CHAPTER III
Results and Discussion

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III.1. Absorption spectra of the captopril with $KMnO_4$ and different dyes

Captopril, as all thiols was expected to undergo some extent oxidative degradation such as the formation of disulphide [177] and this suggests the investigation of an analytical procedure based on the specific reactivity of the thiol group, with regard to obtaining a stability indicating assay method. Captopril was determined spectrophotometrically by oxidation method using molybdophosphoric acid, which is a heteropoly acid of Mo^{VI} as oxidizing agent [21], bromate ($KBrO_3$) using celestine blue as indicator [14] and determined by electro-generated chemiluminescence Mn^{3+} as oxidant in H_2SO_4 medium [43] and Ag^{2+} as the oxidant in acidic media using flow injection chemiluminescence [37].

No attempts have been made to develop a spectrophotometric method for determination of captopril by oxidation with potassium permanganate, using the five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential (1.51 V) and excellent solution stability. $KMnO_4$ was used for quantitative determination of many drugs, as ramipril in basic media [178], codeine and morphine in acidic media [179, 180]. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with five different

dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously. However they are used as indicator to estimate captopril. $KMnO_4$ reacts with captopril, resulting in oxidation depending upon the functional group (-SH) present in captopril, probably a mixture of products, with reproducible data under specified experimental conditions. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product. The remaining dye is measured spectrophotometrically at their corresponding λ_{max}. The absorption spectra of the reaction products of the method show characteristic λ_{max} value, as shown in Fig. 1. Suggested mechanism is:

1- Oxidation of CAP with $KMnO_4$ in sulphuric acid medium by heating in water bath

2- Determination of excess (unreactant) oxidant by measuring the decrease in absorbance of dyes at their λ_{max}

$KMnO_4 + H^+ + CAP \longrightarrow$ oxidation products

$KMnO_4$ (excess) + dye $\longrightarrow$ oxidative dye (colorless products)

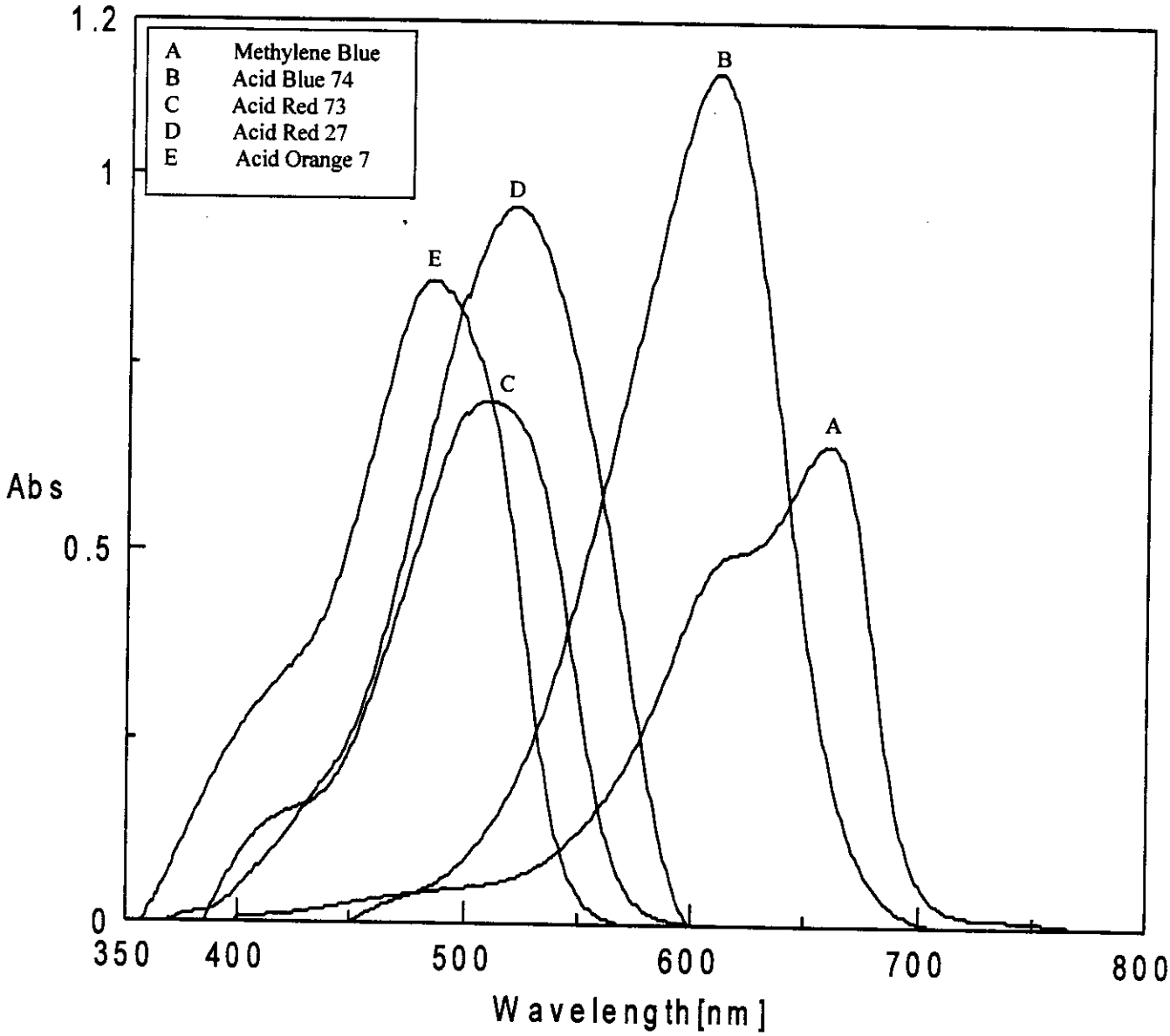
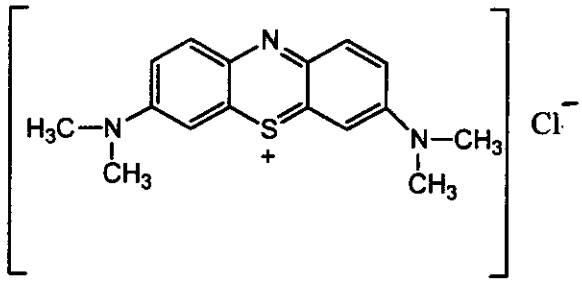
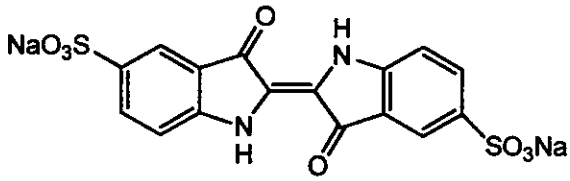


Fig. (1): Absorption spectra for the reaction product of 8.0 μg ml^{-1} of captopril with $KMnO_4$ (5.0 x 10^{-4} M) and dyes (1.0 x 10^{-3} M) except on using methylene blue (1.0 x 10^{-4} M)

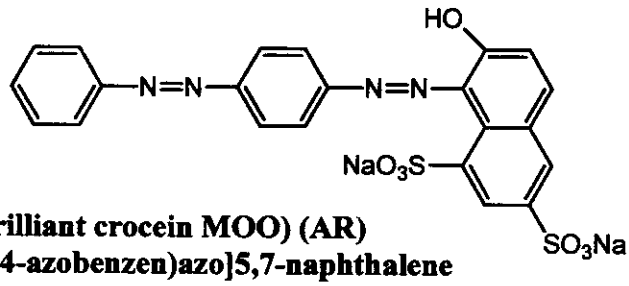
Reagents:



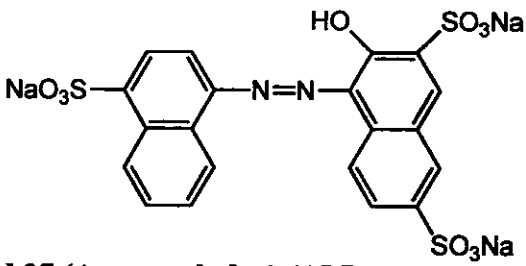
Methylene blue (Basic blue 9) (MB)
3,7-Bis(dimethylamino) phenothiazin-5-ium -chloride
C.I. 52015 CAS 7220-79-3



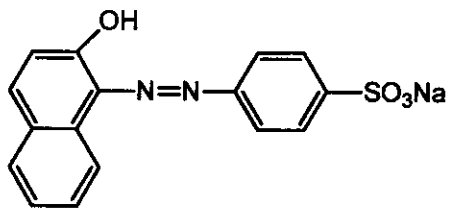
Acid blue 74 (Indigo carmine) (AB)
Disodium 5,5'-indigotin disulphonate
C.I. 73015 CAS 860-22-0



Acid red 73 (Brilliant crocein MOO) (AR)
3-Hydroxy-4-[(4-azobenzen)azo]5,7-naphthalene disulphonic acid disodium salt
C.I. 27290 CAS 5413-75-2



Acid red 27 (Amaranth dye) (AM)
1-(4-sulpho-1-naphthylazo)-2-naphthol-3,6-disulphonic acid trisodium salt
C.I. 16185 CAS 915-67-3



Acid orange 7 (Orange II) (AO)
4-[(2-Hydroxy-1-naphthalenyl)azo] benzensulphonic acid sod. salt
C.I. 15510 CAS 633-96-5

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$ caused the color disturbed. In order to investigate the optimum reaction conditions for the color development of 8.0 μg ml^{-1} (0.8 ml of 100 μg ml^{-1}) of CAP with $KMnO_4$ (5.0×10^{-4} M). The effect of different experimental variable were studied and recorded below

III.1.2. Effect of acid concentration

Different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. The results indicating that the sulphoric acid was the preferable acid with potassium permanganate as an oxidant. To each 10 ml measuring flask, 0.8 ml of the CAP (100 μg ml^{-1}) and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) were added. 1.0 ml of H_2SO_4 (0.2 M) is the optimum concentration of H_2SO_4, and then the solution was diluted to 7.0 ml. After 5.0 min standing time at 50 °C in water bath, the solution was cooled for about 3.0 min; the dye was added, then complete to 10 ml total volume as shown in Fig. 2.

III.1.3. Effect of time and temperature

The effect of time on the oxidation process of CAP was investigated by measuring the absorbance of a solution containing 8.0 μg ml^{-1} of CAP, oxidant and acid solution against blank solution prepared by the same way without drug at λ_{max} 660, 610, 510, 520 and 485 nm using MB, AB, AR, AM and AO, respectively, at various time intervals. Also the effect of temperature was studied by heating the sample solution (oxidant, acid and

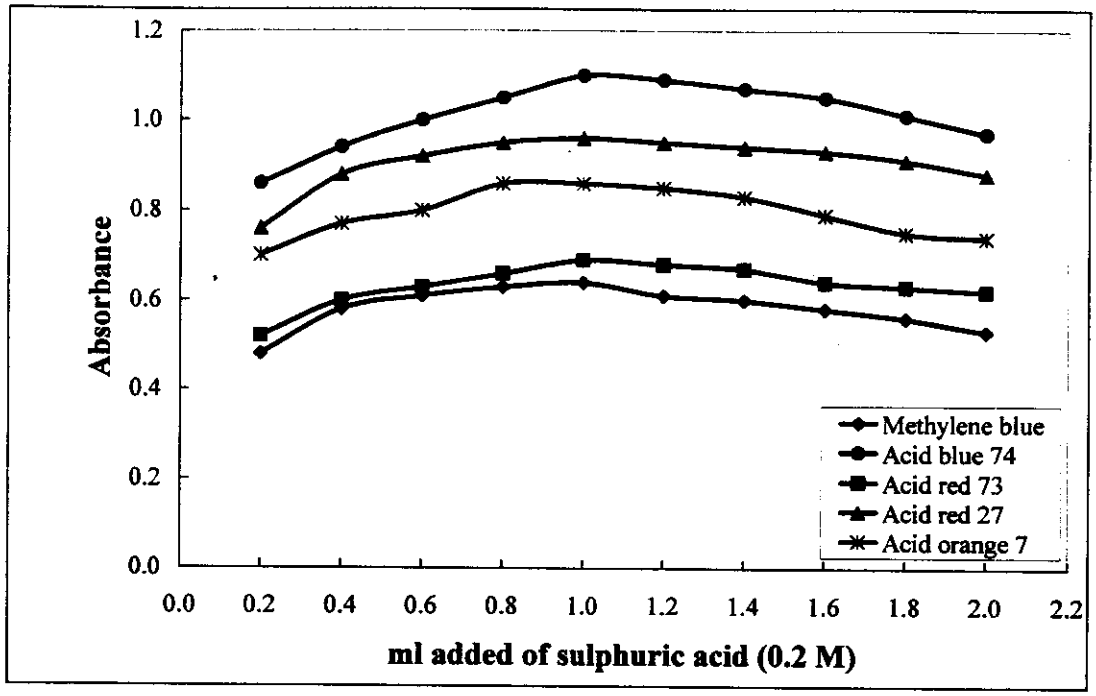


Fig. (2): Effect of ml added of sulphuric acid (0.2 M) on absorbance of 8.0 μg ml^{-1} of captopril with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

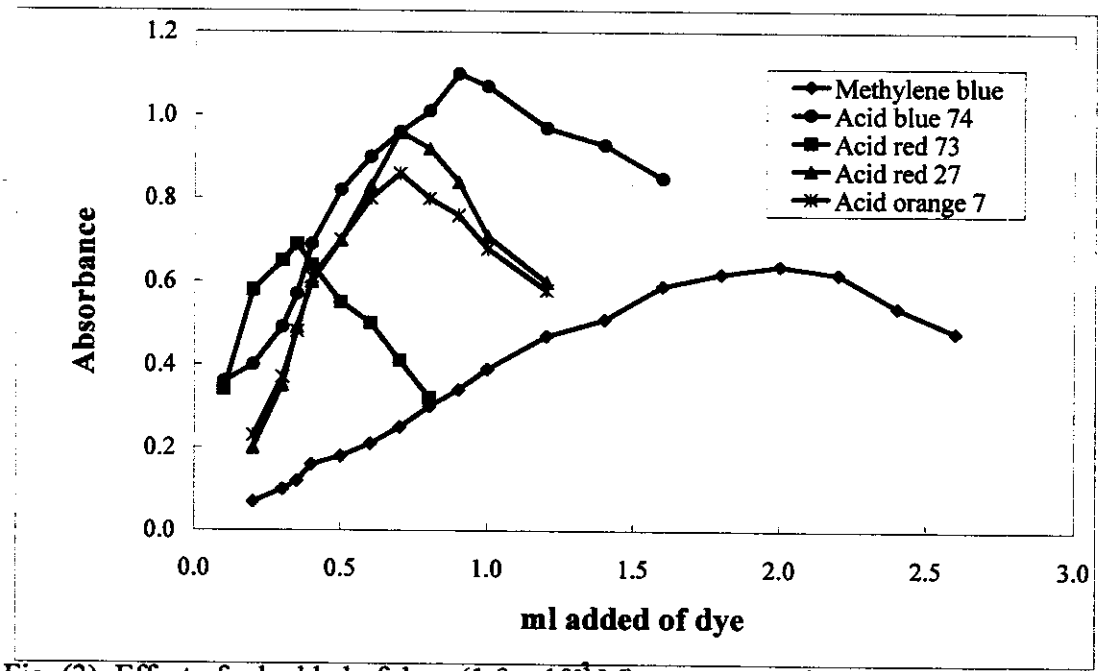


Fig. (3): Effect of ml added of dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M) on absorbance of 8.0 μg ml^{-1} of captopril with $KMnO_4$ (5.0×10^{-4} M)

drug) and the blank (oxidant and acid) at different temperature (30-100 °C) in water bath. The oxidation took place completely after 5.0 min and at 50 °C temperature in water bath. Raising the temperature more than 50 °C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals (1.0-5.0 min). The absorbance indicated that shaken for 2.0 min was sufficient to give reliable results. The produced color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

III.1.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process of CAP was studied by measuring the absorbance of solution prepared by different sequence of additions against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.1.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of the different dyes were added to 8.0 μg ml^{-1} of CAP. The optimum volumes used for production of maximum color intensity are 2.0 ml (1.0 x 10^{-4} M) MB, whereas 0.9, 0.35, 0.7 and 0.7 ml (1.0 x 10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum dye volume as shown in Fig. 3.

