrochloride

Bromocresol Green Sodium salt

Buffer
(pH 2.2)

-NaCl



Fexofenadine hydrochloride -BCG complex

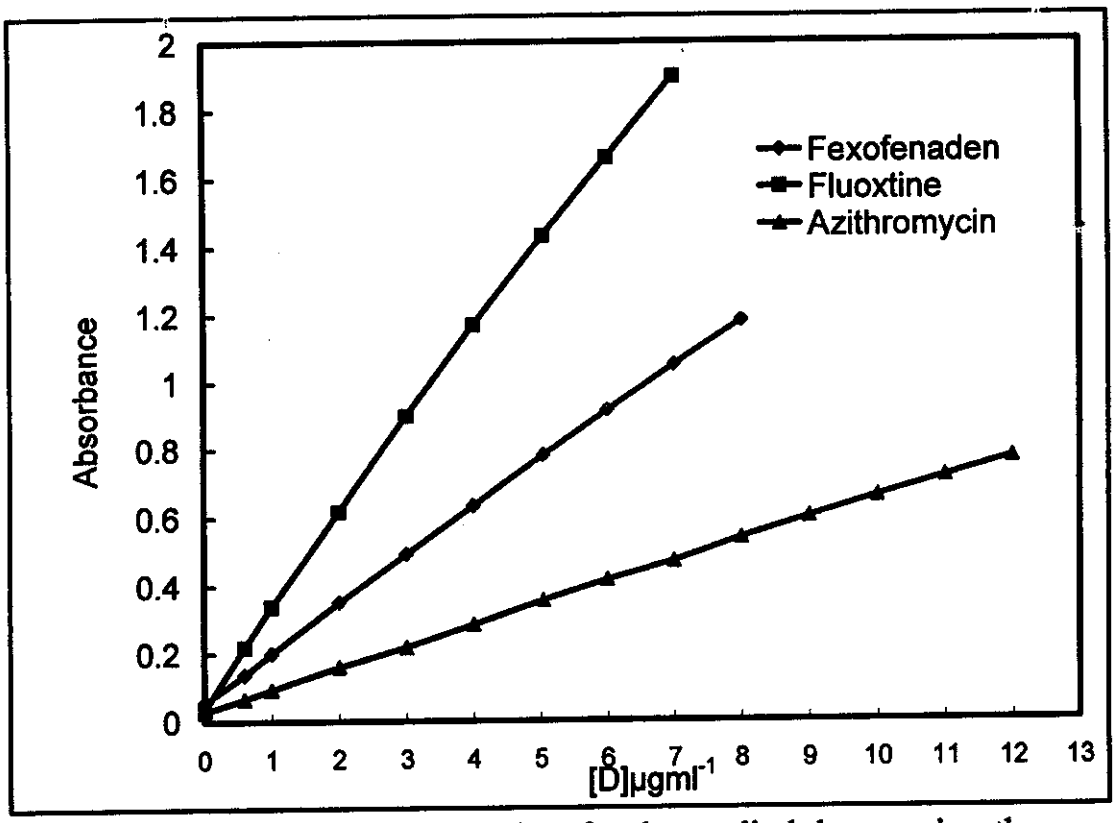


Fig (19): Application of Beer's law for the studied drugs using the optimum volume of (1.0×10^{-4} M) BCG.

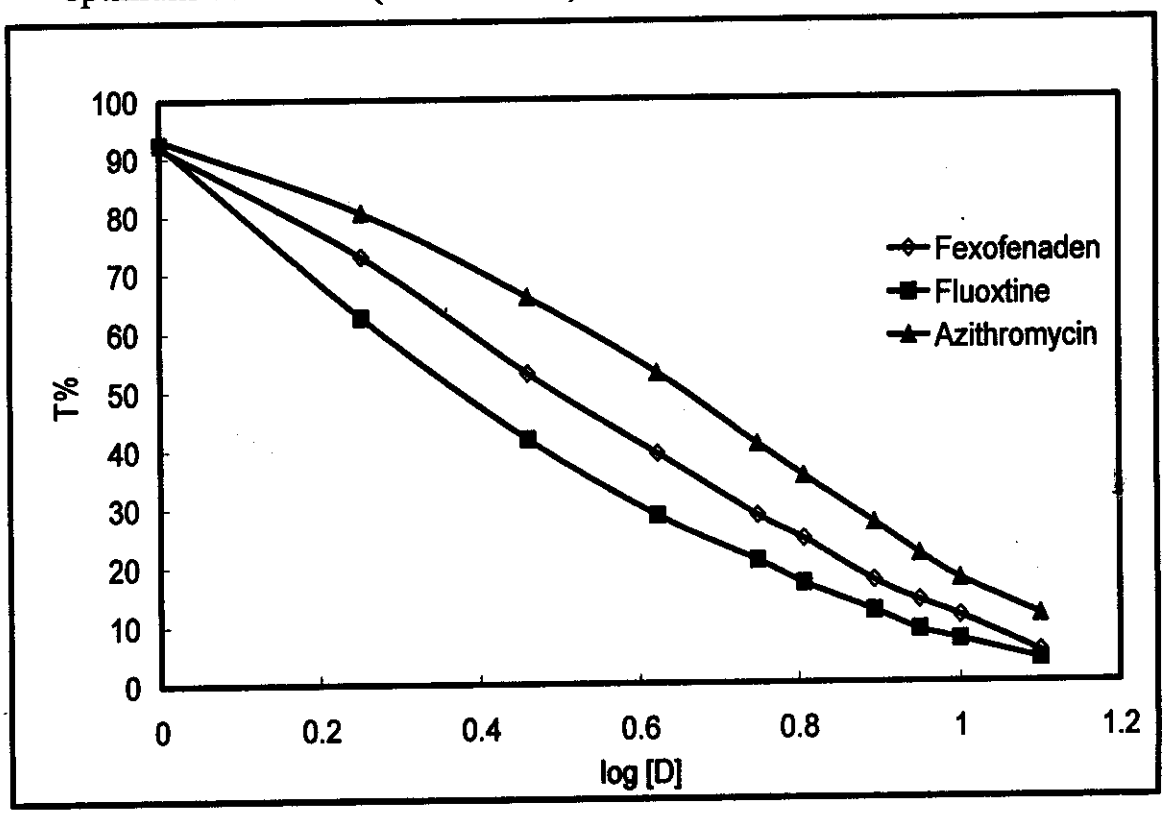


Fig (20): Ringbom plots for the studied drugs solution using the optimum volume of (1.0×10^{-4} M) BCG.

3. 1.3. Absorption spectra of the studied drugs with BTB

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 BTB (1.0×10^{-4} M), the effect of different experimental variables were studied and recorded below.

3. 1. 3. 1. Effect of pH

In order to establish the optimum pH value for each ion-pairs formed, Fexo., Fluox. and Azithr. was allowed to react with the BTB in aqueous type of buffered solution of various pH ranges (2.0 – 12.0). The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr). to measure The absorbance value at $\lambda_{max.}$ The highest absorbance values were obtained at pH 2.4 in case of Fexo., 2.4 in case of Fluox. and 2.2 in case of Azithr. which are selected for ion-pairs formation. These results are shown in Fig. (21). Furthermore, the amount of buffer was examined and found to be 2.0 ml in each case of Fexo., Fluox and Azithr. as shown in Fig. (22). The maximum wavelength corresponding to each ion–pair complex of the drugs with BTB is at 414 nm in case of Fexo., 410 nm in case of Fluox and at 411 nm in case of Azithr. as shown in Fig (23).

3. 1. 3. 2. Effect of time

The effect of time required for complete color development of the ion - pair formed between the studied drugs and BTB 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 in case of Fexo , Fluox, and Azithr which is measured directly against blank prepared by the same way without the drug, before

extraction. The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr), the optimum shaking time is 2.0 min in each case Fexo., Fluox, and Azithr as shown in Fig (24). The formed ion-pairs were found to be stable for more than 24 hours.

3. 1. 3. 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 BCG reagent on the drugs under consideration indicated that chloroform is the best solvent for extraction in the three cases (Fexo., Fluox and Azithr.) ion-pair 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 time.

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

When various concentrations of BTB were added to fixed concentrations of each of Fexo., Fluox and Azithr, it was found that. 2.0 ml of BCG (1.0×10^{-4} M) in the three cases (Fexo., Fluox and Azithr). were found to be sufficient for the production of the maximum and reproducible color intensity. Higher concentration of the reagent was affects in color intensity. The absorbance decreased gradually with increasing reagent concentration as shown in Fig (25).

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

The stoichiometery of the ion-pair complex was established by the molar ratio and continuous variation methods using both variable reagent

BTB (1.0×10^{-4} M) and the drug Fexo., Fluox and Azithr,. The results showed that the stoichiometeric ratio of the complexes 1:1 (reagent: drugs) and the shape of resulting curves indicated that the complex is labile, as shown in Figs. (26&27). Consequently, a large excess of reagent must be always used to enhance the formation of the complex. The stability constant of the complex was calculated by using the data of the mole ratio and job's continuous variation methods applying the equation) given in page 51. The results of the stability constants are recorded in Table (17).

3. 1. 3. 6. Suggested mechanism

The acid dye-drug technique is an ion – pair mechanism in which ion – pair is formed between negative ion produced from ionization of BTB, which is converted into BTB sodium salt in the buffer solution and positive ion of drug e.g. Fexofenadine Hydrochloride shown in Fig (28) the ion-pair exhibits maximum absorbance at λ_{max} 414 nm in case of complex with fexofenadine hydrochloride, 410 nm in case of fluoxetine hydrochloride, and at 411 nm in case of azithromycine.

3. 1. 3. 7. Validity to beer's law

A calibration graph was constructed using standard solution of the studied drugs. Under the optimum conditions, a linear relationship is obtained between the absorbance and concentration of the studied drugs. The concentration ranges listed in Table (17). The correlation coefficient, slopes and intercepts of the calibration data for Fexo., Fluox or Azithr are calculated using the equations given in (page 50, 51). The reproducibility of the method was determined by running six replicate samples, each containing 6.0 µg/ml of Fexo., Fluox and Azithr in the final assay solution. At this concentration, the relative standard deviation was found

to be ≤ 0.881 % for more accurate results of three drugs, Ringbom optimum concentration ranges are calculated and the mean molar absroptivity, Sandell sensitivity, detection and quantification limit are calculated from Beer's law. All this results are recorded in Table (17), while representation curves of the validity to Beer's law and Ringbom plots for BCG are shown in Figs. (29&30)

3. 1. 3. 8. *Accuracy and precision*

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Fexo., Fluox and Azithr were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table (18). The percent standard devotions and the percentage rang of error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

3. 1. 3. 9. *Analytical applications*

The validity of the proposed procedures is tested for determining Fexo., Fluox and Azithr in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t-value and variance ratio F-test with those obtained using official method on the sample of the same batch. The student's t-values obtained at 95% confidence level and five degrees of freedom did not exceed the

theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that their is no significant difference between precision of the proposed and the official method are recorded in Tables (19-21). The accuracy of the proposed method when applied to pharmaceutical preparations is evaluated by applying standard addition technique. In which variable amounts of the drugs (Fexo., Fluox and Azithr.) were added to the previously analyzed portion of pharmaceutical preparations. The results shown in Table (22-24), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystalline cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl p-hdroxy benzoate, propyl p-hydroxybenzoate, hydroxyl ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (17) Analytical data and Characteristics of Colored Product, precision and accuracy of the studied drugs Using BTB.

Parameters	Bromothymol blue		
	Fexofenadine	Fluoxetine	Azithromycine
pH	2.4	2.4	2.2
Wavelength λ_{max} (nm)	414	410	411
Stability constant	6.226	5.8599	6.032
Beer's law limits(µg/ml)	0.5 - 9.0	0.7- 6.0	0.2– 7.5
Ringboom limits (µg/ml)	0.55 – 8.5	0.8 – 5.7	0.3 – 7.1
Regression equation* Slope (b)	0.114	0.15	0.0808
Intercept (a)	0.0775	0.0391	0.0856
Standard deviation (SD)	0.0062	0.0069	0.0039
Correlation Coefficient (r)	0.9995	0.9996	0.9999
Detection limit (µg/ml)	0.0186	0.0207	0.0117
Quantitation limit (µg/ml)	0.062	0.069	0.039
Molar absorptivity x 10^4 ($Lmol^{-1}$ cm^{-1})	6.1	6.5	6.3
Sandell sensitivity((ng cm-2)	0.0084	0.0067	0.0011
RSD %	0.8313	0.8862	0.4829
RE%**	0.2531	0.2816	0.1592

*with respect to A = a + b C where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drugs in (µg/ml)
** Relative Slandered deviation % for six determinations.