III.1.6. Molar ratio method

The molar ratio between oxidant and dye [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable concentrations of dye (1 x 10^{-4} M MB, 1 x 10^{-3} M for AB, AR, AM and

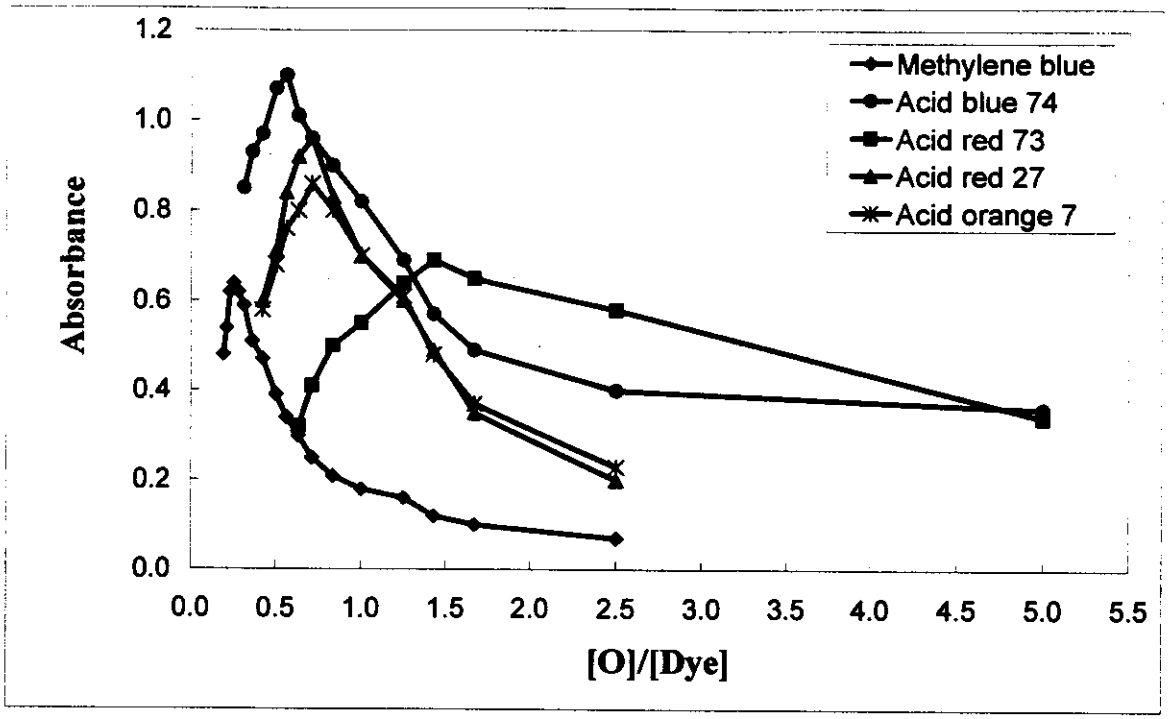


Fig. (4): Molar ratio method [O]/[Dye] for 8.0 μg ml^{-1} captopril using $KMnO_4$ (5.0 x 10^{-4} M) and dyes (1.0 x 10^{-3} M) except on using methylene blue (1.0 x 10^{-4} M)

AO) were added and 8.0 μg ml^{-1} of CAP. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 4. Experimental results showed that the inflection of the two straight lines at 0.25, 0.56, 1.43, 0.71 and 0.71 in case of MB, AB, AR, AM and AO, respectively. Thus the stoichiometric ratio of oxidant to dye are 1.0 : 0.4, 1.0 : 1.79, 1.0 : 0.7, 1.0 : 1.41 and 1.0 : 1.41 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 1.

In order to investigate the molar ratio between CAP and oxidant at the selected conditions, the molar ratio method described by *Yoe* and *Jones* [169] was carried out. In this method 1.0 ml of 5.0 x10^{-4} M $KMnO_4$ is kept constant and variable concentrations (0.1 - 2.5 ml) of CAP 5.0 x 10^{-4} M were added. The absorbance was measured at λ_{max} (660, 610, 510, 520 and 485 nm using MB, AB, AR, AM and AO, respectively) against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 5. Experimental results showed that the inflection of the two straight lines at 1.25, 1.0, 1.1, 0.83 and 0.93 in case of MB, AB, AR, AM and AO, respectively. Thus the molar ratio of CAP to oxidant are 1.0 : 0.8, 1.0 : 1.0, 1.0 : 0.91, 1.0 : 1.2 and 1.0 : 1.08 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 1.

III.1.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of CAP. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration as listed in Table 1. The correlation coefficient slopes and intercepts are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1},

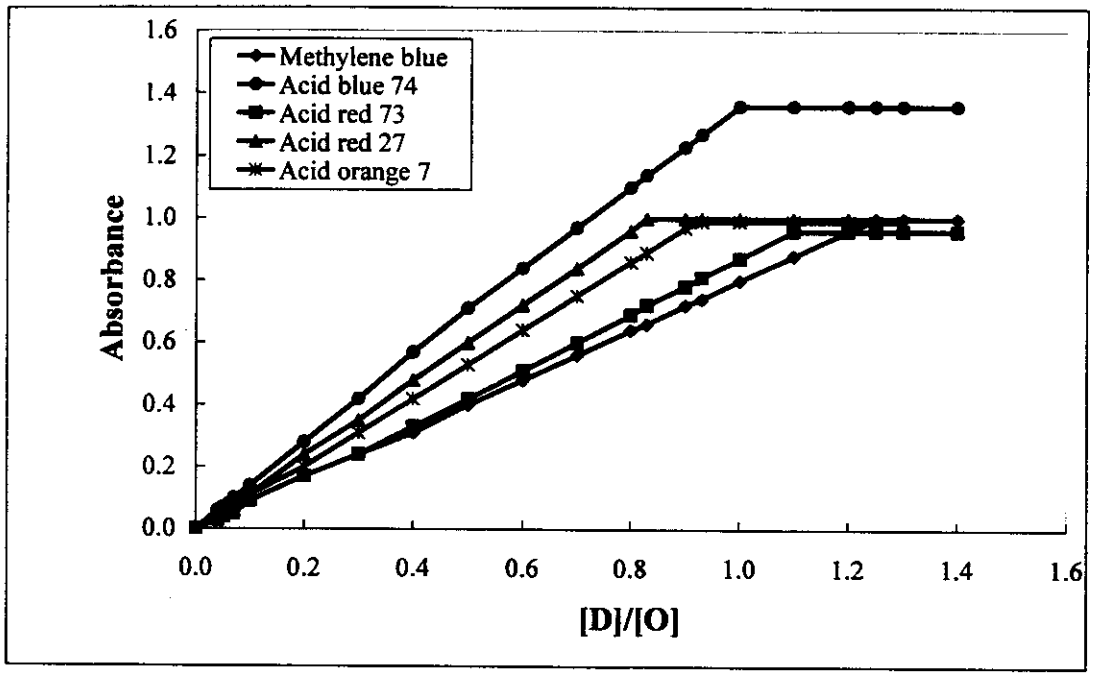


Fig. (5): Molar ratio method for captopril (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

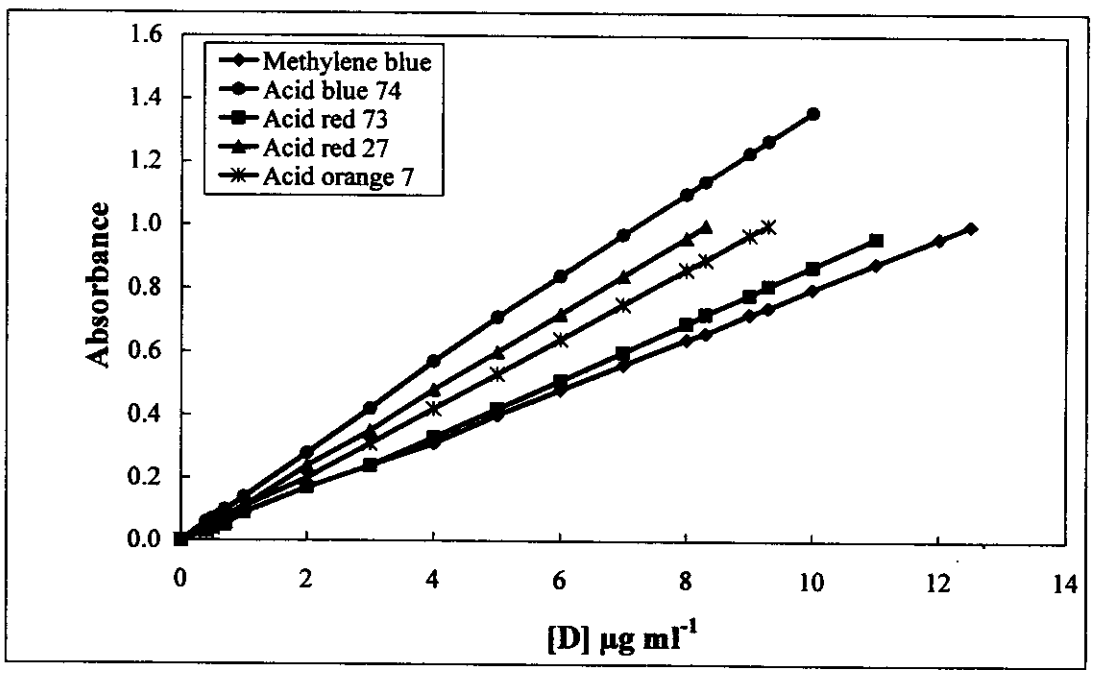


Fig. (6): Validity of Beer's law for reaction product of captopril with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 1, while representative curves on the validity of Beer's law for CAP with the different dyes are shown in Fig. 6. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

III.1.8. Accuracy and precision [182]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of CAP were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2. The percentage standard deviations and the percentage range 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.

III.1.9. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 8.0 μg ml^{-1} of CAP with varying concen0trations of the additives and excipients such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose, these substances reacted with $KMnO_4/H^+$ system producing oxidative dye (colorless products) as shown by higher reading of the blank solutions when dye was

added, other excipients do not interfere. The hydrochlorothiazide present in Capozide tablets not interfere with captopril.

III.1.10. Analytical applications

The validity of the proposed procedures is tested by determining CAP in tablets obtained from local manufacturing companies as mentioned before. The concentration of CAP in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum or absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [170]. The results obtained were compared statistically by the Student's t-test (for accuracy), and variance ratio F-test (for precision) [181] with those obtained by official methods on the sample of the same batch. The Student's t-test values obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as recorded in Tables (3 and 4).

Table (1): Optical and regression characteristics of captopril with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	660	610	510	520	485
Bear's law limits ($\mu g\ ml^{-1}$)	0.4-12.5	0.3-10	0.5-11	0.4-8.3	0.5-9.3
Ringbom limits ($\mu g\ ml^{-1}$)	0.5-12	0.5-9.6	0.6-10.5	0.5-8.0	0.7-9.0
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	1.74×10^4	2.98×10^4	1.87×10^4	2.63×10^4	2.35×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	12.5	7.30	11.63	8.26	9.26
Detection limits ($\mu g\ ml^{-1}$)	0.106	0.063	0.145	0.093	0.134
Quantitation limits ($\mu g\ ml^{-1}$)	0.353	0.211	0.484	0.309	0.446
Regression equation*: Slope (b)	0.080	0.137	0.086	0.121	0.108
Intercept (a)	5.8×10^{-3}	9.7×10^{-3}	-8.2×10^{-3}	-7.5×10^{-3}	-10.8×10^{-3}
Correlation coefficient (r)	0.9998	0.9995	0.9997	0.9996	0.9998
RSD** %	0.78	0.66	0.59	0.81	0.36
Stoichiometric ratio [O]/[Dye]	1.0 : 0.4	1.0 : 1.79	1.0 : 0.7	1.0 : 1.41	1.0 : 1.41
Stoichiometric ratio [D]/[O]	1.0 : 0.8	1.0 : 1.0	1.0 : 0.91	1.0 : 1.2	1.0 : 1.08

* With respect to A = a + b C where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.

** Relative standard deviation for six determinations

Table (2): Evaluation of the accuracy and precision of the proposed procedure of captopril.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	4.0	101.5	0.78	0.81	4.06±0.0327
	6.0	100.7	0.62	0.64	6.04±0.0388
	8.0	100.3	0.50	0.52	8.02±0.0419
Acid blue 74	4.0	100.3	0.64	0.67	4.01±0.0269
	6.0	99.8	0.30	0.31	5.99±0.0188
	8.0	100.2	0.47	0.48	8.01±0.0388
Acid red 73	4.0	100.3	1.00	1.05	4.01±0.0420
	6.0	99.8	0.88	0.92	5.99±0.0553
	8.0	100.6	0.63	0.67	8.06±0.0536
Acid red 27	4.0	99.7	1.11	1.16	3.99±0.0462
	6.0	99.7	0.69	0.73	5.98±0.0434
	8.0	100.5	0.21	0.22	8.04±0.0178
Acid orange 7	4.0	99.5	0.53	0.56	3.98±0.0221
	6.0	100.9	0.54	0.55	6.06±0.0336
	8.0	100.6	0.24	0.26	8.05±0.0207

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

Table (3): Evaluation of the accuracy and precision of the proposed and official procedures for captopril in dosage forms.

Official method			Proposed methods													
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7	
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A1	4.0	98.3	99.1	0.88	2.97	98.7	0.57	1.89	99.7	0.31	3.01	100.3	0.27	3.42	99.3	1.11	3.44
B1	4.0	99.4	100.3	0.67	1.54	99.7	0.79	1.59	100.2	0.42	2.45	99.6	0.63	3.08	100.5	0.48	2.25
A1	6.0	98.6	98.8	0.70	1.23	99.4	0.43	2.44	99.6	0.37	1.66	98.9	0.81	2.64	100.4	0.19	1.99
B1	6.0	99.1	100.1	0.58	2.60	99.5	1.09	3.08	100.2	0.88	2.32	99.8	0.55	1.87	99.7	0.44	3.06
A1	8.0	100.6	100.2	0.29	2.48	100.3	0.46	3.55	99.7	1.66	1.47	99.5	0.49	1.57	99.6	1.37	2.41
B1	8.0	98.8	99.6	0.39	3.39	98.9	0.84	2.33	100.2	0.77	2.58	100.4	0.37	2.04	99.2	1.21	1.87
A2	4.0	99.1	99.3	0.29	3.02	100.2	0.68	2.34	99.7	0.55	2.44	99.6	1.28	2.22	100.1	0.61	2.66
B2	4.0	98.2	99.7	0.85	2.55	99.3	0.45	2.09	100.3	0.62	2.39	100.5	0.99	3.04	99.4	0.27	1.56
A2	6.0	99.2	99.5	0.36	2.66	100.1	0.19	3.14	99.6	0.24	2.99	99.7	0.82	1.65	100.3	0.36	2.14
B2	6.0	98.5	100.1	1.03	1.77	99.4	0.24	1.45	99.7	0.31	3.01	98.9	0.23	1.38	98.8	0.96	2.78
A2	8.0	99.0	99.7	1.76	1.28	100.3	0.74	1.58	99.2	1.02	1.54	99.5	0.43	1.84	99.8	0.49	1.36
B2	8.0	98.8	99.4	0.66	1.64	99.6	0.91	2.35	100.3	1.25	2.36	100.5	0.24	2.99	99.6	1.12	2.12
A3	4.0	99.1	99.8	0.25	2.06	99.4	0.34	1.58	100.3	0.25	2.34	99.6	1.21	2.99	99.5	0.22	1.45
B3	4.0	98.5	99.1	0.36	2.32	99.6	0.24	2.36	99.1	0.34	2.31	99.7	0.35	1.25	100.2	0.34	1.69
A3	6.0	98.8	100.1	0.28	2.14	98.9	0.61	3.04	100.2	0.60	2.08	99.4	0.62	2.41	99.6	0.44	1.13
B3	6.0	99.3	100.3	0.81	1.84	99.6	0.18	2.18	99.8	0.81	1.67	100.3	0.55	2.11	100.5	0.80	2.38
A3	8.0	98.9	99.3	0.39	1.89	99.2	0.21	2.14	100.3	0.21	3.07	99.3	0.84	1.97	99.5	0.17	1.29
B3	8.0	99.4	99.5	0.74	2.36	99.8	0.84	1.57	100.2	0.33	2.14	99.6	0.16	1.21	99.7	1.03	2.47

*Dosage forms

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

A1 Capotril tablet 25 mg/tablet B1 Capotril tablet 50 mg/tablet

A2 Capoten tablet 25 mg/tablet B2 Capoten tablet 50 mg/tablet

A3 Farcopril tablet 25 mg/tablet B3 Farcopril tablet 50 mg/tablet

Table (4): Evaluation of the accuracy and precision of the proposed and official procedures for captopril in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F*- ratio
A	4.0	98.4	99.2	0.34	1.41	99.3	0.32	1.54	98.9	0.28	1.47	100.1	0.25	1.49	99.8	1.37	3.14
	6.0	98.9	100.2	0.36	1.66	99.7	0.55	1.64	99.4	0.80	2.14	99.1	0.91	3.10	100.3	0.55	1.84
	8.0	99.3	99.8	0.81	2.06	100.2	0.19	1.29	100.3	0.44	1.56	99.6	0.14	1.21	99.7	1.02	2.77
B	4.0	98.8	99.1	0.66	1.47	98.9	0.52	2.41	99.0	0.35	1.40	99.2	0.24	1.54	99.4	0.21	1.76
	6.0	99.1	99.3	0.71	1.83	99.2	0.36	2.11	99.4	0.42	2.33	99.6	0.34	2.47	99.5	1.02	3.08
	8.0	99.3	99.5	0.28	1.37	99.4	0.24	1.77	99.7	0.73	2.45	99.8	0.17	2.09	99.3	0.88	2.77
C	4.0	99.1	100.1	0.36	2.15	99.6	1.01	2.38	99.4	0.54	2.61	100.3	0.29	2.56	99.5	0.66	2.30
	6.0	99.4	100.3	0.24	1.38	99.8	0.25	1.99	99.6	0.32	1.92	99.8	0.64	2.99	100.4	0.91	2.88
	8.0	98.7	99.8	0.31	1.44	100.1	0.64	2.97	99.5	0.70	2.90	100.2	0.73	3.11	99.6	0.34	1.65

• Dosage forms

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

A Hypopress 25 mg/tablet

B Lotnsine 25 mg/tablet

C Capozide 50 mg captopril/25 mg hydrochlorothiazide/tablet

III.2.2. Absorption spectra of the amlodipine besylate with $KMnO_4$ and different dyes

No attempts have been made to develop a spectrophotometric method for determination of amlodipine besylate (ADB) by oxidation with potassium permanganate, using the five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential and excellent solution stability. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with five different dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously. However they are used as indicator to estimate ADB. $KMnO_4$ reacts with ADB, resulting in oxidation depending upon the functional group ($-NH_2$, -NH) present in ADB, probably a mixture of products, with reproducible data under specified experimental conditions. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product). The remaining dye is measured spectrophotometrically at their corresponding λ_{max}. The absorption spectra of the reaction products show characteristic λ_{max} value, as shown in Fig. 7.

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$ caused the color to fade. The effect of different experimental variable were studied and recorded below

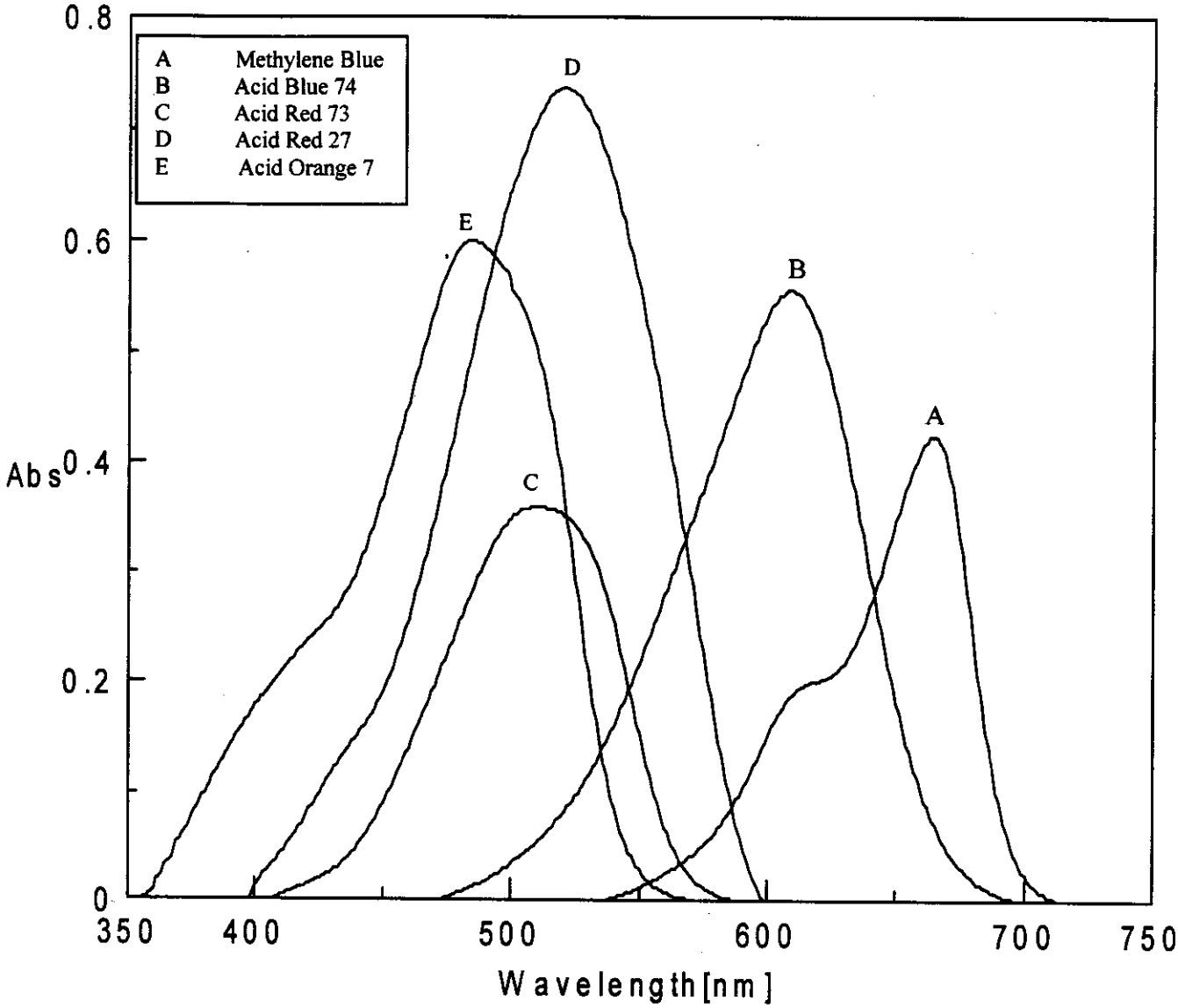


Fig. (7): Absorption spectra for the reaction product of 10 μg ml^{-1} of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.2.2. Effect of acid concentration

Different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. H_2SO_4 was preferable with using the $KMnO_4$ oxidant. To each 10 ml measuring flask, 1.0 ml of the ADB (100 μg ml^{-1}) and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) was added, 1.0 ml of H_2SO_4 (0.2 M) is the optimum concentration of acid, then the solution was diluted to 6.0 ml. After 5.0 min standing time at 50 °C in water bath, the solution was cooled for about 3.0 min; dye was added, then complete to 10 ml total volume. The absorbance was measured against a blank solution prepared by the same way without drug in the same acid concentration, as shown in Fig. 8.

III.2.3. Effect of time and temperature

The time required to complete the oxidation process of ADB in the proposed concentration range was investigated by measuring the absorbance of a solution containing 10 μg ml^{-1} of the drug, oxidant and acid solution against blank solution prepared by the same way without drug at various time intervals. Also the effect of temperature was studied by heating the sample solution (oxidant, acid and drug) and the blank (oxidant and acid) at different temperature (30-100 °C) in water bath. The reaction took place completely after 5.0 min, and at 50 °C temperature in water bath. Raising the temperature more than 50 °C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals (1.0-5.0 min). The absorbance indicated that shaken for 1.0 min (3.0 min in case of AR and AM dye) was sufficient to give reliable results. The color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

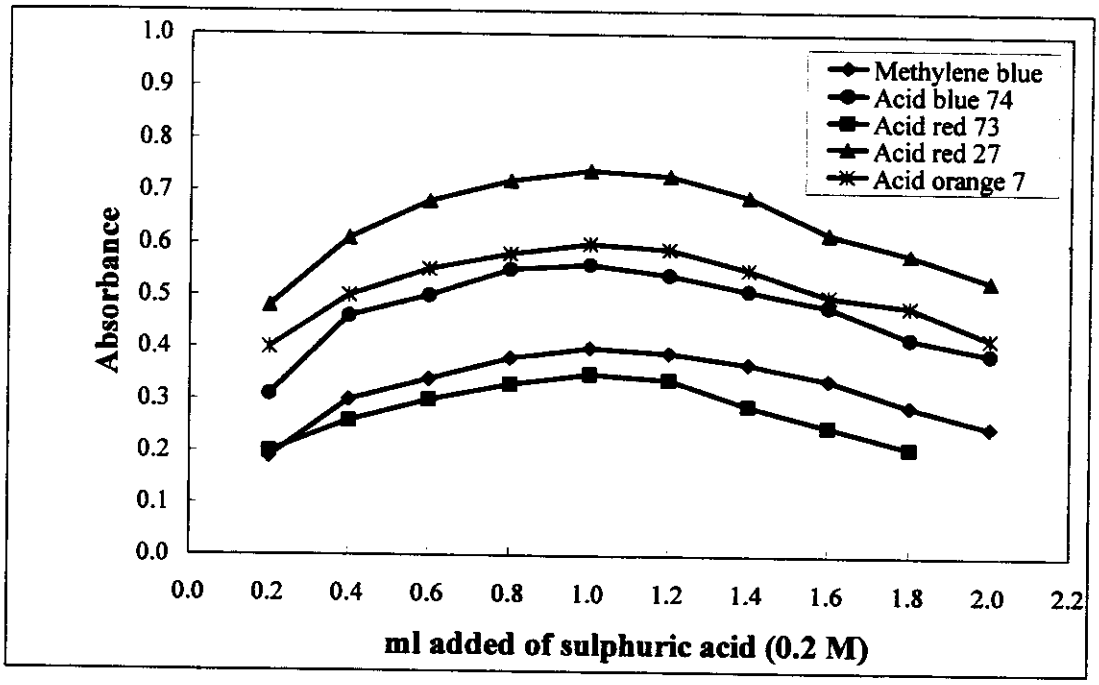


Fig.(8): Effect of ml added of sulphuric acid (0.2 M) on absorbance of 10 μg ml^{-1} of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M) and dyes(1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

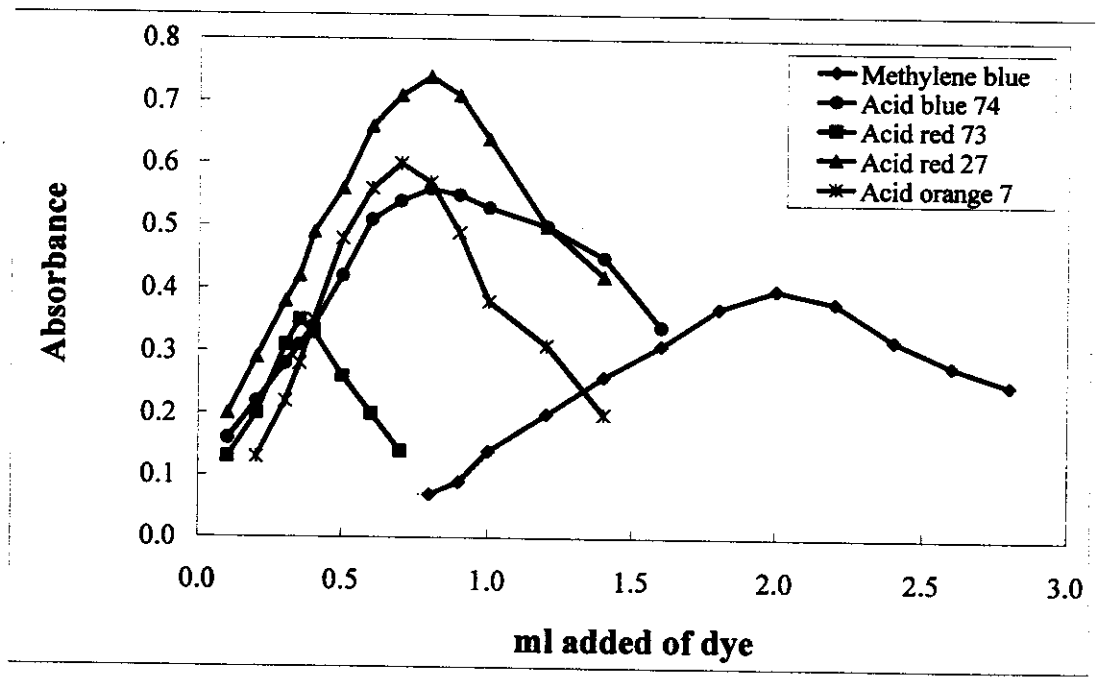


Fig.(9): Effect of ml added of dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M) on absorbance of 10 μg ml^{-1} of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M)

III.2.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process of ADB was studied by measuring the absorbance of solution prepared by different sequence of additions at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.2.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of different dyes were added to 10 μg ml^{-1} of ADB. The optimum volumes used for production of maximum and reproducible color intensity are 2.0 ml (1.0 x 10^{-4} M) MB as shown in Fig. 12, whereas 0.8, 0.35, 0.8 and 0.7 ml (1.0 x 10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum dye volume as shown in Fig. 9.