Table (18): Evaluation of the accuracy and precision of the proposed method Using BTB .

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[a] (%)	RE (%)	Confidence[b] Limits
Fexofenadine Hydrochloride	2	2.02	100.10	1.4595	1.391	2.02 ± 0.0281
	4	4.01	100.25	1.4939	0.426	4.01± 0.0571
	6	5.97	99.50	0.6713	0.639	5.97± 0.0382
Fluoxetine Hydrochloride	3	3.01	100.33	1.4639	0.940	3.01± 0.0420
	5	4.98	99.60	0.6342	0.604	4.98± 0.0301
	6	6.03	100.50	0.9048	0.863	6.03± 0.0520
Azithromycine	2.5	2.52	100.80	1.715	1.634	2.52± 0.0412
	4	3.99	99.75	0.7626	0.726	3.99± 0.0290
	5.5	5.48	99.60	0.5418	0.538	5.48± 0.0283

[a] Relative standard deviation for six determinations.
[b] 95% confidence limits and five degrees of freedom.

Table (19):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fexo. In it's pharmaceutical forms using BTB.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Allerfen tab. 60 mg per tab	60	60.03	100.05	60	60.06	100.10	0.976	2.961
Telfast tab. 120 mg per tab.	120	119.97	99.97	120	120.05	100.04	0.948	2.861
Fexon tab 180 mg per tab.	180	179.96	99.98	180	180.07	100.04	0.839	1.923
Fastofen tab. 120 mg per tab.	120	119.98	99.98	120	120.01	100.01	1.281	1.962
Histafree tab. 120 mg per tab.	120	119.96	99.97	120	119.98	99.98	0.639	2.841
Fastel tab. 120 mg per tab.	120	119.94	99.95	120	120.03	100.03	0.875	2.317

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (20):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fluox. In it's pharmaceutical forms using BTB.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Flutine cap. 20 mg per cap	20	19.97	99.85	20	20.03	100.15	0.826	2.856
Prozac cap. 20 mg per cap.	20	19.99	99.95	20	19.97	99.85	0.957	2.116
Depreban cap. 20 mg per cap.	20	19.96	99.80	20	20.04	100.20	1.182	1.966
Fluozac cap. 10 mg per cap.	10	9.99	99.90	10	9.99	99.90	1.528	1.864
Florosin cap. 20 mg per cap.	20	19.97	99.85	20	20.01	100.05	0.957	0.947
Octazac cap. 20 mg per cap.	20	19.98	99.90	20	20.05	100.25	0.829	1.693

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (21):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Azithr. In it's pharmaceutical forms using BTB.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Azalide cap. 200 mg per cap.	200	199.94	99.97	200	200.06	100.03	0.974	2.831
Aziwok susp. 250 mg .	250	249.93	99.97	250	250.04	100.02	0.825	2.634
Xithrone cap. 250 mg per cap.	250	250.02	100.01	250	249.97	99.99	1.163	1.728
Zithromax cap. 250 mg per cap.	250	250.01	100.00	250	249.96	99.98	1.529	0.982
Zisrocin cap. 500 mg per cap.	500	499.94	99.99	500	500.07	100.01	0.847	1.927
Zithrokan cap. 500 mg per cap.	500	500.02	100.00	500	500.04	100.01	0.834	1.834

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table(22): Determination of Fexo. in its pharmaceutical dosage forms applying standard addition technique using BTB.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Allerfen tab. 60 mg per tab.	2.0	0.0	1.97	98.50
		2.0	4.05	101.25
		3.0	5.06	101.20
		5.0	7.07	101.00
Telfast tab. 120 mg per tab.	2.0	0.0	2.04	102.00
		2.0	4.06	101.50
		3.5	5.49	99.82
		4.0	6.07	101.16
Fexon tab 180 mg per tab.	2.5	0.0	2.54	101.60
		2.5	5.07	101.40
		3.5	6.03	100.50
		4.0	6.57	101.07
Fastofen tab. 120 mg per tab.	2.0	0.0	1.95	97.50
		3.0	5.07	101.40
		4.0	6.04	100.66
		5.0	7.07	101.00
Histafree tab. 120 mg per tab.	3.0	0.0	3.05	101.66
		2.0	4.95	99.00
		3.0	5.95	99.16
		3.5	6.45	99.23
Fastel tab. 120 mg per tab.	2.0	0.0	1.96	98.00
		2.0	4.06	101.50
		4.0	6.03	100.50
		5.0	7.08	101.14

* : Average of six determinations.

Table(23): Determination of Fluox. in its pharmaceutical dosage forms applying standard addition technique using BTB.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Flutine cap. 20 mg per cap.	2.0	0.0	2.04	102.00
		2.0	4.07	101.75
		3.5	5.57	101.27
		4.5	6.56	100.92
Prozac cap. 20 mg per cap.	2.0	0.0	1.96	98.00
		2.5	4.56	101.33
		3.5	5.55	100.91
		4.5	6.53	100.46
Depreban cap. 20 mg per cap.	2.0	0.0	2.03	101.50
		2.0	4.01	100.25
		3.0	5.07	101.40
		4.0	6.06	101.00
Fluozac cap. 10 mg per cap.	2.0	0.0	2.03	101.50
		2.0	4.05	101.25
		3.5	5.57	101.27
		4.5	6.54	100.61
Florosin cap. 20 mg per cap.	2.0	0.0	1.95	97.50
		2.0	4.02	100.50
		3.5	5.47	99.45
		4.5	6.46	99.38
Octazac cap. 20 mg per cap.	2.0	0.0	2.03	101.50
		2.5	4.48	99.55
		3.5	5.46	99.27
		4.5	6.53	100.46

* : Average of six determinations.

Table(24): Determination of Azithr. in its pharmaceutical dosage forms applying standard addition technique using BTB.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Azalide cap. 200 mg per cap.	2.0	0.0	2.03	101.50
		2.0	4.03	100.75
		3.0	4.96	99.20
		4.0	6.07	101.16
Aziwok susp. 250 mg .	1.5	0.0	1.47	98.00
		2.5	4.05	101.25
		3.5	4.95	99.00
		4.5	6.07	101.16
Xithrone cap. 250 mg per cap.	1.5	0.0	1.53	102.00
		2.5	4.05	101.25
		3.5	5.07	101.40
		4.5	6.03	100.50
Zithromax cap. 250 mg per cap.	2.0	0.0	1.96	98.00
		2.0	3.95	98.75
		3.0	5.06	101.20
		4.0	6.06	101.00
Zisrocin cap. 500 mg per cap.	2.0	0.0	1.97	98.50
		2.5	4.54	100.88
		3.5	5.54	100.72
		4.0	6.07	101.16
Zithrokan cap. 500 mg per cap.	1.5	0.0	1.53	102.00
		2.5	4.05	101.25
		3.5	5.06	101.20
		4.5	6.03	100.50

* : Average of six determinations.

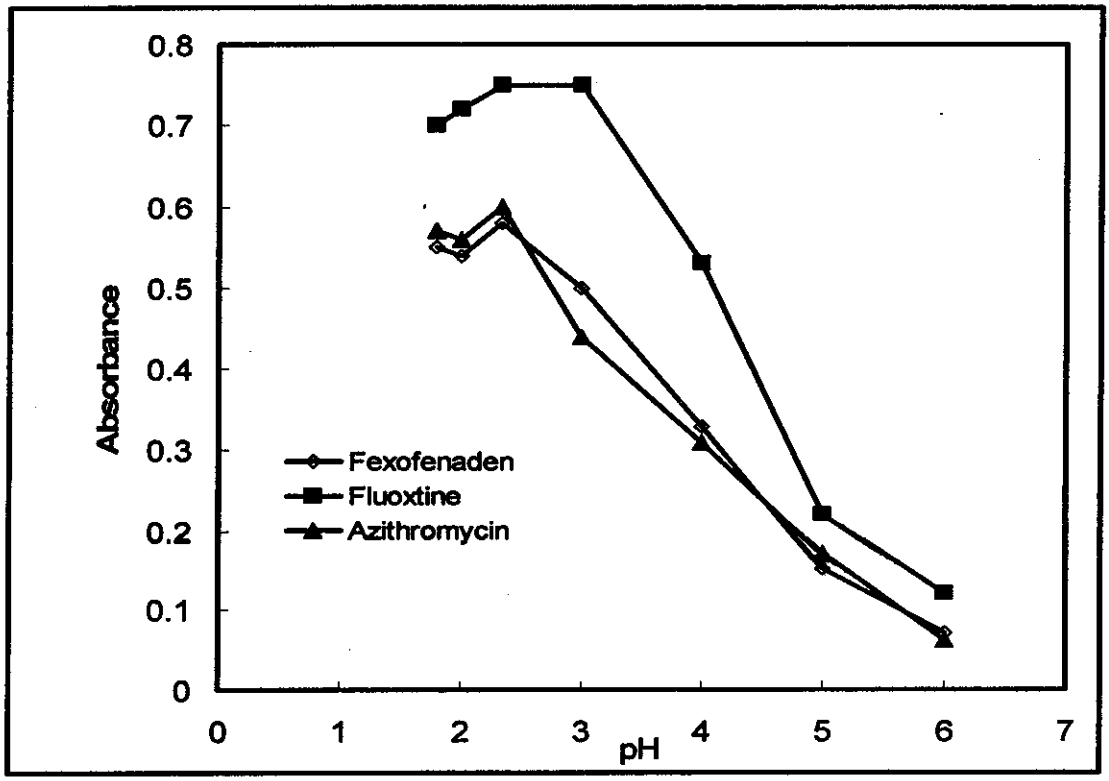


Fig. (21): Effect of pH on the absorbance of the studied drugs using (1.0×10^{-4} M) BTB.

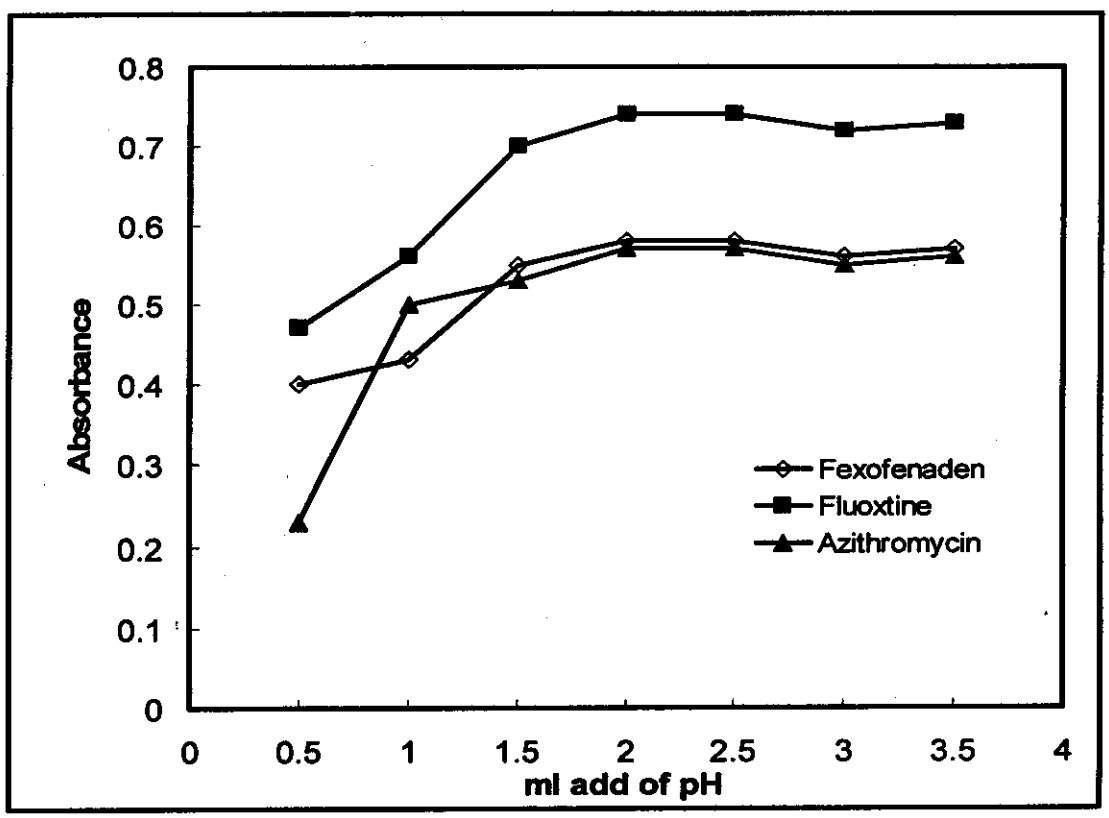


Fig (22): Effect of ml added of pH on the absorbance of the Studied drugs (1.0×10^{-4} M) BTB.

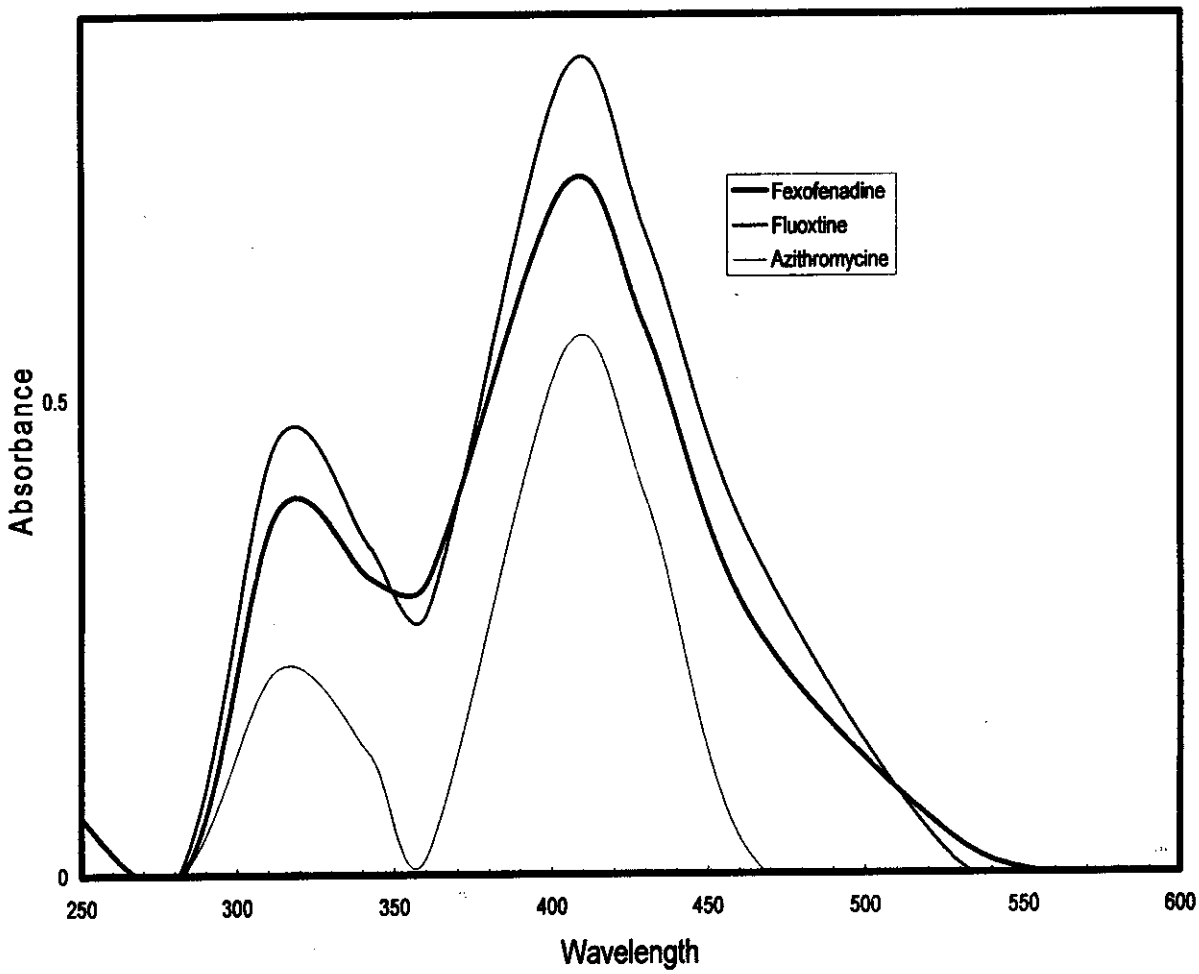


Fig (23): Absorption spectra of ion-pair complex of 10 µg/ml of the studied drugs with acid dye BTB (1.0×10^{-4} M).

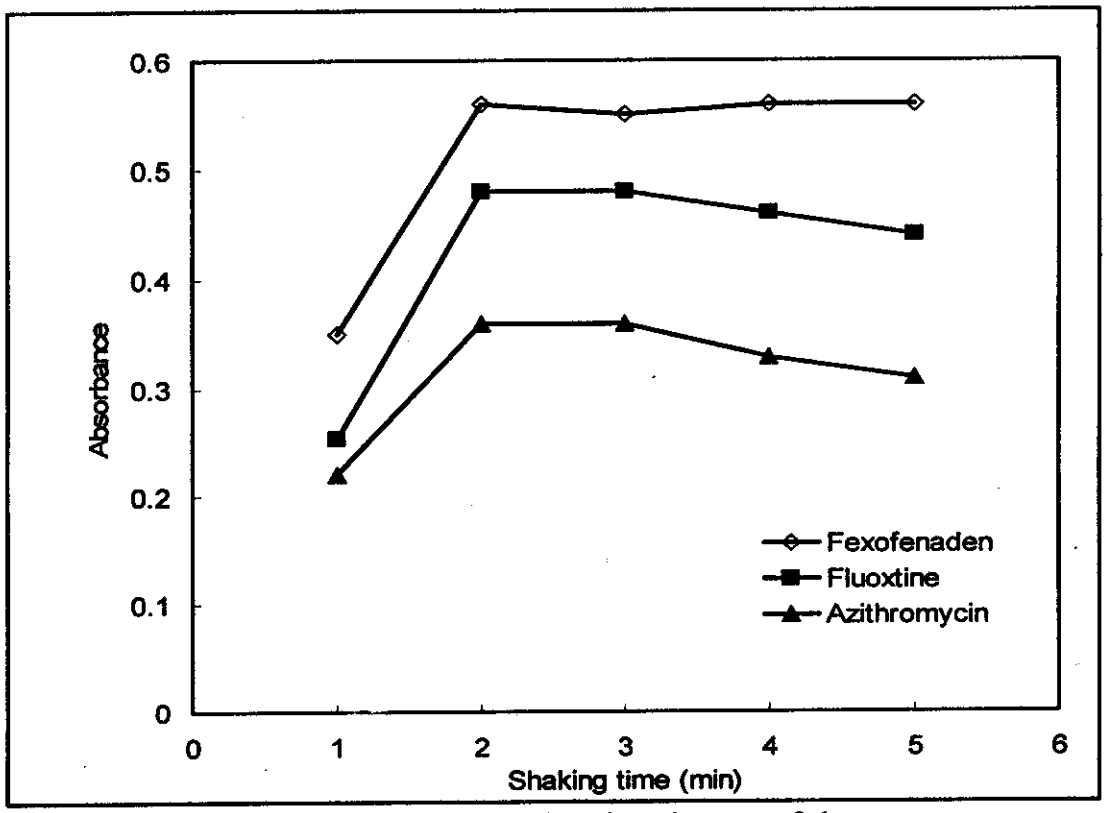


Fig (24): Effect of shaking time on the absorbance of the studied drugs solution using (1.0 x 10^{-4} M) BTB.

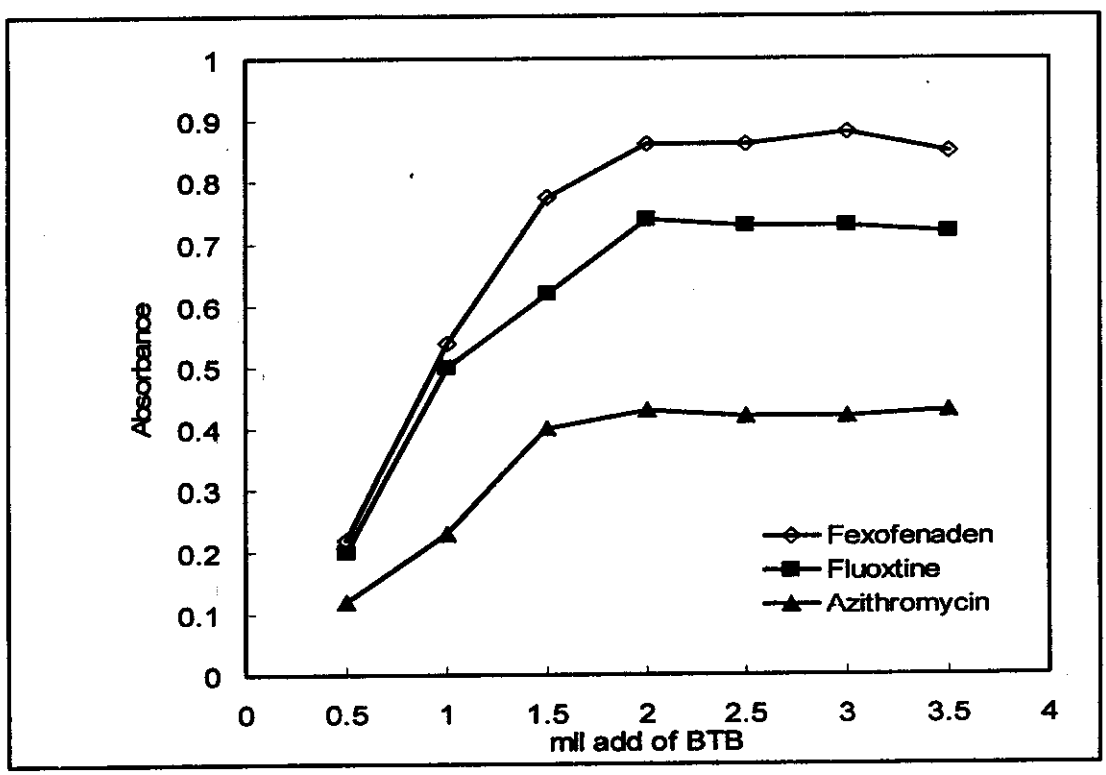


Fig. (25): Effect of reagent concentration on the absorbance of the studied drugs solution using (1.0 x 10^{-4} M) BTB.

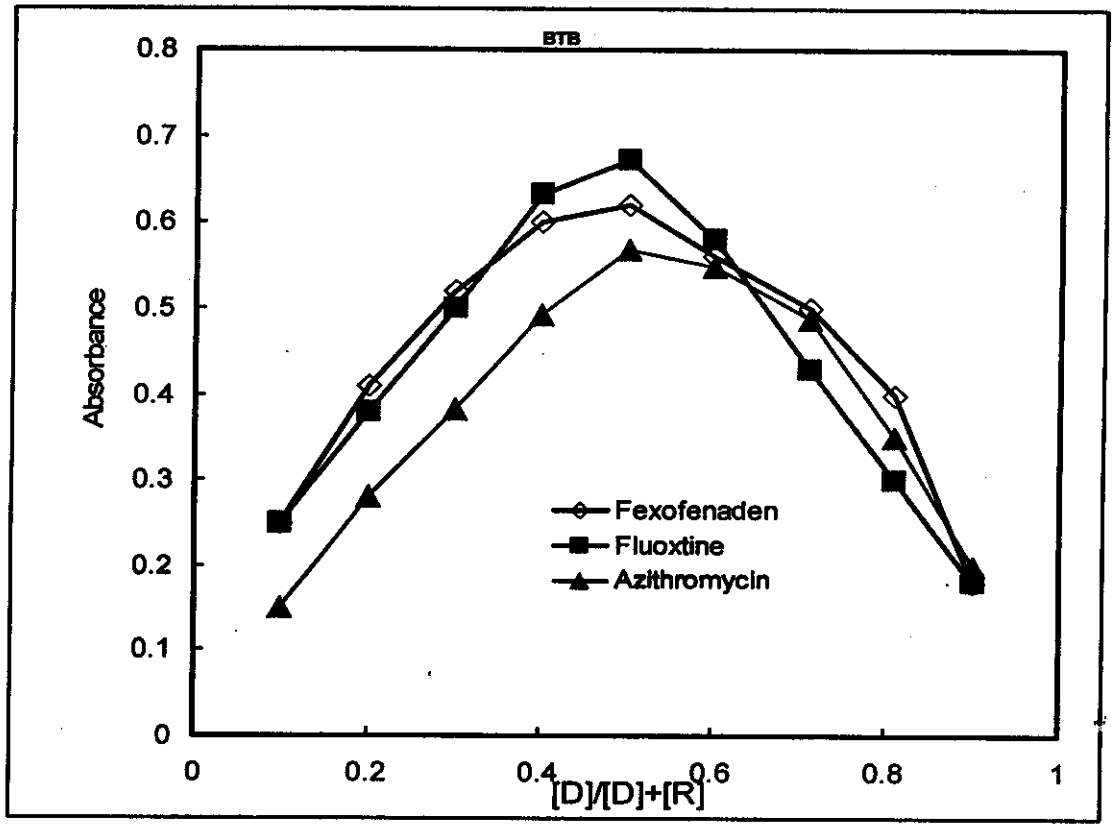


Fig. (26) : continuous variation using BTB (1.0×10^{-4} M) reagent with (1.0×10^{-4} M) of the Drugs under consideration.