III.2.6. Molar ratio method

The stoichiometry of [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable volumes of dye (1 x 10^{-4} M MB, 1 x 10^{-3} M for AB, AR, AM and AO) were added and 10 μg ml^{-1} of ADB. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 10. Experimental results showed that the inflection of the two straight lines at 0.25, 0.63, 1.43, 0.63 and 0.71 in case of MB, AB, AR, AM and AO, respectively. Thus the Stoichiometric ratio of oxidant to dye are 1.0 : 0.4, 1.0 : 1.59, 1.0 : 0.7, 1.0 : 1.59 and 1.0 : 1.41 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 5.

The stoichiometry of the reaction between ADB and the oxidant at the selected conditions was established by the molar ratio method [169]. In this method 1.0 ml of 5.0 x10^{-4} M $KMnO_4$ is kept constant and variable

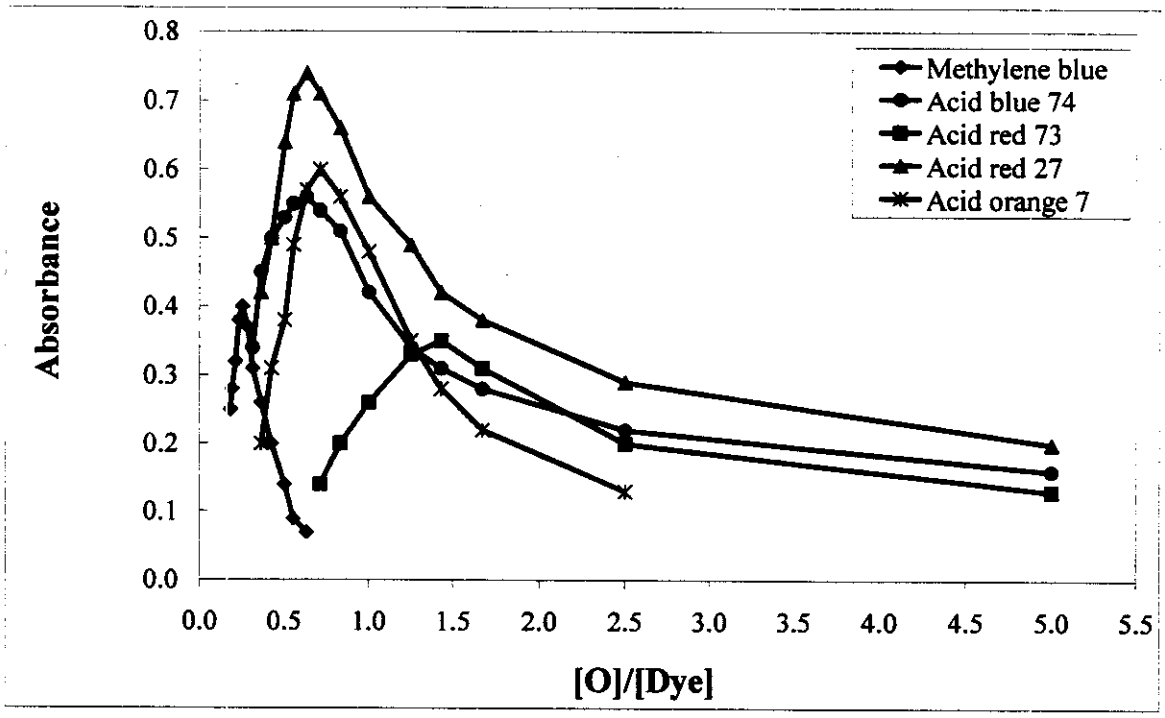


Fig. (10): Molar ratio method [O]/[Dye] for 10 μg ml^{-1} of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

concentrations (0.1-2.5 ml) of ADB (5.0 x 10^{-4} M) were added. The absorbance was measured at λ_{max} (663, 609, 511, 520 and 484 nm using MB, AB, AR, AM and AO, respectively) against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 11. Experimental results showed that the inflection of the two straight lines at 1.8, 1.0, 1.8, 0.44 and 0.7 in case of MB, AB, AR, AM and AO, respectively. Thus molar ratios of ADB to oxidant are 1.0 : 0.56, 1.0 : 1.0, 1.0 : 0.56, 1.0 : 2.27 and 1.0 : 1.43 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 5.

III.2.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of ADB. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration as listed in Table 5. The correlation coefficient slopes and intercepts are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1}, against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 5, while representative curves on the validity of Beer's law for ADB with different dyes are shown in Fig. 12. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

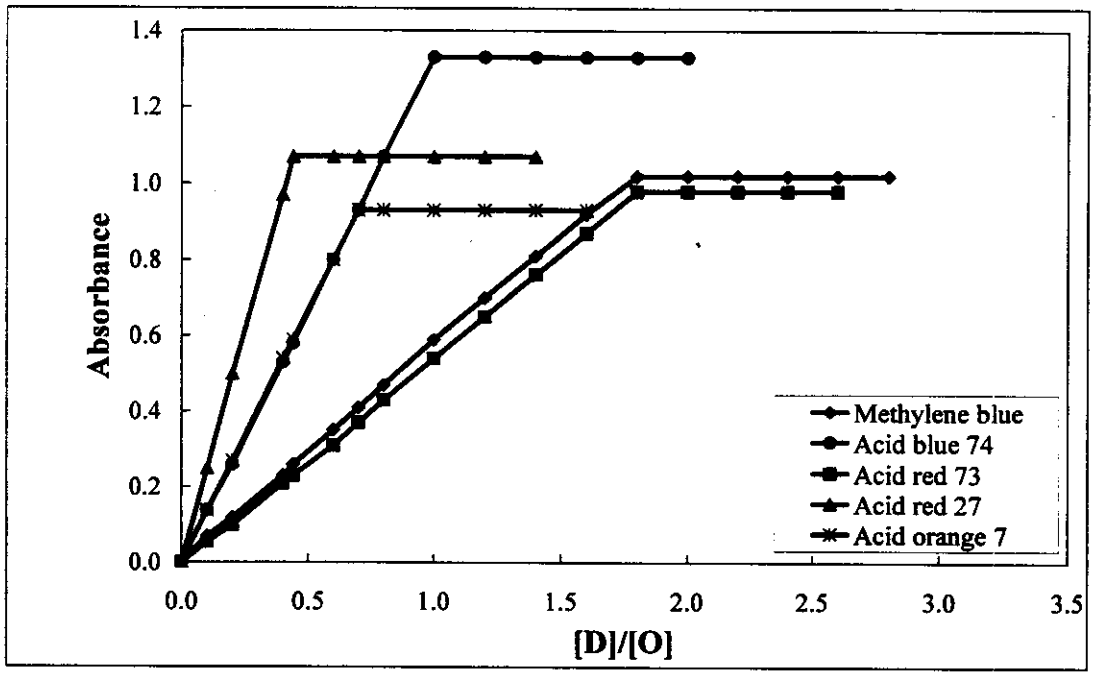


Fig. (11): Molar ratio method for amlodipine besylate (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

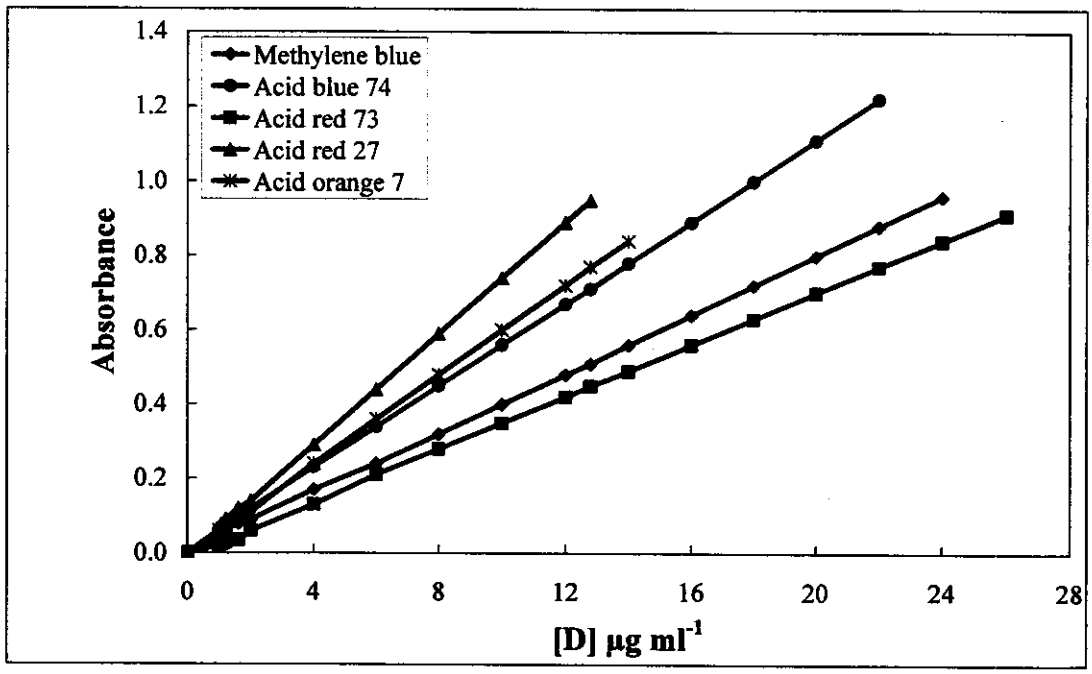


Fig. (12): Validity of Beer's law for reaction product of amlodipine besylate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.2.8. Accuracy and precision [182]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of ADB were prepared and analyzed in six replicates. The results are summarized in Table 6. The % standard deviations and the % error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

III.2.9. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 10 μg ml^{-1} of ADB with varying concentration of the additives and excipients such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose 2-fold, and there are not interference for other excipients.

III.2.10. Analytical applications

The validity of the proposed procedures is tested by determining ADB in tablets obtained from local manufacturing companies as mentioned before. The concentration of ADB in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum or absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [171]. The results obtained were compared statistically by the Student's t-test and variance ratio F-test with those obtained by official methods on the sample of the same batch. The Student's t-test values obtained at 95% confidence level and five degree of freedom did not

exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as recorded in Table 7.

Table (5): Optical and regression characteristics of amlodipine besylate with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	663	609	511	520	484
Bear's law limits ($\mu g\ ml^{-1}$)	1.0-24	0.9-22	1.2-26	0.9-12.8	1.0-14
Ringbom limits ($\mu g\ ml^{-1}$)	1.2-22.4	1.1-20	1.4-24.5	1.0-12.3	1.3-13.2
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2.25×10^4	3.12×10^4	2.01×10^4	4.22×10^4	3.42×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	25.19	18.15	28.17	13.44	16.58
Detection limits ($\mu g\ ml^{-1}$)	0.277	0.249	0.329	0.239	0.272
Quantitation limits ($\mu g\ ml^{-1}$)	0.923	0.831	1.096	0.798	0.907
Regression equation*: Slope (b)	0.0397	0.0551	0.0355	0.0744	0.0603
Intercept (a)	5.3×10^{-3}	8.5×10^{-3}	-9.9×10^{-3}	-4.6×10^{-3}	-3.1×10^{-3}
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9996	0.9999
RSD** %	0.66	1.01	0.82	0.51	0.73
Stoichiometric ratio [O]/[Dye]	1.0 : 0.4	1.0 : 1.59	1.0 : 0.7	1.0 : 1.59	1.0 : 1.41
Stoichiometric ratio [D]/[O]	1.0 : 0.56	1.0 : 1.0	1.0 : 0.56	1.0 : 2.27	1.0 : 1.43

* With respect to $A = a + b\ C$ where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.
** Relative standard deviation for six determinations

Table (6): Evaluation of the accuracy and precision of the proposed procedure of amlodipine besylate.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	8.0	100.1	0.86	0.90	8.01±0.0724
	10	100.2	0.89	0.93	10.02±0.0933
	12	99.9	0.53	0.55	11.99±0.0661
Acid blue 74	8.0	99.8	0.74	0.78	7.98±0.0619
	10	100.5	0.46	0.48	10.05±0.0483
	12	99.7	0.38	0.39	11.96±0.0472
Acid red 73	8.0	100.3	0.65	0.68	8.02±0.0546
	10	99.6	0.71	0.95	9.96±0.0745
	12	99.8	0.40	0.42	11.98±0.0504
Acid red 27	8.0	99.5	0.70	0.74	7.96±0.0588
	10	99.9	0.88	0.92	9.99±0.0923
	12	100.3	0.67	0.71	12.04±0.0850
Acid orange 7	8.0	100.4	0.80	0.84	8.03±0.0672
	10	100.6	0.77	0.80	10.06±0.0808
	12	100.6	0.33	0.34	12.07 ±0.0409

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

Table (7): Evaluation of the accuracy and precision of the proposed and official procedures for amlodipine besylate in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F*- ratio
A	8.0	98.9	99.2	0.27	1.36	99.8	0.58	2.11	100.1	0.82	2.85	99.5	0.90	2.58	99.7	0.57	1.89
	10	99.1	99.9	0.39	1.99	99.5	0.64	2.43	100.2	0.91	3.01	99.4	0.67	1.97	99.6	0.83	2.65
	12	98.8	100.1	0.45	2.31	99.3	0.37	1.65	99.8	0.27	2.14	99.4	0.81	2.33	99.5	0.59	1.97
B	8.0	98.8	99.7	0.91	2.68	99.2	0.65	1.97	100.2	0.37	1.66	99.6	0.38	1.85	99.5	0.35	1.87
	10	99.3	99.8	0.38	2.15	99.7	0.34	1.35	99.5	0.94	2.21	100.6	0.24	1.65	101.1	0.94	2.39
	12	99.4	100.2	1.02	3.12	99.6	0.80	2.69	100.4	0.62	1.84	99.9	0.15	1.27	99.7	1.08	2.93
C	8.0	99.5	99.8	0.58	1.45	100.1	0.62	2.11	99.6	0.36	1.14	100.4	0.48	2.12	99.7	0.54	1.58
	10	99.3	99.7	1.41	2.25	99.4	0.81	2.35	100.3	0.46	1.22	99.8	0.27	1.82	100.5	0.58	2.10
	12	98.9	99.5	0.85	1.66	99.3	0.27	1.24	99.7	0.62	1.25	100.2	0.64	1.99	99.4	0.86	2.31
D	8.0	99.4	100.5	0.19	1.21	99.6	0.37	1.27	99.7	0.34	1.64	99.8	0.39	1.77	100.2	0.95	1.89
	10	98.6	99.4	0.93	2.97	100.1	0.61	1.96	99.6	0.61	1.68	99.7	0.54	1.94	99.3	0.18	1.11
	12	99.5	100.2	0.64	2.39	99.9	0.40	1.54	99.8	0.97	3.22	100.2	0.89	2.64	100.4	1.03	2.28
E	8.0	99.4	100.3	0.92	1.58	99.8	0.36	1.89	99.6	0.64	1.91	99.5	0.54	1.85	100.1	0.34	2.11
	10	98.7	99.3	0.14	1.34	101.1	0.24	1.64	100.2	0.38	1.32	99.7	0.34	1.43	99.4	0.98	2.54
	12	99.5	99.9	0.53	1.19	100.4	0.90	2.25	99.7	0.94	2.14	100.1	0.32	1.21	100.4	1.21	2.68

* Dosage forms

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

A Norvasc 5 mg/tablet B Amilo 5 mg/tablet C Alkapress 5 mg/tablet

D Amlodipin 5 mg/tablet E Myodura 5 mg/tablet

III.3. Absorption spectra of the diltiazem HCl with $KMnO_4$ and different dyes

Diltiazem HCl (DIL) was determined colorimetrically by oxidation by Fe^{3+} in acidic medium [100] or by metavanadate in H_2SO_4 medium [95]. No attempts have been made to develop a spectrophotometric method for determination of DIL by oxidation with potassium permanganate, using five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential and excellent solution stability. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with five different dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously. However they are used as indicator to estimate DIL. $KMnO_4$ reacts with DIL, resulting in oxidation depending upon the functional group (-N-, -S-) present in DIL, probably a mixture of products, with reproducible data under specified experimental conditions. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product). The remaining dye is measured spectrophotometrically at their corresponding λ_{max}. The absorption spectra of the reaction products of the method show characteristic λ_{max} value, as shown in Fig. 13.