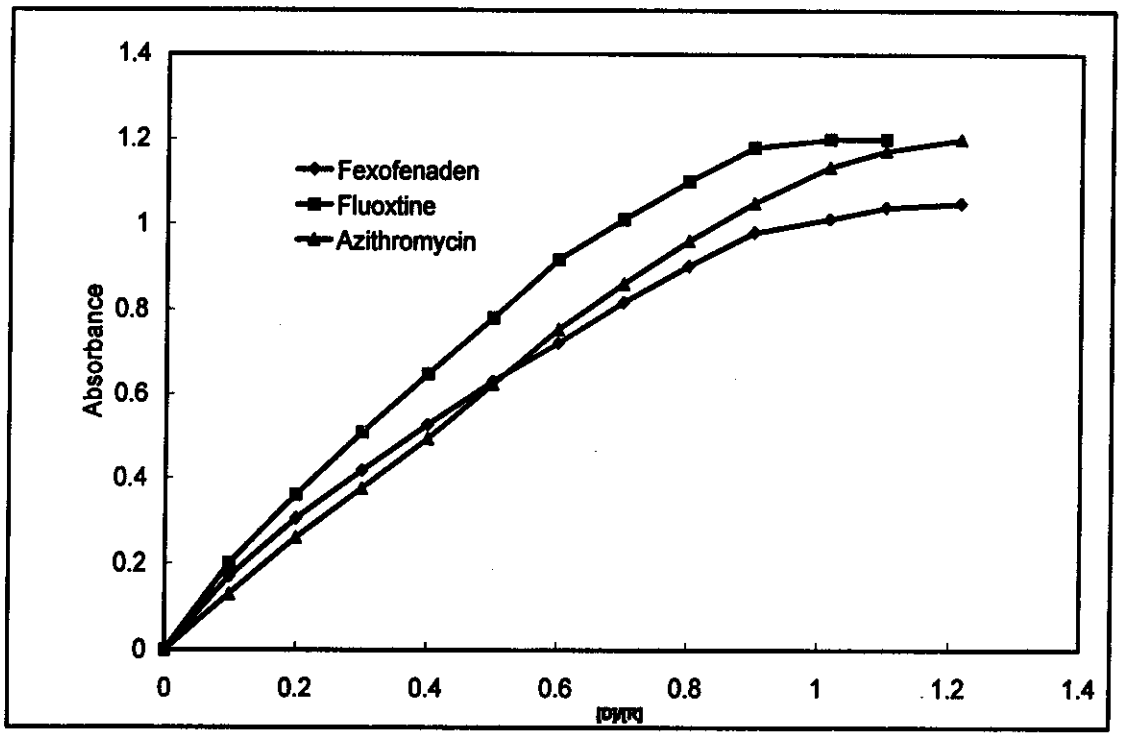
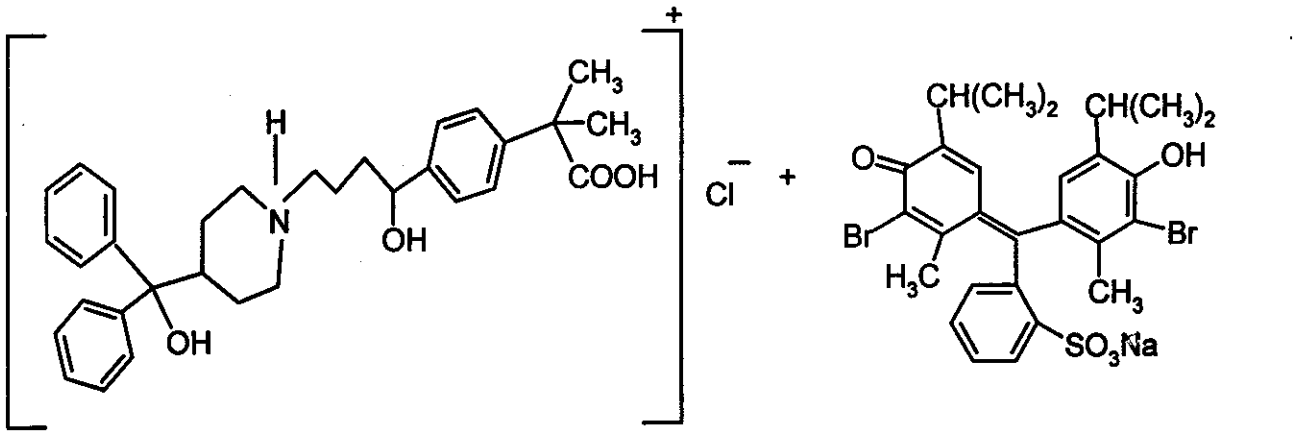


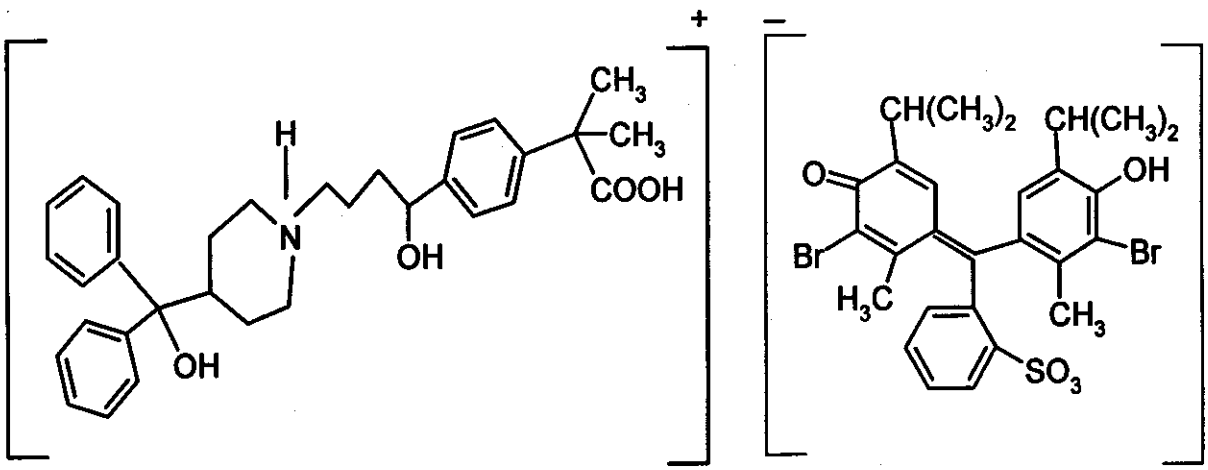
Fig. (27): Mole ratio for BTB - Drugs (1.0×10^{-4} M) under consideration.

Fig (28): proposed mechanism of the reaction between Fexofenadine hydrochloride and BTB sodium salt.



Fexofenadine hydrochloride Bromothymol blue Sodium salt

Buffer (pH 2.4) -NaCl



Fexofenadine hydrochloride -BTB complex

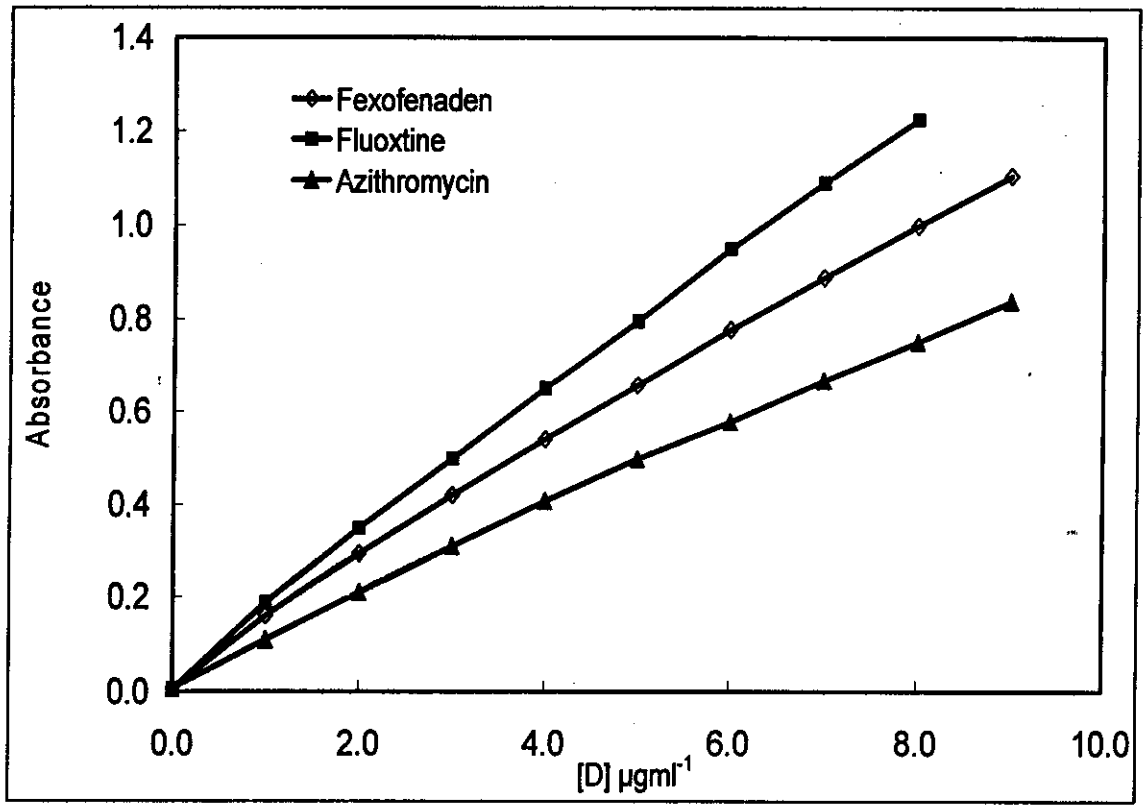


Fig (29): Application of beer's law for the studied drugs using the optimum volume of (1.0×10^{-4} M) BTB.

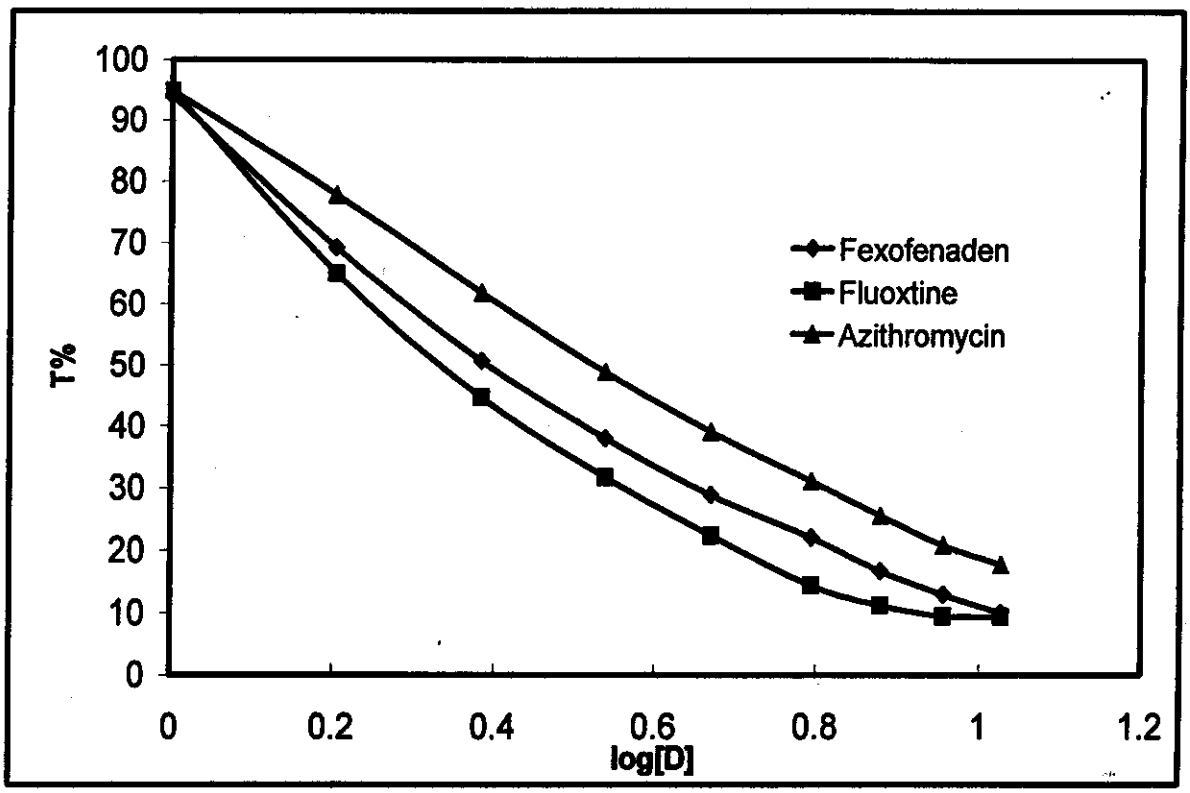


Fig (30): Ringbom plots for the studied drugs solution using the optimum volume of (1.0×10^{-4} M) BTB.

3. 1. 4. Absorption spectra of the studied drugs with BCP

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 BCP (1.0 x 10^{-3} M), the effect of different experimental variables were studied and recorded below.

3. 1. 4. 1. Effect of pH

In order to establish the optimum pH value for each ion-pairs formed, between the studied drugs BCP and the investigated. Fexo., Fluox. and Azithr. was allowed to react with the BCP in aqueous type of buffered solution of various pH ranges (2.0 – 12.0). The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr). to measure The absorbance value at $\lambda_{max.}$ The highest absorbance values were obtained at pH 2.4 in case of Fexo., 2.4 in case of Fluox. and 2.2 in case of Azithr. which are selected for ion-pairs formation. These results are shown in Fig. (31). Furthermore, the amount of buffer was examined and found to be 2.0 ml in each case of Fexo., Fluox and 3ml in case of Azithr. As shown in Fig. (32). The maximum wavelength corresponding to each ion–pair complex of the drugs with BCP is at 414 nm in case of Fexo., 410 nm in case of Fluox and at 411 nm in case of Azithr. As shown in Fig (33).

3. 1. 4. 2. Effect of time

The effect of time required for complete color development of the ion - pair formed between the studied drugs and BCP 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 in case of Fexo and Fluox, which is measured directly against

blank prepared by the same way without the drug, whereas in case of Azithr 3.0 min are quite sufficient to obtain maximum color intensity, before extraction. The formed ion-pair was extracted with chloroform in three cases (Fexo., Fluox and Azithr), the optimum shaking time is 2.0 min in each case Fexo., Fluox, and 2.0 min in case of Azithr as shown in Fig (34). The formed ion-pairs were found to be stable for more than 12 hours.

3. 1. 4. 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 BCP reagent on the drugs under consideration indicated that chloroform is the best solvent for extraction in the three cases (Fexo., Fluox and Azithr.)ion-pair. 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 2.0 ml of the solvent in one time.

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

When various concentrations of BCP were added to fixed concentrations of each of Fexo., Fluox and Azithr, it was found that 2.0 ml of BCG (1.0×10^{-4} M) in the three cases (Fexo., Fluox and Azithr). were found to be sufficient for the production of the maximum and reproducible color intensity. The higher concentration of the reagent was affects in color intensity the absorbance decreased gradually with increasing reagent concentration as in shown in Fig (35).

3. 1. 4. 5. composition of the ion – pair complex and the stability constant of it.

The stoichiometery of the ion-pair complex was established by the mole ratio and continuous variation methods using both variable reagent BCP (1.0×10^{-4} M) and the drug Fexo., Fluox and Azithr. The results showed that the stoichiometeric ratio of the complexes 1:1 (reagent: drugs) and the shape of resulting curves indicated that the complex is labile, as shown in Figs. (36&37). Consequently, a large excess of reagent must be always used to enhance the formation of the complex. The stability constant of the complex was calculated by using the data of the mole ratio and job's continuous variation methods applying the equation given in page (50,51). The results of the stability constants are recorded in Table (25).

3. 1. 4. 6. Suggested mechanism

The acid dye-drug technique is an ion – pair mechanism in which ion – pair is formed between negative ion produced from ionization of BCP, which is converted into BCP sodium salt in the buffer solution and positive ion of drug e.g. Fexofenadine Hydrochloride shown in Fig (38). the ion–pair formed exhibits maximum absorbance at $\lambda_{max.}$ 414 nm in case of complex with Fexofenadine hydrochloride, 410 nm in case of complex with Fluoxetine Hydrochloride and at 411 nm in case of complex with Azithromycine.

the soiled ion-pair complex confirmed by CHN and IR spectra in Micro Analytical Center Faculty of science Cairo University and it was found as in table (25).

Table (25): CHN of solid ion – pair complex

	C%	H%	N%
Found	56.61	4.80	2.00
Calculated	53.667	3.98	1.63

3. 1. 4. 7. Validity to beer's law

A calibration graph was constructed using standard solution of the studied drugs. Under the optimum conditions, a linear relationship is obtained between the absorbance and concentration of the studied drugs in the concentration ranges listed in Table (25). The correlation coefficient, slopes and intercepts of the calibration data for Fexo., Fluox or Azithr are calculated using the equations given in (page 50, 51). The reproducibility of the method was determined by running six replicate samples, each containing 5.0 µg/ml of Fexo., Fluox and Azithr in the final assay solution. At this concentration, the relative standard deviation was found to be ≤ 0.951 % for more accurate results, Ringbom optimum concentration ranges are calculated and the mean molar absroptivity, Sandell sensitivity, detection and quantification limit are calculated from Beer's law. All this results are recorded in Table (25), which representation curves of the validity to Beer's law and Ringbom plot for BCP are shown in Figs. (39&40)

3. 1. 4. 8. Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Fexo., Fluox and Azithr were prepared and analyzed in six replicates. The

analytical results obtained from this investigation are summarized in Table (26). The percent standard devotions and the percentage rang of error at 95% confidence level were calculated. The results can be considered as very satisfactory, at least for the level of concentrations examined.

3. 1. 4. 9. Analytical applications

The validity of the proposed procedures is tested for determining Fexo., Fluox and Azithr in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t-value and variance ratio F-test with those obtained using official method on the sample of the same batch. The student's t-values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that their is no significant difference between precision of the proposed and the official method Table (27-29). The accuracy of the proposed method when applied to pharmaceutical preparations is evaluated by applying standard addition technique. In which variable amounts of the drugs (Fexo., Fluox and Azithr.) were added to the previously analyzed portion of pharmaceutical preparations. The results shown in Tables (30-32), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystalline cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate,

methyl p-hdroxy benzoate, propyl p-hydroxy benzoate, hydroxyl ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

3. 1. 4. 10. Infrared spectra of solid complexes:

A substantial part of the knowledge concerning the mode of bonding in ion – pair complex can be gained by applying infrared spectrophotometry either in absorption or reflactance arrangements. An insight in the type of bonds formed between the reagent and Drugs can be achieved by careful investigation of the spectrum of the complex compound essentially in comparison to that of the free reagent.

The Ir - spectra of the solid ion – pair produced from the complexes of reagent with drug under investigation of stoichiometeric ratio (1:1) are studied. The spectra of solid complex are shown in Fig. (41).

An examination of the ir-spectra of the solid complex show that, the band due to γ- (OH) or γ-N-H stretching vibration are expected to appear 3424 cm^{-1}. This confirmed by band at 1328 cm^{-1} corresponding to the in-plane bending modes of γ-OH group. The ν_{CH} bands for aromatic system and aliphatic group appear at 2960 and 2775 cm^{-1} respectively. The band observed at 1617 and 1517 cm^{-1} are attributed to stretching vibrations of C=O and C=N groups, respectively. Also the ir spectra show band at 1248 that corresponding to stretching vibration of C-O.

Table. (26) Analytical data and characteristics of colored product ,precision and accuracy of the studied drugs using BCP.

Parameters	Bromocresol purple		
	Fexofenadine	Fluoxetine	Azithromycine
PH	2.4	2.2	2.2
Wavelength λ_{max} (nm)	411	408	408
Stability constant	5.6879	5.797	5.472
Beer's low limits(µg/ml)	0.5 - 6.0	0.5- 8.0	0.4– 11.0
Ringbom limits (µg/ml)	0.83 – 5.76	0.9 – 7.72	1 – 10.84
Regression equation* slope (b)	0.15	0.2245	0.123
Intercept (a)	0.0602	0.0867	0.0071
Slandered deviation (SD)	0.0052	0.0086	0.0040
Correlation coefficient (r)	0.988	0.997	0.997
Detection limit (µg/ml)	0.0156	0.0258	0.0120
Quantitation limit (µg/ml)	0.0052	0.0086	0.0040
Molar Absorptivity ($10^{4)}$ ($1mol^{-1}$ cm^{-1})	6.9	5.6	9.4
Sandell sensitivity((μg cm^{-1})	0.0076	0.0078	0.008
Error**%	0.2123	0.3511	0.1633
RSD %	0.9446	0.9523	0.9850

*with respect to A=a+ bC where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drugs in (µg/ml)
** Average of six determinations.

Table (27): Evaluation of the accuracy and precision of the proposed method Using BCP.

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD [a] (%)	RE (%)	Confidence [b] Limits
Fexofenadine Hydrochloride	2	2.03	101.50	0.5065	0.4828	2.03 ± 0.0098
	4	4.07	101.75	0.9023	0.8599	4.07 ± 0.0350
	6	6.05	100.83	0.7318	0.6975	6.05 ± 0.0422
Fluoxetine Hydrochloride	3	3.01	100.33	0.5961	0.5681	3.01 ± 0.0171
	5	5.04	100.8	0.6682	0.6369	5.04 ± 0.0321
	6	5.96	99.33	0.9277	0.8842	5.96 ± 0.0527
Azithromycine	2.5	2.49	99.60	0.9439	0.8996	2.49 ± 0.0224
	4	3.97	99.25	1.1443	1.0907	3.97 ± 0.0433
	6	5.98	99.67	0.7457	0.7107	5.98 ± 0.0425

[a] Relative standard deviation for six determinations.
[b] 95% confidence limits and five degrees of freedom.

Table (28):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fexo. In it's pharmaceutical forms using BCP.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Allerfen tab. 60 mg per tab	60	60.02	100.03	60	60.05	100.08	0.937	1.573
Telfast tab. 120 mg per tab.	120	119.94	99.95	120	119.97	99.97	0.894	1.732
Fexon tab 180 mg per tab.	180	179.94	99.97	180	180.04	100.02	0.629	1.927
Fastofen tab. 120 mg per tab.	120	120.02	100.02	120	120.06	100.05	0.926	2.731
Histafree tab. 120 mg per tab.	120	119.96	99.97	120	119.97	99.97	1.261	2.163
Fastel tab. 120 mg per tab.	120	120.03	100.03	120	120.06	100.05	1.042	2.957

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table (29):Evaluation of the accuracy and precision of the proposed and official methods for the determination of Fluox. In it's pharmaceutical forms using BCP.