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$

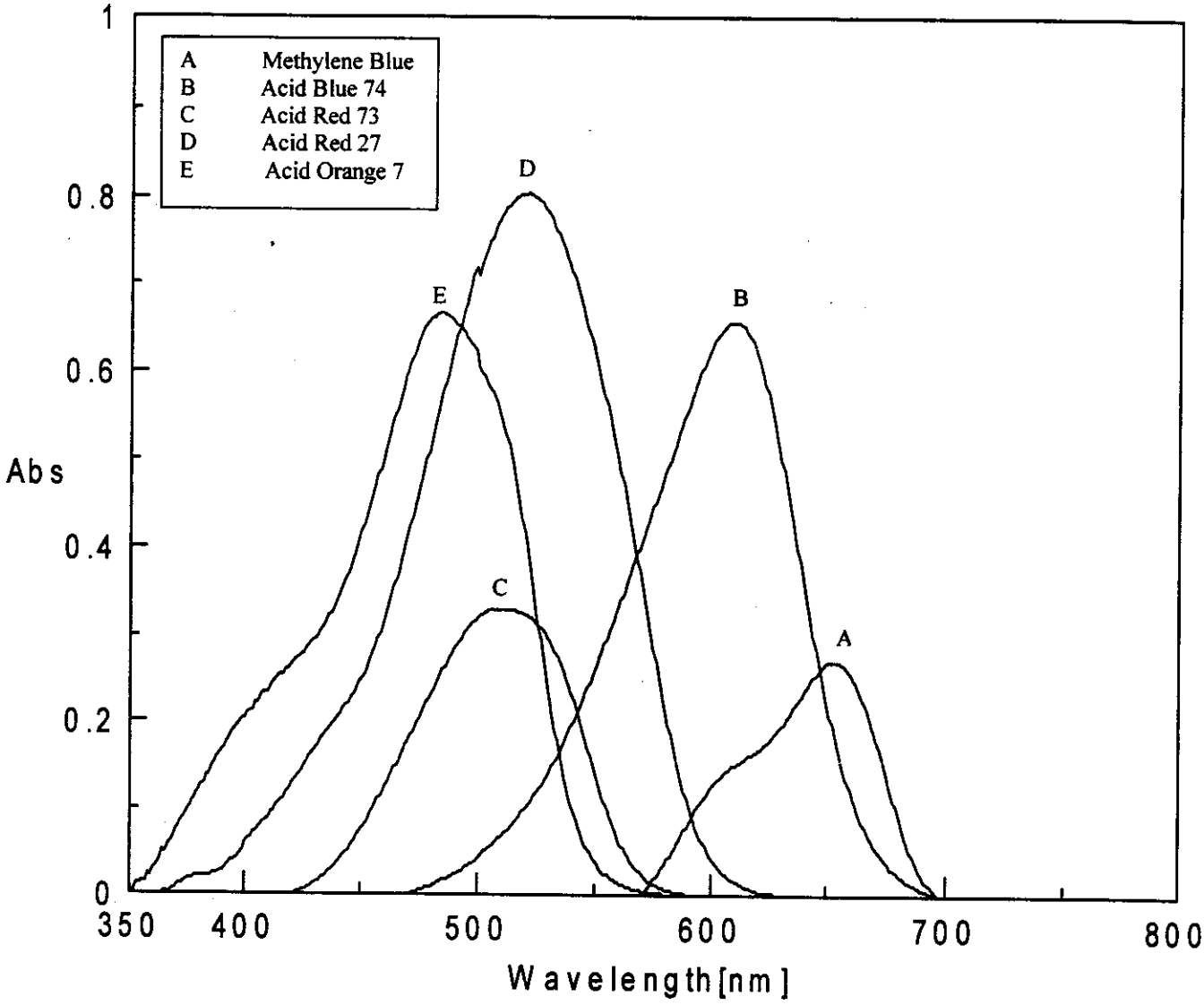


Fig. (13): Absorption spectra for the reaction product of 5.0 μg ml^{-1} of diltiazem HCl with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

caused the color to fade. The effect of different experimental variable were studied and recorded below

III.3.2. Effect of acid concentration

Different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. Sulphuric acid was preferable with using the $KMnO_4$ oxidant. To each 10 ml measuring flask, 0.5 ml of the DIL (100 μg ml^{-1}) equivalent to 5.0 μg ml^{-1} and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) was added, 0.5 ml of H_2SO_4 (2.0 M) is the optimum concentration of acid, then the solution was diluted to 7.0 ml. After 10 min standing time at 70 °C in water bath, the solution was cooled for about 3.0 min, dye was added and then complete to 10 ml total volume as shown in Fig. 14.

III.3.3. Effect of time and temperature

The effect of time on the oxidation process of DIL was investigated by measuring the absorbance of a solution containing 5.0 μg ml^{-1} (0.5 ml of 100 μg ml^{-1}) of the drug, oxidant and acid solution against blank solution prepared by the same way without drug at various time intervals. Also the effect of temperature was studied by heating the sample solution and the blank at different temperature. (30-100 °C) in water bath. The reaction took place completely after 10 min and at 70 °C in water bath. Raising the temperature more than 70 °C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals. The absorbance indicated that shaken for 2.0 min was sufficient to give reliable results. The color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

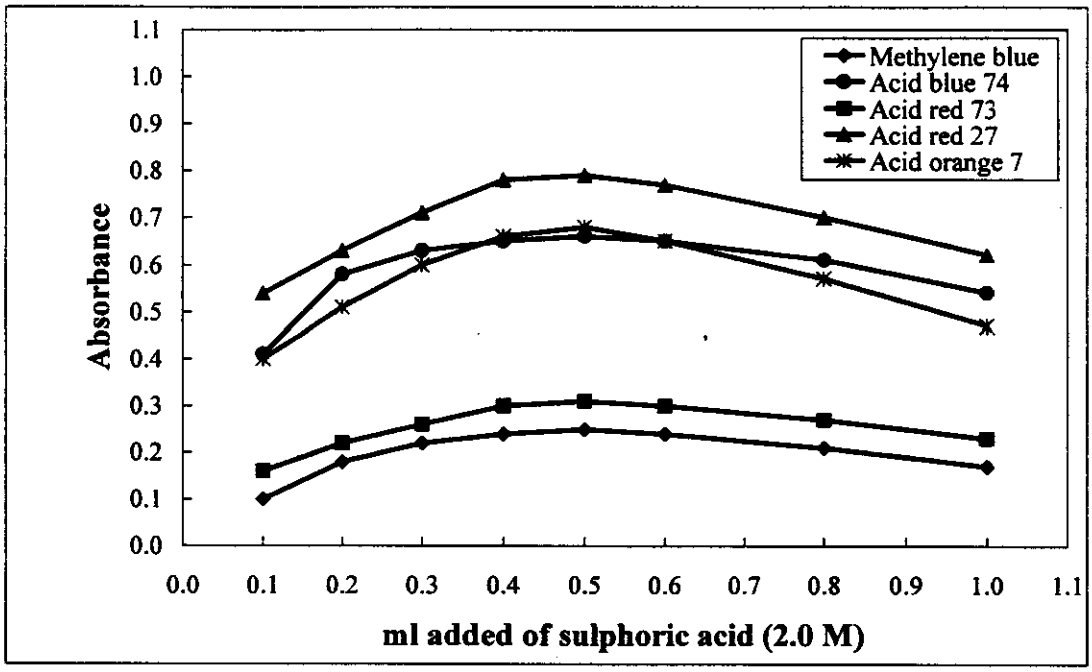


Fig. (14): Effect of ml added of sulphuric acid (2.0 M) on absorbance of 5.0 μg ml^{-1} of diltiazem HCl with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

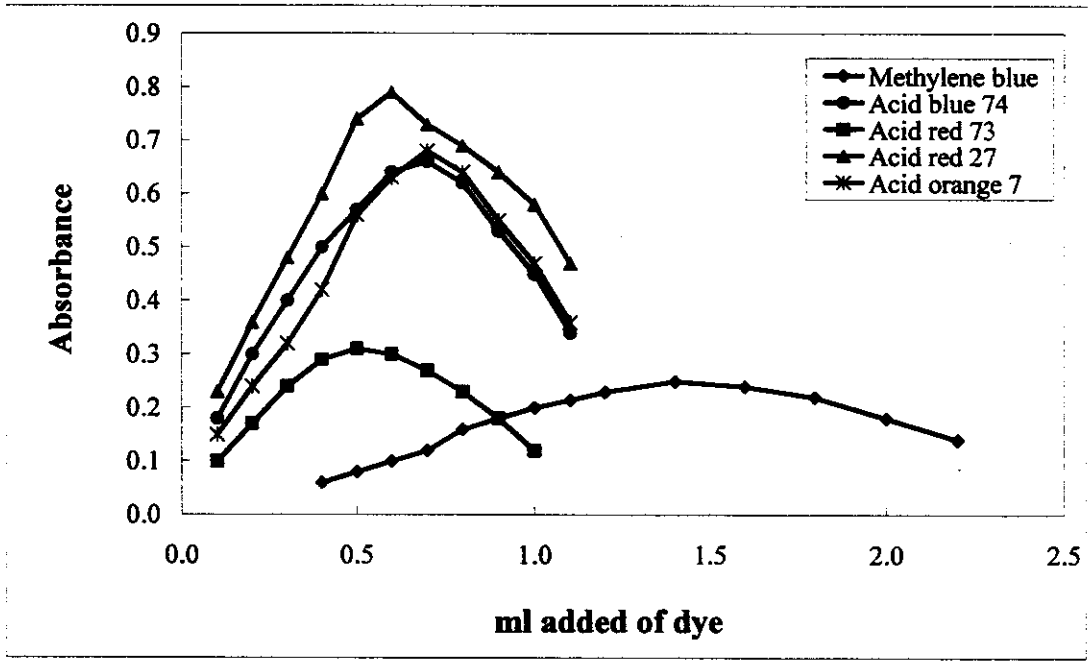


Fig. (15): Effect of ml added of dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M) on absorbance of 5.0 μg ml^{-1} of diltiazem HCl with $KMnO_4$ (5.0×10^{-4} M)

III.3.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process was studied by measuring the absorbance of solution prepared by different sequence of additions at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.3.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of the five different dyes were added to the proposed concentration of DIL 5.0 μg ml^{-1}. The optimum volumes used for production of maximum and reproducible color intensity are 1.4 ml (1.0 x 10^{-4} M) MB, whereas 0.7, 0.5, 0.6 and 0.7 ml (1.0 x 10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum dye volume as shown in Fig. 15.

III.3.6. Molar ratio method

The stoichiometry of [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable concentrations of dye (1 x 10^{-4} M MB, 1 x 10^{-3} M for AB, AR, AM and AO) were added and 5.0 μg ml^{-1} of DIL. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 16. Experimental results showed that the inflection of the two straight lines at 0.36, 0.71, 1.0, 0.83 and 0.71 in case of MB, AB, AR, AM and AO, respectively. Thus the stoichiometry ratio of oxidant to dye are 1.0 : 0.28, 1.0 : 1.41, 1.0 : 1.0, 1.0 : 1.49 and 1.0 : 1.59 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 8.

The molar ratio between DIL and oxidant at the selected conditions was investigated [169]. In this method 1.0 ml of 5.0 x10^{-4} M $KMnO_4$ is kept constant and variable concentrations (0.1-2.5 ml) of DIL 5.0 x 10^{-4} M

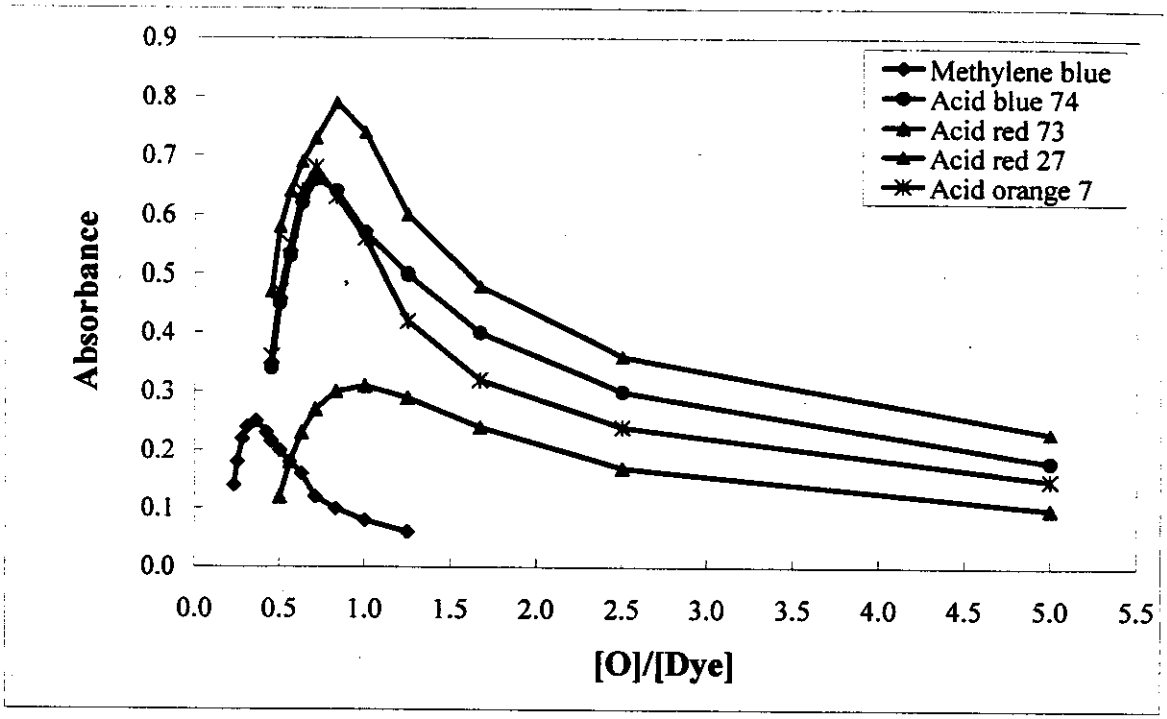


Fig. (16): Molar ratio method [O]/[Dye] for 5.0 μg ml^{-1} of diltiazem HCl with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

were added. The absorbance was measured at λ_{max} (654, 609, 510, 521 and 484 nm using MB, AB, AR, AM and AO, respectively) against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 17. Experimental results showed that the inflection of the two straight lines at 0.9, 0.42, 0.5, 0.4 and 0.31 in case of MB, AB, AR, AM and AO, respectively. Thus molar ratios of DIL to oxidant are 1.0 : 1.11, 1.0 : 2.38, 1.0 : 2.0, 1.0 : 2.50 and 1.0 : 3.23 in case of MB, AB, AR, AM and AO, respectively.