Dosage forms	Official method			Proposed method				
	Taken mg	**Found* mg**	**Recovery (%)**	**Taken mg**	**Found* mg**	**Recovery (%)**	**t** value**	**F****
Flutine cap. 20 mg per cap	20	19.97	99.85	20	20.05	100.25	0.836	2.843
Prozac cap. 20 mg per cap.	20	19.99	99.95	20	20.02	100.10	0.926	2.359
Depreban cap. 20 mg per cap.	20	19.96	99.80	20	20.03	100.15	0.957	1.947
Fluozac cap. 10 mg per cap.	10	9.97	99.70	10	10.01	100.10	1.035	1.976
Florosin cap. 20 mg per cap.	20	20.01	100.05	20	20.04	100.20	1.837	0.9487
Octazac cap. 20 mg per cap.	20	20.04	100.20	20	20.01	100.05	0.837	1.759

* : Average of six determinations.
** : Theoretical values for t- and F- values for five degree of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

Table(31): Determination of Fexo in its pharmaceutical dosage forms applying standard addition technique using BCP.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Allerfen tab. 60 mg per tab.	2.0	0.0	2.03	101.50
		2.0	4.06	101.50
		3.0	5.06	101.20
		5.0	7.08	101.14
Telfast tab. 120 mg per tab.	2.0	0.0	1.95	97.50
		2.0	4.03	100.75
		3.5	5.52	100.36
		4.0	6.06	101.00
Fexon tab 180 mg per tab.	2.5	0.0	2.46	98.40
		2.5	5.05	101.00
		3.5	6.05	100.83
		4.0	6.53	100.46
Fastofen tab. 120 mg per tab.	2.0	0.0	1.96	98.00
		3.0	5.04	100.80
		4.0	6.07	101.16
		5.0	7.06	100.85
Histafree tab. 120 mg per tab.	3.0	0.0	3.02	100.66
		2.0	5.03	100.60
		3.0	6.07	101.16
		3.5	6.54	100.61
Fastel tab. 120 mg per tab.	2.0	0.0	2.04	102.00
		2.0	4.05	101.25
		4.0	5.96	99.33
		5.0	7.05	100.71

* : Average of six determinations.

Table(32): Determination of Fluox in its pharmaceutical dosage forms applying standard addition technique using BCP.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Flutine cap. 20 mg per cap.	2.0	0.0	1.95	97.50
		2.0	3.95	98.75
		3.5	5.54	100.73
		4.5	4.55	70.00
Prozac cap. 20 mg per cap.	2.0	0.0	2.02	101.00
		2.5	4.53	100.66
		3.5	5.52	100.36
		4.5	6.51	100.15
Depreban cap. 20 mg per cap.	2.0	0.0	2.01	100.50
		2.0	4.02	100.50
		3.0	5.04	100.80
		4.0	6.05	100.83
Fluozac cap. 10 mg per cap.	2.0	0.0	1.96	98.00
		2.0	3.95	98.75
		3.5	5.53	100.54
		4.5	6.53	100.46
Florosin cap. 20 mg per cap.	2.0	0.0	1.96	98.00
		2.0	3.96	99.00
		3.5	5.53	100.54
		4.5	6.52	100.30
Octazac cap. 20 mg per cap.	2.0	0.0	1.96	98.00
		2.5	4.53	100.66
		3.5	5.52	100.36
		4.5	6.52	100.30

* : Average of six determinations.

Table(33): Determination of Azithr in its pharmaceutical dosage forms applying standard addition technique using BCP.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Azalide cap. 200 mg per cap.	2.0	0.0	1.96	98.00
		2.0	4.03	100.75
		3.0	5.06	101.20
		4.0	6.07	101.16
Aziwok susp. 250 mg .	1.5	0.0	1.52	101.33
		2.5	4.03	100.75
		3.5	5.06	101.20
		4.5	6.07	101.16
Xithrone cap. 250 mg per cap.	1.5	0.0	1.52	101.33
		2.5	4.02	100.50
		3.5	5.05	101.00
		4.5	6.05	100.83
Zithromax cap. 250 mg per cap.	2.0	0.0	1.95	97.50
		2.0	4.03	100.75
		3.0	4.95	99.00
		4.0	5.95	99.16
Zisrocin cap. 500 mg per cap.	2.0	0.0	1.98	99.00
		2.5	4.52	100.44
		3.5	5.52	100.36
		4.0	6.02	100.33
Zithrokan cap. 500 mg per cap.	1.5	0.0	1.52	101.33
		2.5	4.03	100.75
		3.5	4.95	99.00
		4.5	5.97	99.50

* : Average of six determinations.

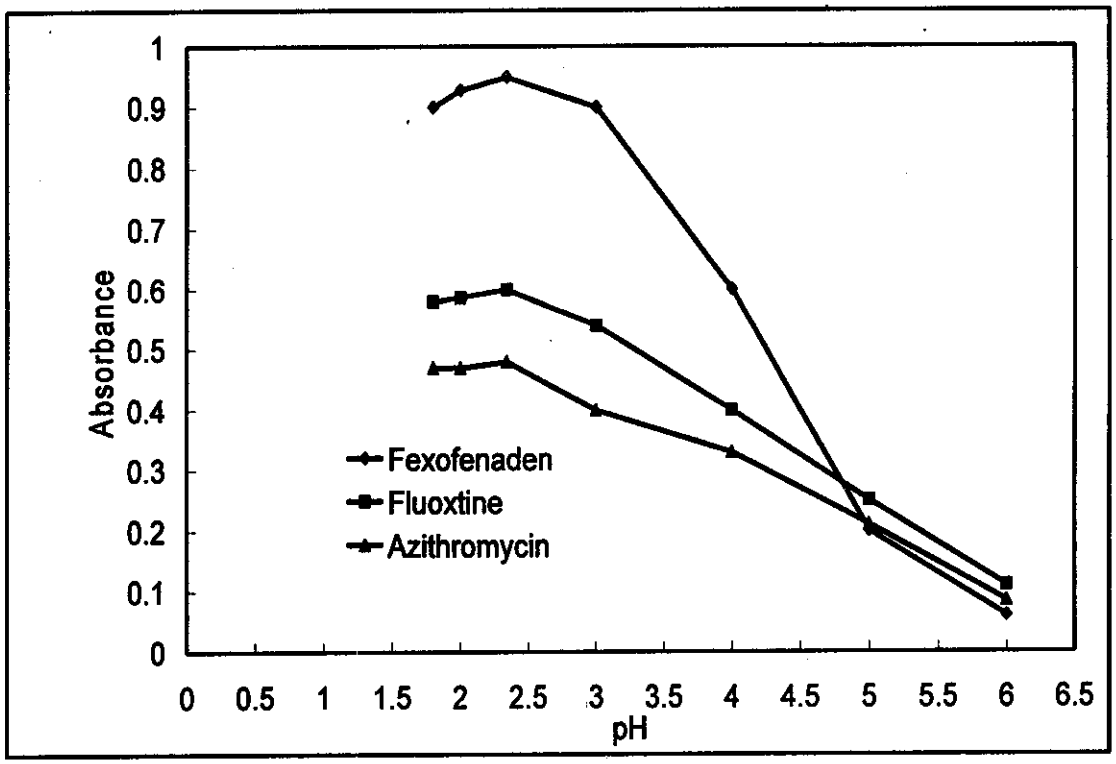


Fig (31): effect of pH on the absorbance of the studied drugs using ($1.0x\ 10^{-4}$M) BCP.

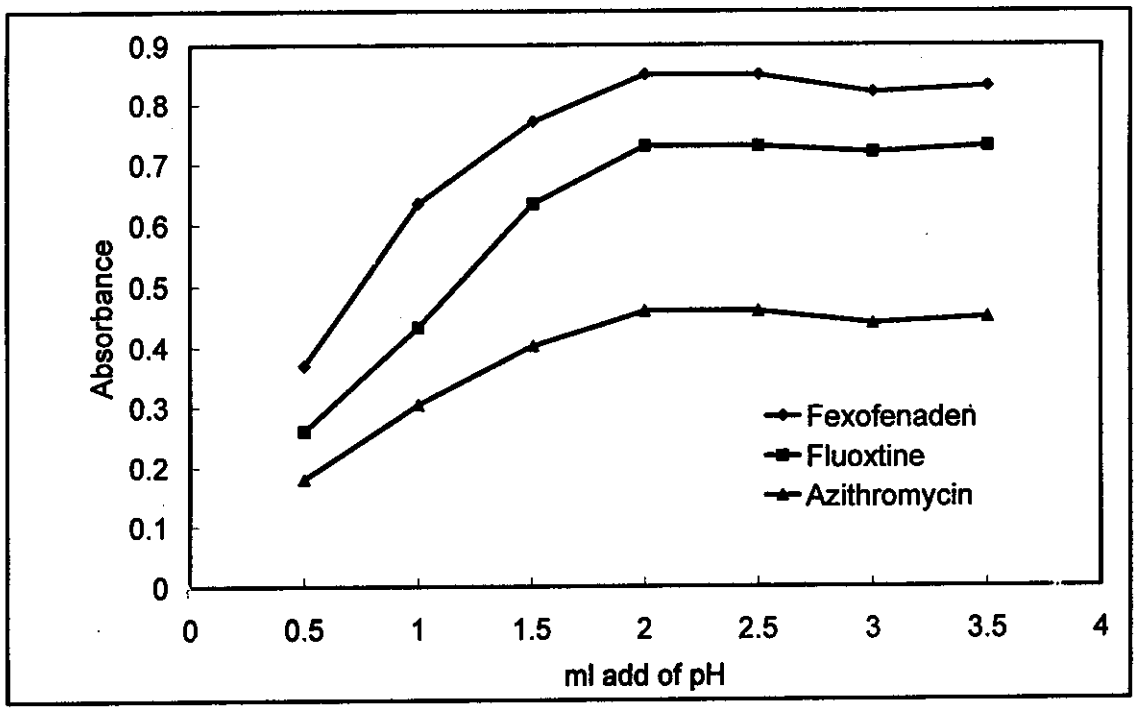


Fig (32): Effect of ml added of pH on the absorbance of the Studied drugs using ($1.0x\ 10^{-4}$ M) BCP.

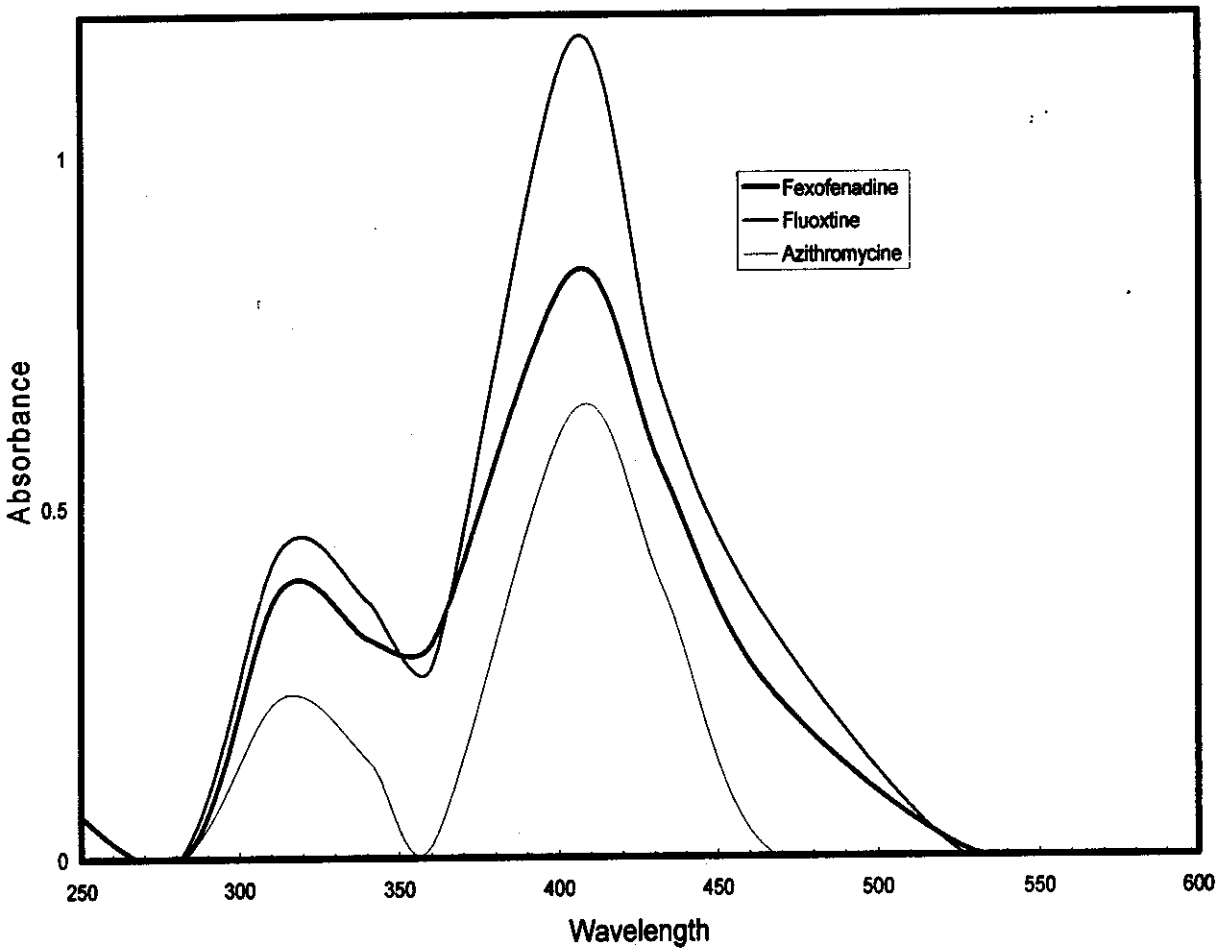


Fig (33): Absorption spectra of ion-pair complex of 5.0 μg/ml of the studied drugs with acid dye BCP (1.0×10^{-4} M).

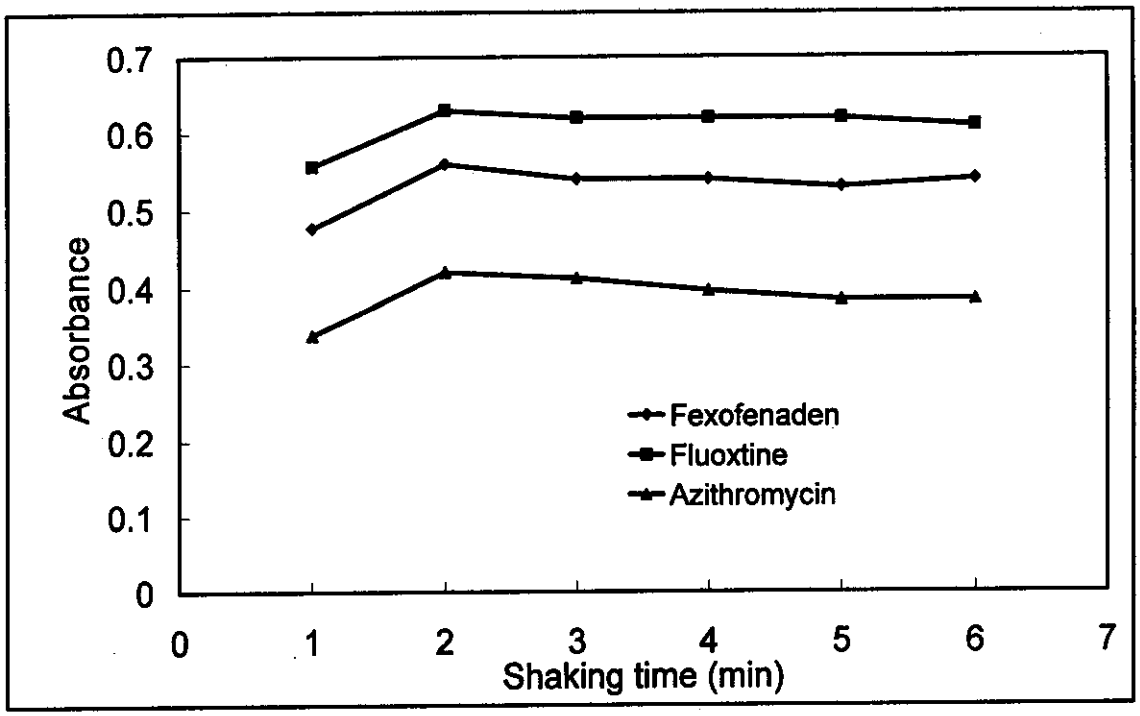


Fig (34): Effect of shaking time on the absorbance of the studied drugs solution using (1.0×10^{-4} M) BCP.

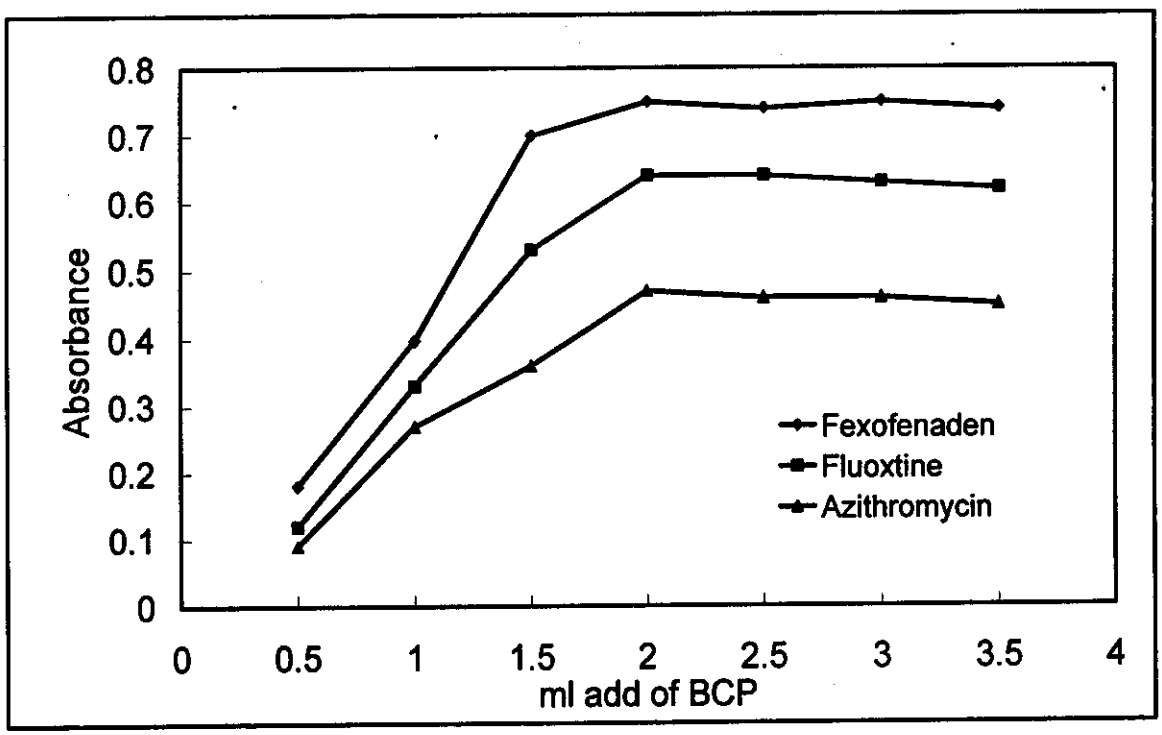


Fig. (35): Effect of reagent concentration on the absorbance of the studied drugs solution using (1.0×10^{-4} M) BCP.

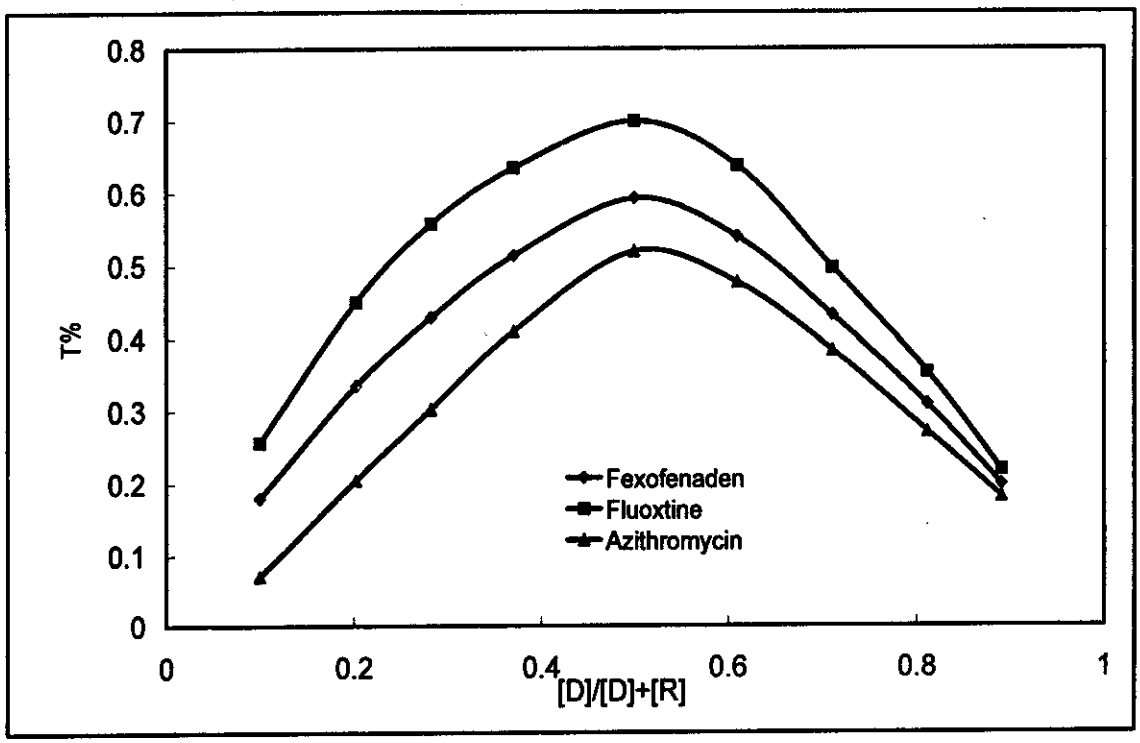


Fig. (36) : continuous variation using BCP (1.0×10^{-4} M) reagent with (1.0×10^{-4} M) of the Druges under consideration.