III.3.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of DIL. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration. The correlation coefficient, slopes, intercepts, are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1}, against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 8, while representative curves on the validity of Beer's law are shown in Fig. 18. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

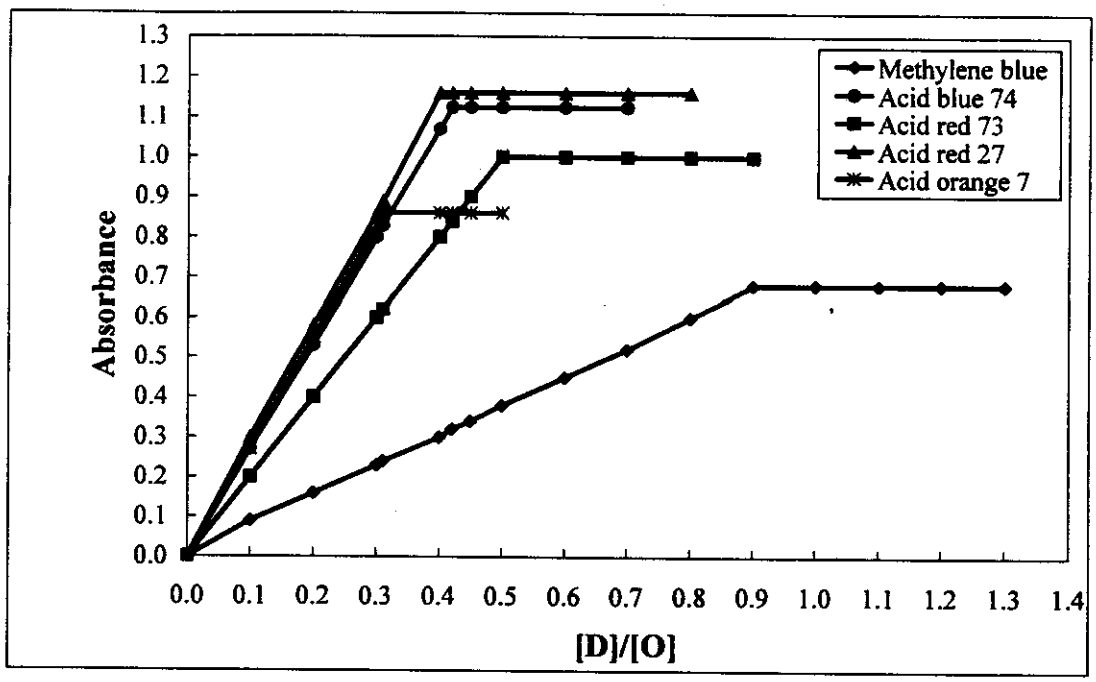


Fig. (17): Molar ratio method for diltiazem HCl (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

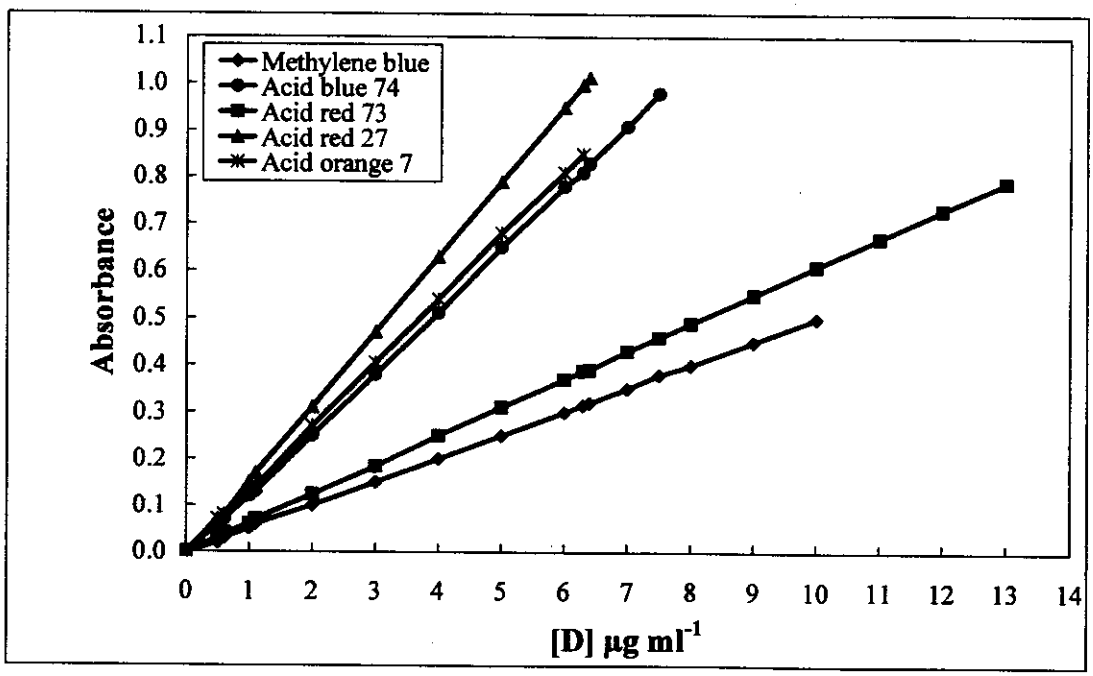


Fig. (18): Validity of Beer's law for reaction product of diltiazem HCl with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.3.8. Accuracy and precision [182]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of DIL were prepared and analyzed in six replicates. The results are summarized in Table 9. The % standard deviations and the % error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

III.3.9. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 5.0 μg ml^{-1} of DIL with varying concentration of the additives and excipients such as glucose, lactose, fructose, calcium, hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose 2-fold, and there are not interference for other excipients.

III.3.10. Analytical applications

The validity of the proposed procedures is tested by determining DIL in tablets obtained from local manufacturing companies as mentioned before. The concentration of DIL in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum or absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [172]. The results obtained were compared statistically by the Student's t-test and variance ratio F-test with those obtained by official methods on the sample of the same batch. The Student's t-test values obtained at 95% confidence level and five degree of freedom did not

exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as recorded in Table 10.

Table (8): Optical and regression characteristics of diltiazem HCl with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	654	609	510	521	484
Bear's law limits ($\mu g\ ml^{-1}$)	0.7-10	0.6-7.5	0.5-13	0.4-6.4	0.4-6.3
Ringbom limits ($\mu g\ ml^{-1}$)	0.8-9.8	0.7-7.2	0.6-12.6	0.6-6.2	0.5-5.8
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2.26×10^4	5.95×10^4	2.76×10^4	7.15×10^4	6.04×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	19.96	7.58	16.33	6.31	7.46
Detection limits ($\mu g\ ml^{-1}$)	0.196	0.164	0.124	0.083	0.087
Quantitation limits ($\mu g\ ml^{-1}$)	0.653	0.547	0.412	0.277	0.291
Regression equation*: Slope (b)	0.0501	0.1320	0.0612	0.1585	0.1340
Intercept (a)	-2×10^{-4}	-8.8×10^{-3}	-9.0×10^{-4}	-8.3×10^{-3}	-1.7×10^{-3}
Correlation coefficient (r)	0.9998	0.9997	0.9998	0.9998	0.9997
RSD** %	0.55	0.48	0.61	0.31	0.59
Stoichiometric ratio [O]/[Dye]	1.0 : 0.28	1.0 : 1.41	1.0 : 1.0	1.0 : 1.2	1.0 : 1.41
Stoichiometric ratio [D]/[O]	1.0 : 1.11	1.0 : 2.38	1.0 : 2.0	1.0 : 2.5	1.0 : 3.33

* With respect to $A = a + b\ C$ where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.

** Relative standard deviation for six determinations

Table (9): Evaluation of the accuracy and precision of the proposed procedure of diltiazem HCl.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	3.0	99.0	0.52	0.55	2.97±0.0163
	4.0	97.8	0.74	0.78	3.91±0.0304
	5.0	100.6	0.83	0.88	5.03±0.0441
Acid blue 74	3.0	100.3	0.69	0.73	3.01±0.0219
	4.0	99.5	0.94	0.99	3.98±0.0394
	5.0	98.2	0.65	0.68	4.91±0.0336
Acid red 73	3.0	101.7	0.56	0.58	3.05±0.0178
	4.0	98.5	1.30	1.36	3.94±0.0536
	5.0	97.0	0.74	0.78	4.85±0.0378
Acid red 27	3.0	100.3	0.69	0.73	3.01±0.0219
	4.0	100.8	0.71	0.75	4.03±0.0302
	5.0	98.6	0.56	0.59	4.93±0.0290
Acid orange 7	3.0	99.7	1.00	1.05	2.99±0.0315
	4.0	97.5	0.48	0.50	3.99±0.0199
	5.0	100.8	0.44	0.46	5.04±0.0233

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

Table (10): Evaluation of the accuracy and precision of the proposed and official procedures for diltiazem HCl in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A	3.0	99.3	100.3	0.25	1.34	99.4	0.65	1.85	99.8	0.45	1.54	99.6	0.15	1.23	100.4	0.54	1.88
	4.0	98.9	99.4	0.36	1.87	99.5	0.85	2.34	100.3	0.34	1.46	99.2	0.64	1.87	99.7	0.20	1.64
	5.0	99.4	99.7	0.27	1.65	100.1	0.67	2.10	99.5	0.94	1.65	100.2	0.52	1.64	99.8	0.37	1.25
B	3.0	99.3	99.7	0.92	2.30	100.3	0.54	1.68	99.8	1.24	2.89	100.2	0.37	1.35	99.6	0.16	1.37
	4.0	99.4	100.3	0.17	1.25	99.7	0.37	1.24	99.7	0.67	1.54	100.5	0.64	1.58	99.8	0.28	1.15
	5.0	98.7	99.3	0.34	1.23	99.6	0.94	2.14	99.5	1.01	2.68	99.7	0.76	1.98	99.8	0.47	1.96
C	3.0	99.5	100.4	0.38	2.31	99.6	0.64	1.89	99.8	0.34	1.38	99.9	0.25	1.48	99.7	0.74	1.65
	4.0	98.8	99.6	0.64	2.65	99.4	0.27	1.65	99.4	0.84	2.39	100.6	1.19	2.58	101.1	0.87	1.99
	5.0	99.4	100.1	0.25	1.64	99.8	1.21	3.05	100.2	0.57	1.58	99.5	1.22	2.98	99.6	0.64	1.58
D1	3.0	98.6	99.1	0.98	2.87	98.9	0.57	1.89	99.7	0.31	3.01	100.2	0.27	3.42	100.2	1.11	3.44
D2	3.0	99.4	100.3	0.68	1.54	99.7	0.79	1.59	100.2	0.42	2.45	99.6	0.63	3.08	100.5	0.48	2.25
D1	4.0	98.6	98.8	0.75	1.35	99.4	0.43	2.44	99.6	0.38	1.66	98.9	0.81	2.64	100.4	0.19	1.99
D2	4.0	99.2	100.1	0.68	2.80	99.5	1.09	3.08	100.2	0.88	2.32	99.8	0.55	1.87	99.7	0.44	3.06
D1	5.0	100.1	100.2	0.38	2.38	100.3	0.46	3.55	99.7	1.66	1.47	99.5	0.49	1.57	99.6	1.37	2.41
D2	5.0	99.2	99.6	0.49	3.91	99.5	0.84	2.33	100.2	0.77	2.58	100.4	0.37	2.04	99.2	1.21	1.87

* Dosage forms

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

A Altiazem 60 mg/tablet B Delaytiazem SR 90 mg/capsule

C Tildiem 60 mg/tablet

D1 Peltiam 120 mg/tablet D2 Peltiam 240 mg/tablet

III.4. Absorption spectra of the atenolol with $KMnO_4$ and different dyes

No attempts have been made to develop a spectrophotometric method for determination of atenolol (ATL) by oxidation with potassium permanganate, using five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential and excellent solution stability. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with five different dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously. However they are used as indicator to estimate atenolol. $KMnO_4$ reacts with ATL, resulting in oxidation depending upon the functional group (-NH, $-NH_2$) present in ATL, probably a mixture of products, with reproducible data under specified experimental condition. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product). The remaining dye is measured spectrophotometrically at their corresponding λ_{max} (658, 609, 509, 520 and 485 nm using MB, AB, AR, AM and AO, respectively) as shown in Fig. 19.

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$ caused the color to fade. The effect of different experimental variable were studied and recorded below

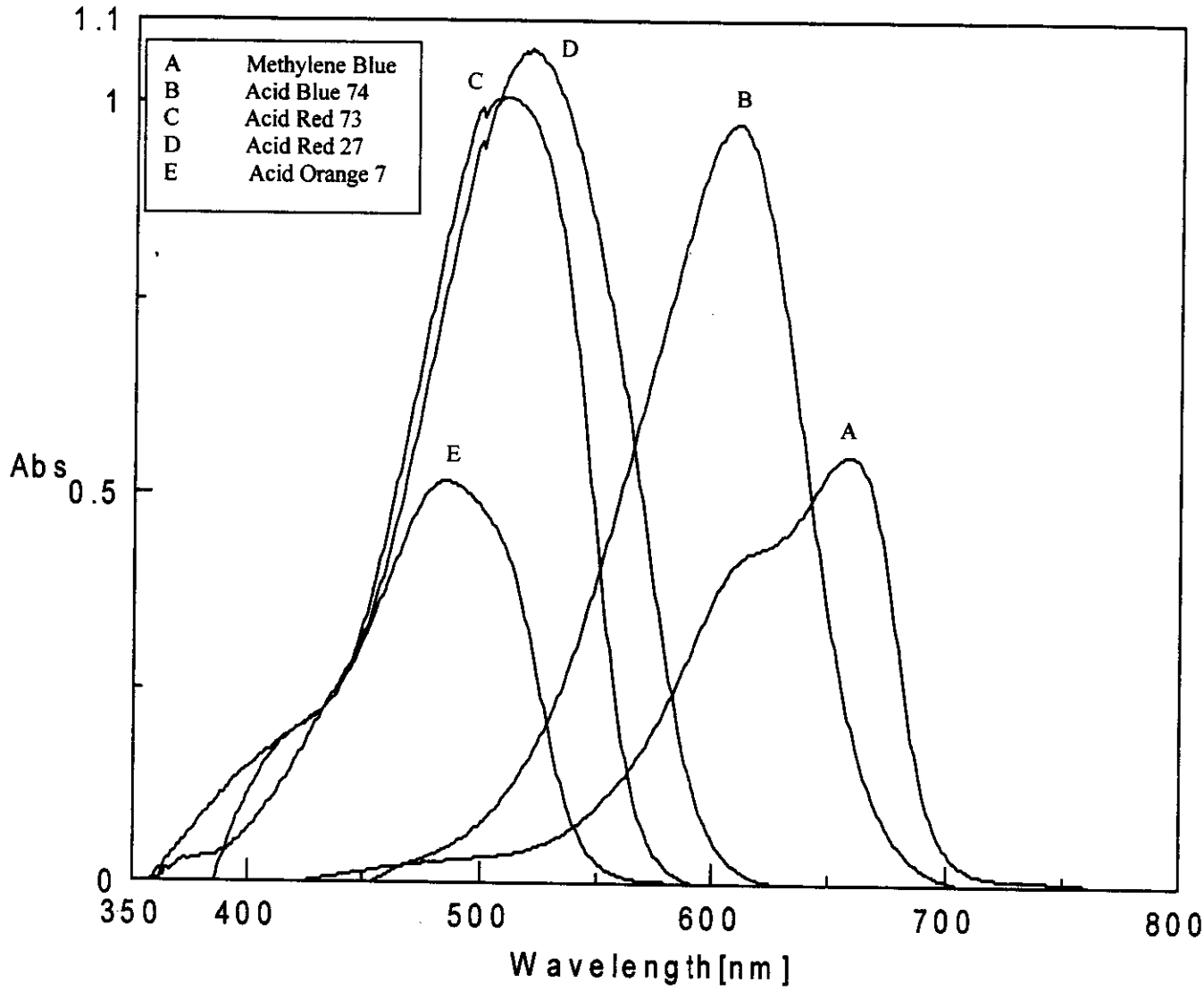


Fig. (19): Absorption spectra for the reaction product of 3.5 μg ml^{-1} of atenolol with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.4.2. Effect of acid concentration

Different types of acid were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. H_2SO_4 was preferable with using the $KMnO_4$ oxidant. To each 10 ml measuring flask 3.5 μg ml^{-1} (0.35 ml of 100 μg ml^{-1}) of the ATL and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) was added, 0.5 ml of H_2SO_4 (2.0 M) is the optimum concentration of acid, then the solution was diluted to 7.0 ml. After 10 min standing time at 80 °C in water bath, the solution was cooled for about 3.0 min; dye was added, then complete to 10 ml total volume as shown in Fig. 20.

III.4.3. Effect of time and temperature

The effect of time on the oxidation process of ATL was investigated by measuring the absorbance of a solution containing 3.5 μg ml^{-1} the drug, oxidant and acid solution against blank solution prepared by the same way without drug at λ_{max} 658, 609, 509, 520 and 485 nm using MB, AB, AR, AM and AO, respectively, at various time intervals. Also the effect of temperature was studied by heating the sample solution and the blank at different temp. (30-100 °C) in water bath. The reaction took place completely after 10 min, and at 80 °C temperature in water bath. Raising the temperature more than 80 °C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals (1.0-5.0 min). The absorbance indicated that shaken for 1.0 min was sufficient to give reliable results. The produced color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

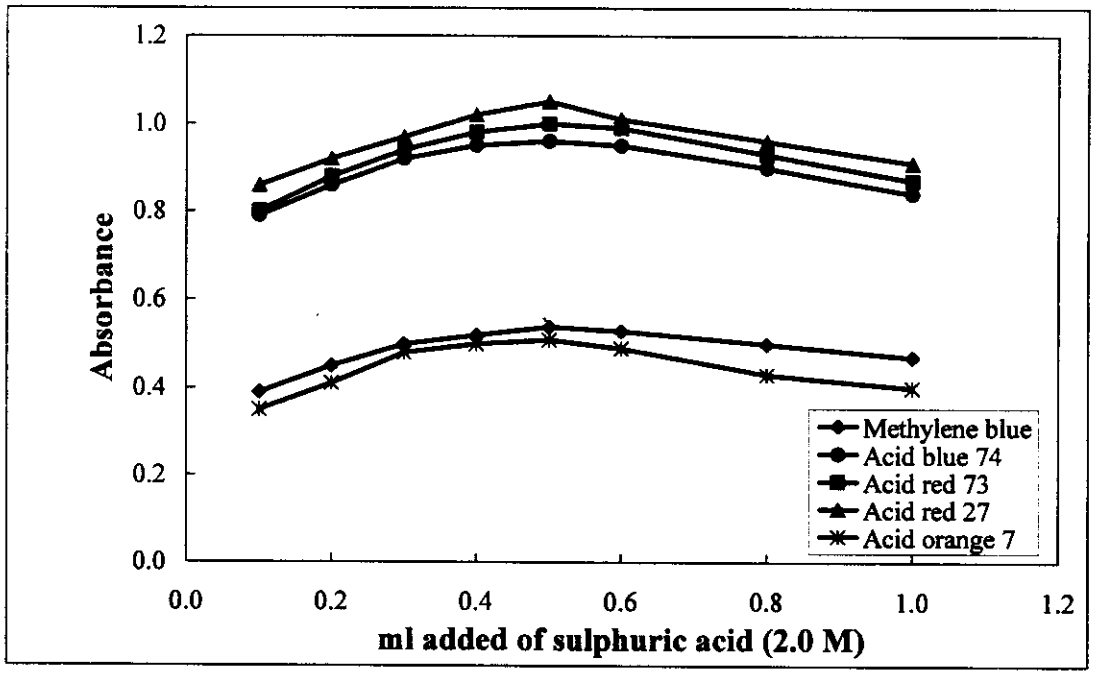


Fig. (20): Effect of ml added of sulphuric acid (2.0 M) on absorbance of 3.5 μg ml^{-1} of atenolol with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

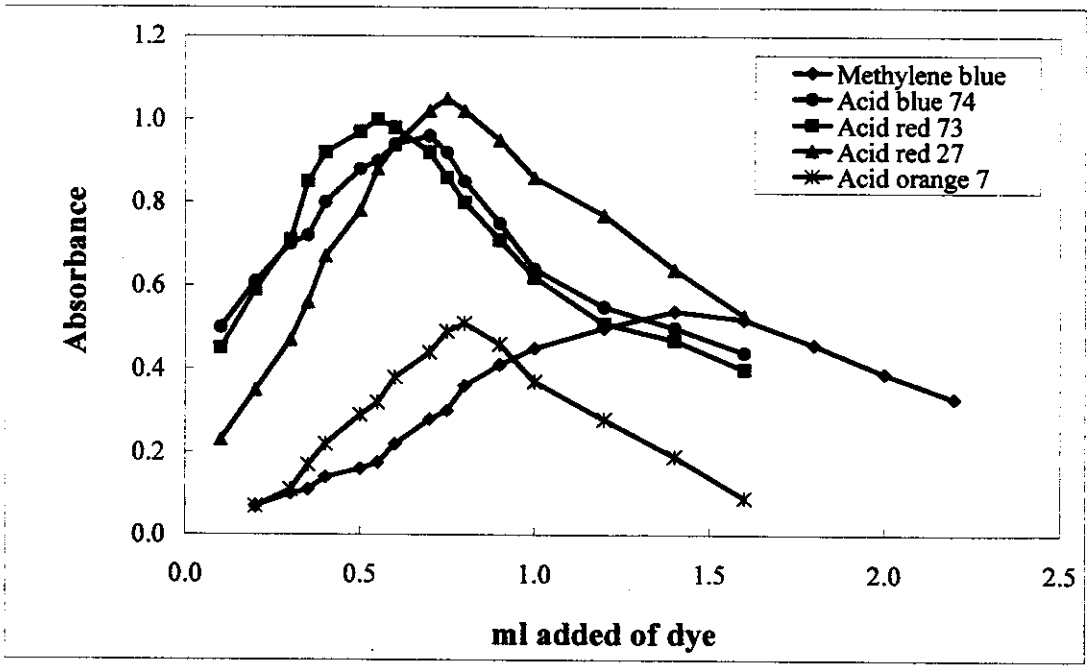


Fig. (21): Effect of ml added of dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M) on absorbance of 3.5 μg ml^{-1} of atenolol with $KMnO_4$ (5.0×10^{-4} M)

III.4.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process was studied by measuring the absorbance of solution prepared by different sequence of additions at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.4.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of the five different dyes were added to the proposed concentration of ATL (3.5 μg ml^{-1}). The optimum volumes used for production of maximum and reproducible color intensity are 1.4 ml (1.0 x 10^{-4} M) MB as shown in Fig. 26, whereas 0.7, 0.55, 0.75 and 0.7 ml (1.0 x10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum dye volume as shown in Fig. 21.

III.4.6. Molar ratio method

The molar ratio between oxidant and dye [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable concentrations of dye (1 x 10^{-4} M MB, 1 x 10^{-3} M for AB, AR, AM and AO) were added and 3.5 μg ml^{-1} of ATL. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 22. Experimental results showed that the inflection of the two straight lines at 0.36, 0.71, 0.91, 0.67 and 0.63 in case of MB, AB, AR, AM and AO, respectively. Thus the stoichiometric ratio of oxidant to dye are 1.0 : 0.28, 1.0 : 1.41, 1.0 : 1.1, 1.0 : 1.49 and 1.0 : 1.59 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 11.

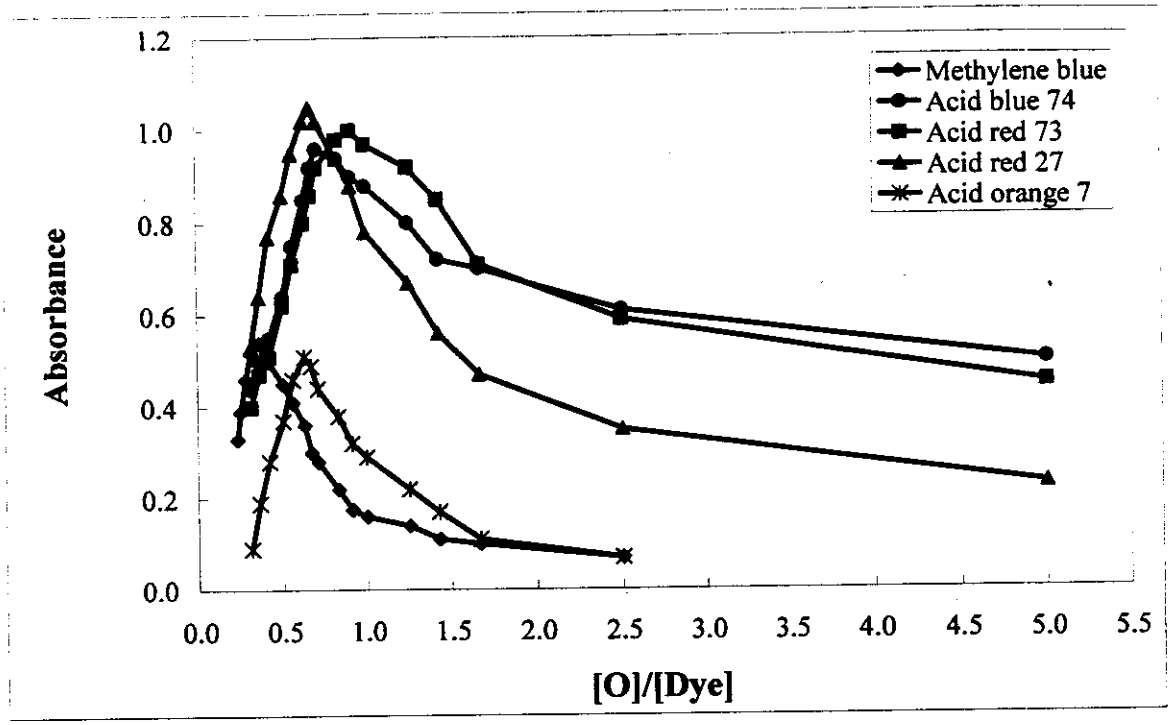


Fig. (22): Molar ratio method [O]/[Dye] for 3.5 μg ml^{-1} of atenolol with $KMnO_4$ (5.0 x 10^{-4} M) and dyes (1.0 x 10^{-3} M) except on using methylene blue (1.0 x 10^{-4} M)

In order to investigate the molar ratio between ATL and oxidant at the selected conditions, the molar ratio method described by *Yoe* and *Jones* [169] was carried out. In this method 1.0 ml of 5.0 $x10^{-4}$ M $KMnO_4$ is kept constant and variable concentrations (0.1-2.5 ml) of ATL 5.0 x 10^{-4} M were added. The absorbance was measured at λ_{max} (658, 609, 509, 520 and 485 nm using MB, AB, AR, AM and AO, respectively) against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 23. Experimental results showed that the inflection of the two straight lines at 0.39, 0.39, 0.40, 0.33 and 0.44 in case of MB, AB, AR, AM and AO, respectively. Thus molar ratios of ATL to oxidant are 1.0 : 2.56, 1.0 : 2.56, 1.0 : 2.50, 1.0 : 3.03 and 1.0 : 2.27 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 11.

III.4.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of ATL. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration as listed in Table 11. The correlation coefficient, slopes, intercepts, are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1}, against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 11, while representative curves on the validity of Beer's law for ATL with the different dyes are shown in Fig. 24. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements

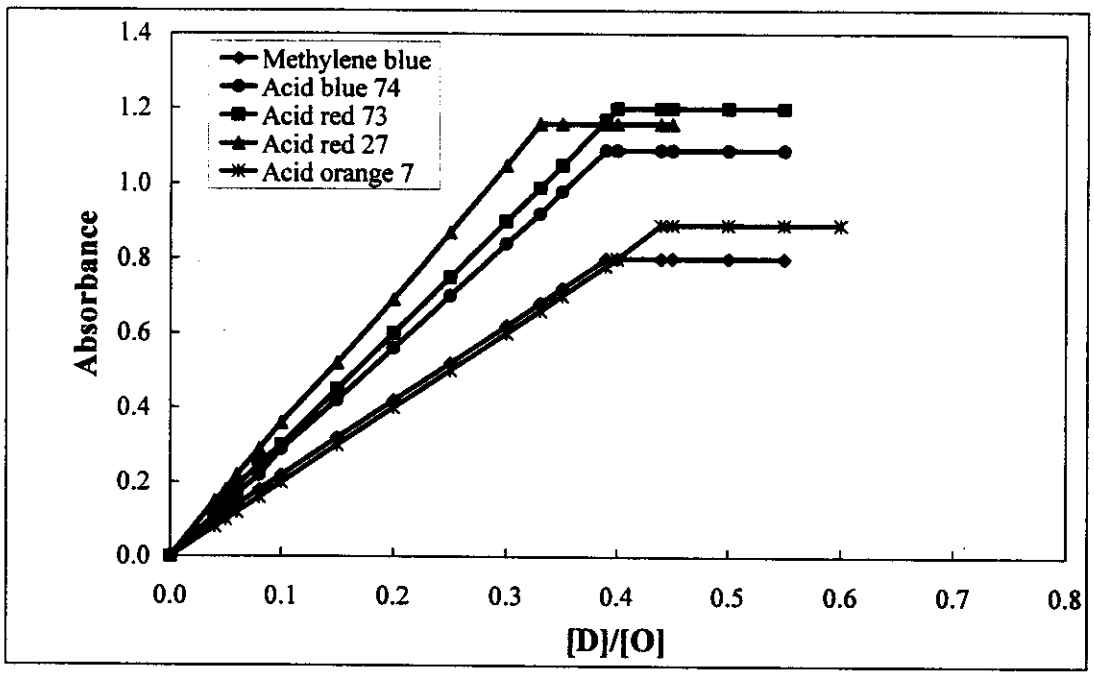


Fig. (23): Molar ratio method for atenolol (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