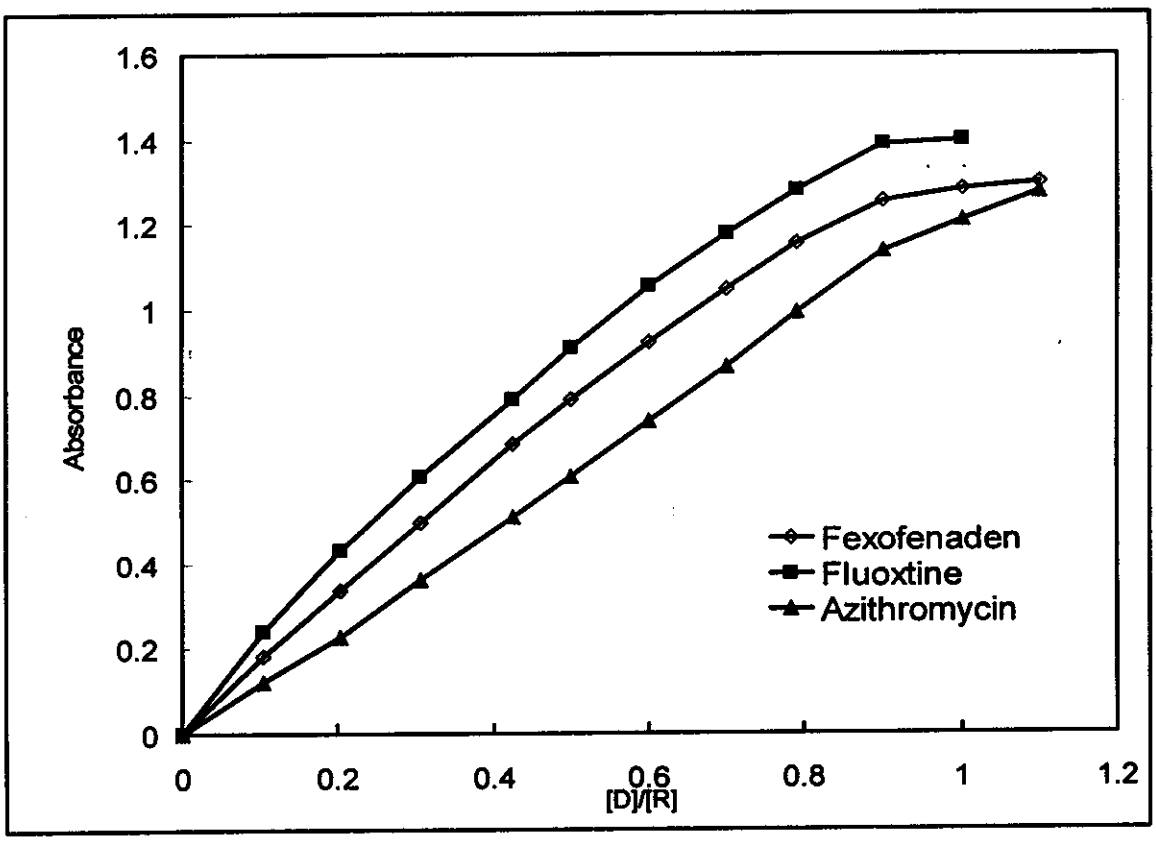
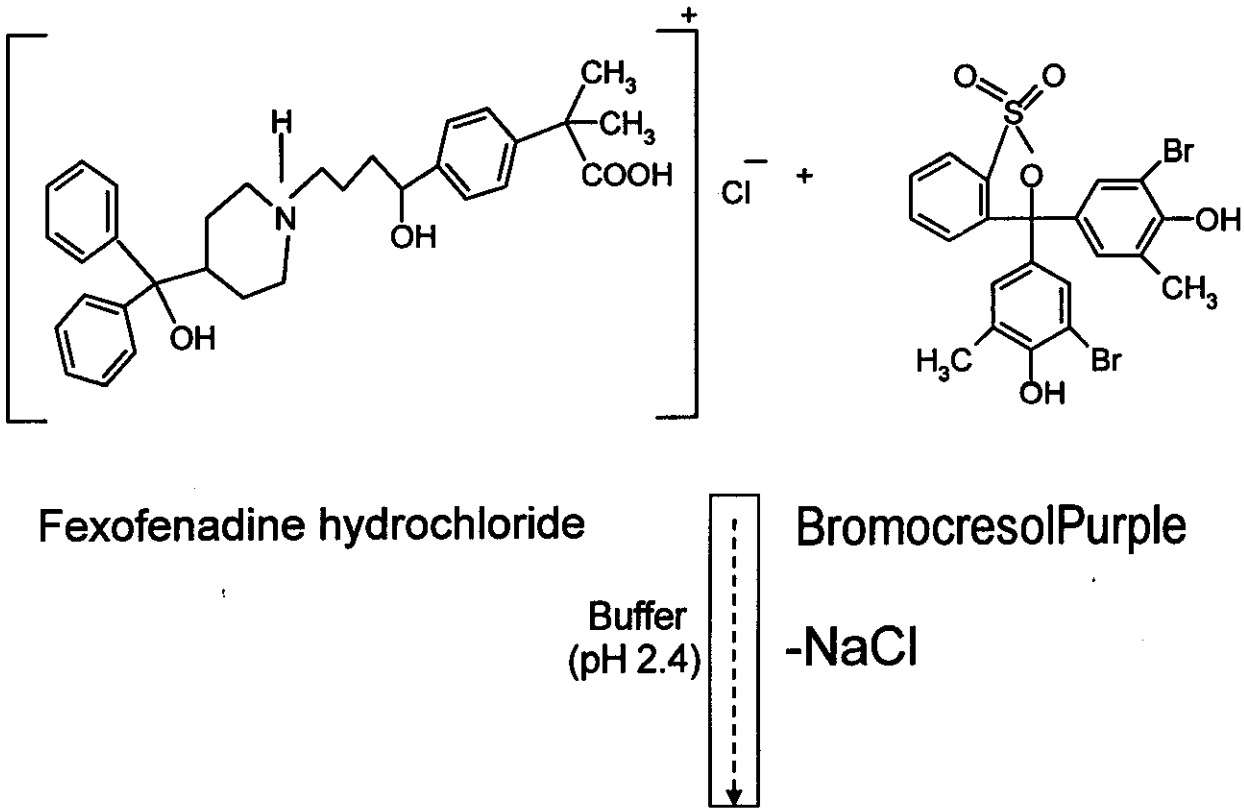
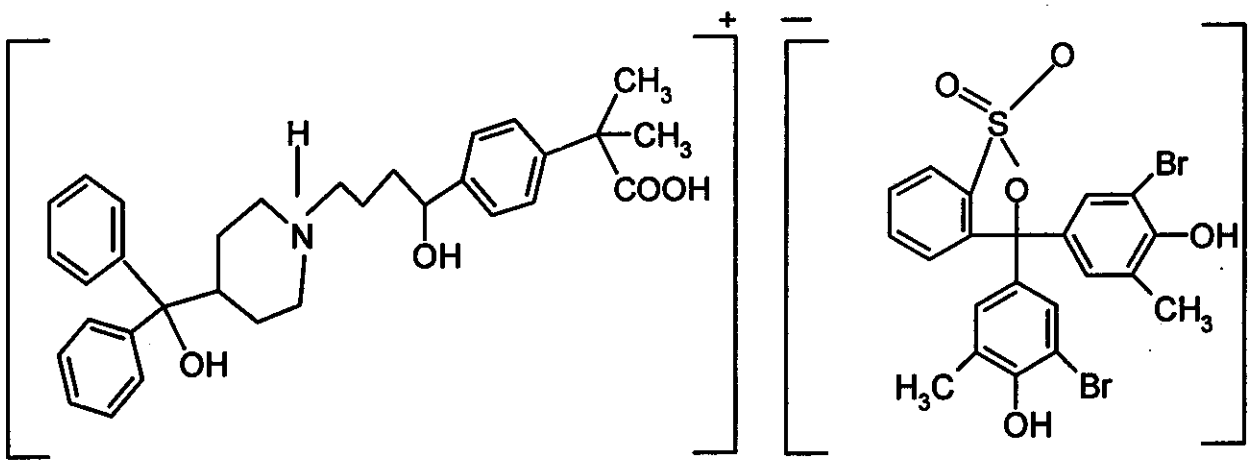


Fig. (37): Mole ratio for BCP - Drugs (1.0×10^{-4} M) under consideration.

Fig (38): proposed mechanism of the reaction between Fexofenadine hydrochloride and BromoCresol purple sodium salt.





Fexofenadine hydrochloride -BCP complex

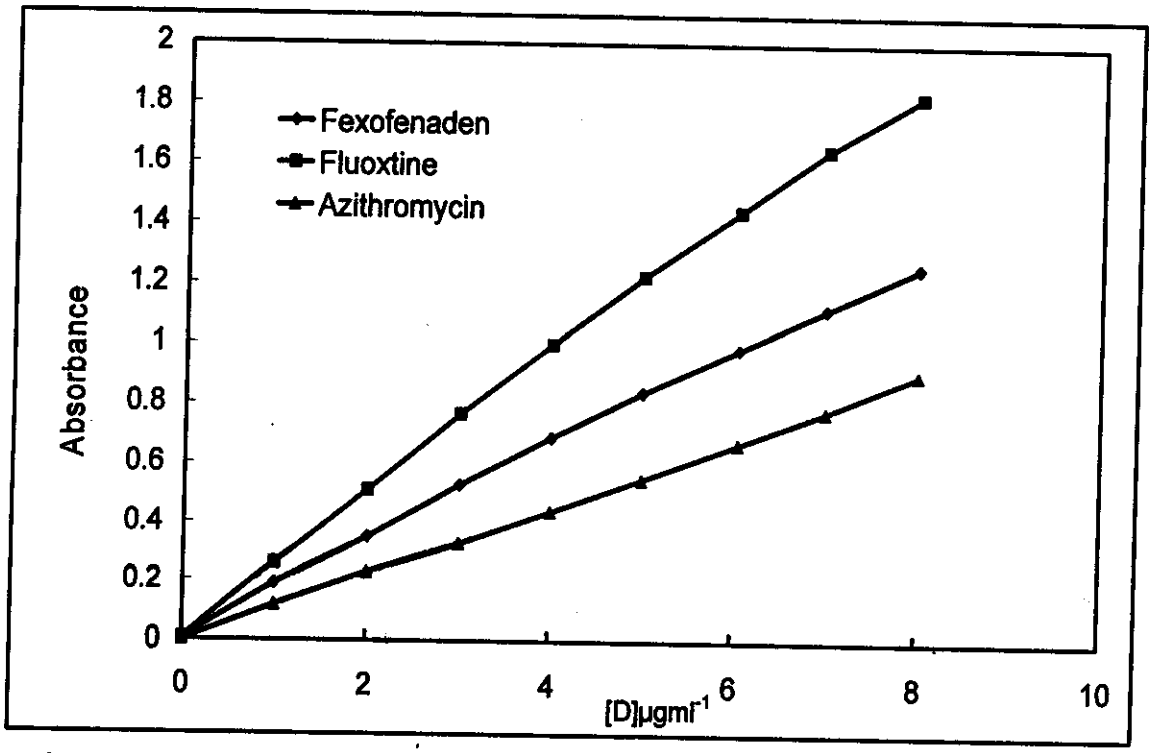


Fig (39): Application of Beer's law for the studied drugs using the optimum volume of (1.0×10^{-4} M) BCP.

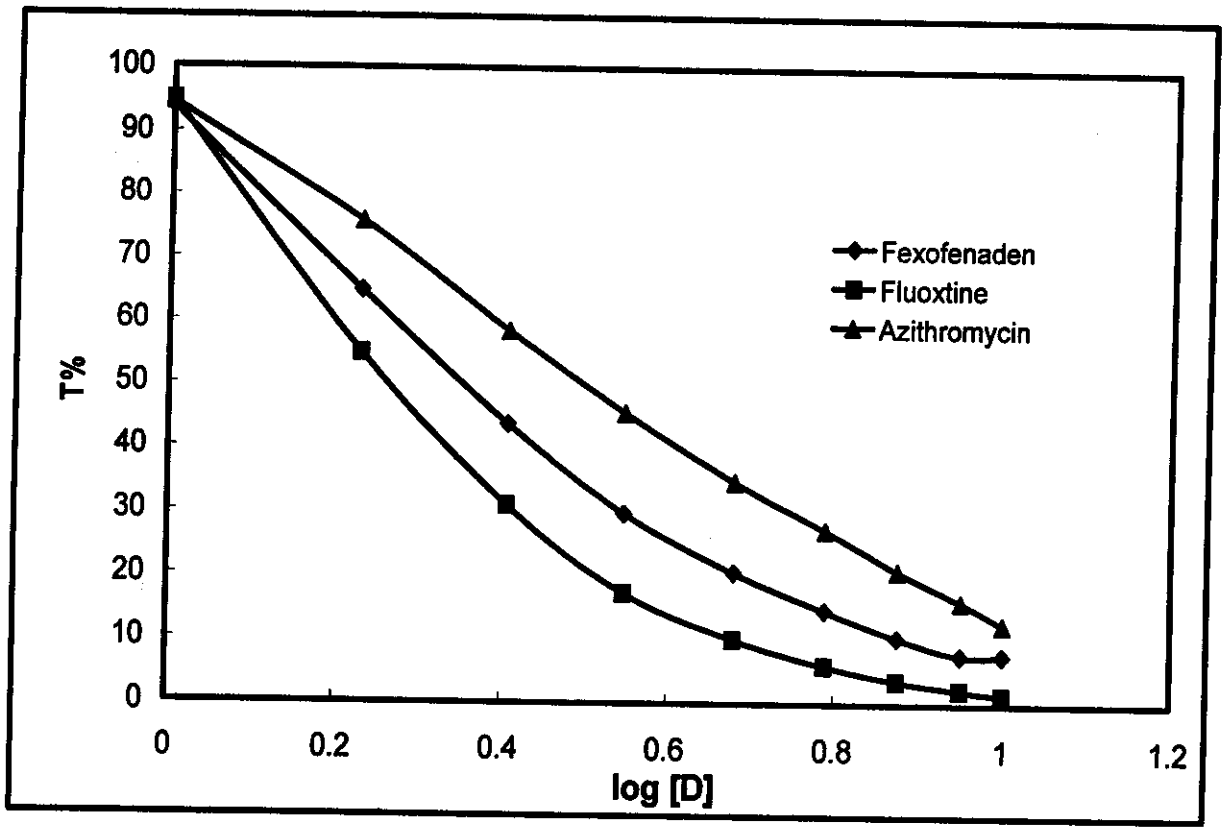


Fig (40): Ringbom plots for the studied drugs solution using the optimum volume of (1.0×10^{-4} M) BCP.