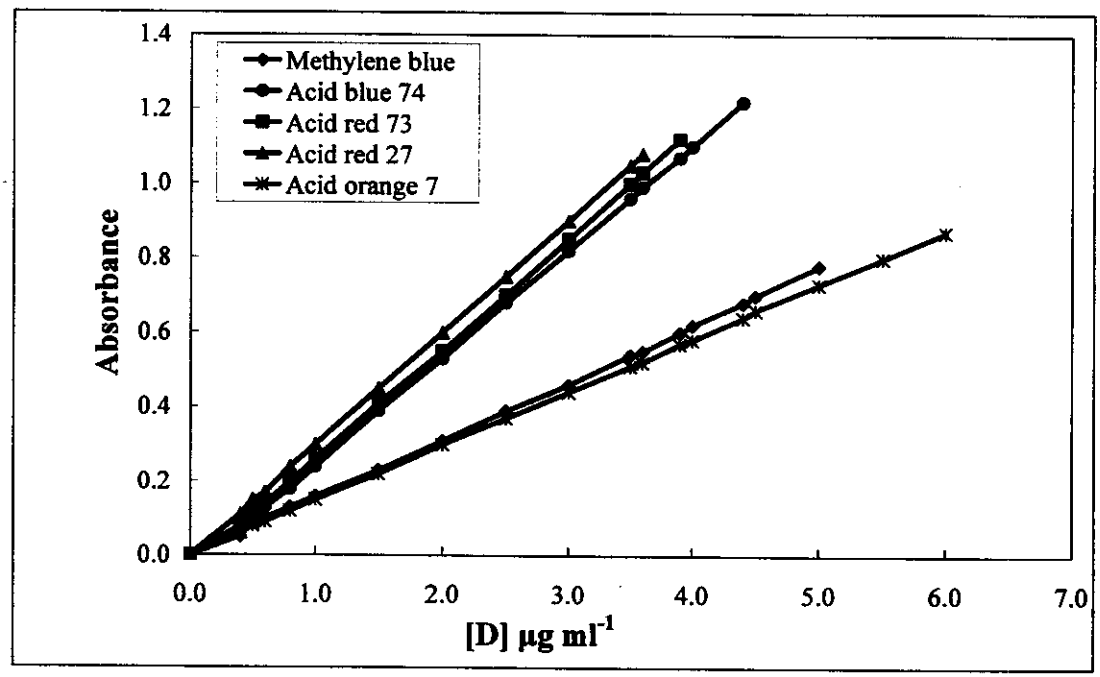


Fig. (24): Validity of Beer's law for reaction product of atenolol with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue(1.0×10^{-4} M)

obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

III.4.8. *Accuracy and precision [182]*

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of ATL were prepared and analyzed in six replicates. The results are summarized in Table 12. The % standard deviations and the % error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

III.4.9. Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 3.5 μg ml^{-1} of ATL with varying concentration of the additives and excipients such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose 2-fold, and there are not interference for other excipients.

III.4.9. Analytical applications

The validity of the proposed procedures are tested by determining ATL in tablets obtained local from manufacturing companies as mentioned before. The concentration of ATL in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [173]. The

results obtained were compared statistically by the Student's t-test and variance ratio F-test with those obtained by official methods on the sample of the same batch. The Student's t-test values obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as shown in Tables (13 and 14).

Table (11): Optical and regression characteristics of atenolol with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	658	609	509	520	485
Bear's law limits ($\mu g\ ml^{-1}$)	0.3-5.0	0.2-4.4	0.2-3.9	0.1-3.6	0.3-6.0
Ringbom limits ($\mu g\ ml^{-1}$)	0.4-4.8	0.4-4.3	0.3-3.7	0.3-3.5	0.4-5.6
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	4.11×10^4	7.38×10^4	7.80×10^4	8.06×10^4	3.88×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	6.48	3.61	3.42	3.30	6.86
Detection limits ($\mu g\ ml^{-1}$)	0.072	0.056	0.0.45	0.018	0.078
Quantitation limits ($\mu g\ ml^{-1}$)	0.239	0.187	0.149	0.061	0.259
Regression equation*: Slope (b)	0.1543	0.2772	0.2928	0.3028	0.1457
Intercept (a)	0.0021	- 0.0272	- 0.0279	- 0.0070	0.0067
Correlation coefficient (r)	0.9997	0.9997	0.9996	0.9998	0.9999
RSD** %	0.48	0.29	0.39	0.52	0.68
Stoichiometric ratio [O]/[Dye]	1.0 : 0.28	1.0 : 1.41	1.0 : 1.1	1.0 : 1.49	1.0 : 1.59
Stoichiometric ratio [D]/[O]	1.0 : 2.56	1.0 : 2.56	1.0 : 2.5	1.0 : 3.03	1.0 : 2.27

* With respect to $A = a + b\ C$ where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.
** Relative standard deviation for six determinations

Table (12): Evaluation of the accuracy and precision of the proposed procedure of atenolol.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	2.0	99.0	0.40	0.42	1.98±0.0084
	3.0	99.3	0.27	0.31	2.98±0.0093
	3.5	97.4	0.24	0.26	3.41±0.0087
Acid blue 74	2.0	98.0	0.49	0.51	1.96±0.0100
	3.0	100.3	0.33	0.34	3.01±0.0102
	3.5	98.6	0.25	0.26	3.45±0.0092
Acid red 73	2.0	101.5	0.94	0.98	2.03±0.0199
	3.0	98.7	0.51	0.53	2.96±0.0157
	3.5	101.1	0.28	0.29	3.54±0.0104
Acid red 27	2.0	99.5	0.49	0.52	1.99±0.0103
	3.0	101.3	0.32	0.33	3.04±0.0101
	3.5	98.0	0.29	0.30	3.46±0.0105
Acid orange 7	2.0	100.5	0.79	0.84	2.01±0.0168
	3.0	100.3	0.66	0.70	3.01±0.0210
	3.5	100.3	0.43	0.45	3.51±0.0157

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

Table (13): Evaluation of the accuracy and precision of the proposed and official procedures for atenolol in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A1	2.0	98.5	99.2	0.39	3.11	100.1	0.68	2.39	99.7	0.56	2.94	99.7	1.38	2.72	100.1	0.68	2.76
B1	2.0	98.2	99.5	0.84	2.55	99.4	0.49	2.19	99.3	0.68	2.39	100.5	0.98	3.04	99.7	0.27	1.56
A1	3.0	99.2	99.5	0.38	2.66	100.3	0.19	3.14	99.6	0.24	2.79	99.8	0.82	1.65	100.3	0.36	2.24
B1	3.0	99.1	100.3	1.21	1.78	99.4	0.24	1.45	99.7	0.31	3.01	99.8	0.23	1.38	98.9	0.96	2.78
A1	3.5	99.3	99.7	1.38	1.38	100.3	0.75	1.59	99.2	1.02	1.59	99.5	0.43	1.84	99.8	0.46	1.66
B1	3.5	98.9	99.4	0.67	1.64	99.6	0.97	2.45	100.4	1.25	2.26	100.5	0.24	2.99	99.6	1.12	2.92
A2	2.0	98.5	100.3	0.58	2.37	99.7	0.67	1.88	99.7	0.44	1.48	99.8	0.28	1.48	99.7	0.84	1.67
	3.0	98.9	99.5	0.74	2.66	99.5	0.37	1.68	99.4	0.84	2.39	100.6	1.19	2.59	101.1	0.97	1.98
	3.5	99.2	100.4	0.28	1.64	99.8	1.24	3.05	100.2	0.59	1.57	99.7	1.26	2.98	99.6	0.68	1.68
A3	2.0	98.7	99.8	0.29	2.06	99.4	0.39	1.98	99.7	0.55	2.34	99.6	0.27	3.42	99.8	1.81	3.24
B3	2.0	99.4	99.5	0.46	2.42	99.6	0.24	2.38	100.1	0.38	2.31	99.7	0.63	3.08	100.5	0.48	2.27
A3	3.0	98.6	100.1	0.26	2.94	99.9	0.71	3.14	100.2	0.60	2.28	99.4	0.81	3.24	100.4	0.19	1.89
B3	3.0	99.2	100.3	0.81	1.88	99.6	0.18	2.18	99.8	0.81	1.67	100.3	0.55	1.87	99.7	0.44	3.16
A3	3.5	99.3	99.3	0.49	1.89	99.2	0.21	1.12	100.3	0.21	3.07	99.3	0.49	1.57	99.6	1.37	2.47
B3	3.5	98.9	99.5	0.74	2.36	99.8	0.84	1.57	100.2	0.33	2.14	99.6	0.37	2.04	99.2	1.21	1.39

* Dosage forms

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

A1 Ateno 50 mg/tablet B1 Ateno 100 mg/tablet

A2 Atenolol 50 mg/tablet

A3 Blokium 50 mg/tablet B3 Blokium 100 mg/tablet

Table (14): Evaluation of the accuracy and precision of the proposed and official procedures for atenolol in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A1	2.0	98.5	100.5	0.39	2.41	100.3	0.66	1.99	99.8	0.44	1.38	99.9	0.29	1.78	99.8	0.75	1.68
	3.0	99.1	99.6	0.66	2.68	99.4	0.27	1.67	99.4	0.84	2.39	100.5	1.19	2.59	101.0	0.87	1.97
	3.5	99.4	99.8	0.35	1.66	99.8	1.21	3.15	100.3	0.58	1.58	99.5	1.32	2.91	99.6	0.64	1.58
A2	2.0	98.6	99.4	0.98	2.87	99.9	0.57	1.87	99.7	0.91	2.84	100.2	0.29	3.47	100.2	1.11	3.44
B2	2.0	99.4	100.3	0.68	1.54	99.7	0.79	1.99	100.3	0.42	2.45	99.7	0.73	3.18	100.5	0.48	2.25
A2	3.0	98.6	98.9	0.75	1.35	99.5	0.43	2.44	99.6	0.38	1.66	98.9	0.81	2.64	100.4	0.19	1.99
B2	3.0	99.2	100.6	0.68	1.80	99.6	1.09	3.08	100.2	0.88	2.32	99.8	0.55	1.87	99.7	0.44	3.06
A2	3.5	100.1	100.2	0.38	2.38	100.3	0.47	3.65	99.7	1.66	1.47	99.5	0.49	1.57	99.6	1.37	2.41
B2	3.5	90.0	99.6	0.49	3.11	99.5	0.85	2.33	100.2	0.77	2.58	100.4	0.37	2.04	99.2	1.21	1.87

* Dosage forms

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

A1 Tensolol 100 mg/tablet

A2 Tenormin 50 mg/tablet B2 Tenormin 100 mg/tablet

III.5. Absorption spectra of the lisinopril dihydrate with $KMnO_4$ and different dyes

No attempts have been made to develop a spectrophotometric method for determination of lisinopril dihydrate (LIS) by oxidation with potassium permanganate, using five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential and excellent solution stability. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with five different dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously. However they are used as indicator to estimate LIS. $KMnO_4$ reacts with LIS, resulting in oxidation depending upon the functional group (-NH, $-NH_2$) present in LIS, probably a mixture of products, with reproducible data under specified experimental condition. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product). The remaining dye is measured spectrophotometrically at their corresponding λ_{max}. The absorption spectra of the reaction products of the method show characteristic λ_{max} (665, 610, 509, 521 and 485 nm using MB, AB, AR, AM and AO, respectively) value, as shown in Fig. 25.

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$ caused the color to fade. The effect of different experimental variable were studied and recorded below

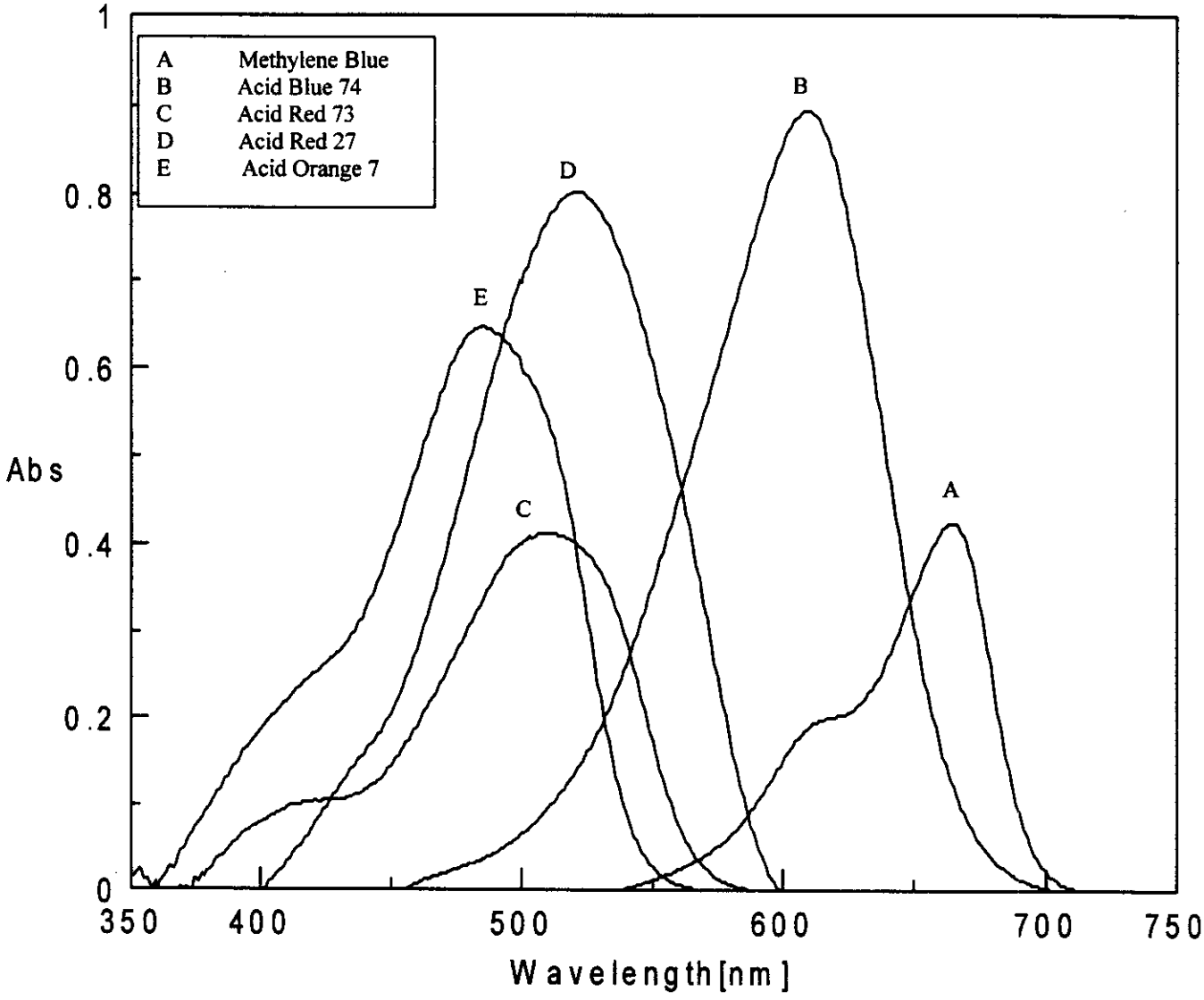


Fig. (25): Absorption spectra for the reaction product of 5.0 μg ml^{-1} of lisinopril dihydrate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.5.2. Effect of acid concentration

Different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. H_2SO_4 was preferable with using the $KMnO_4$ oxidant. To each 10 ml measuring flask, 5.0 μg ml^{-1} LIS (0.5 ml of 100 μg ml^{-1}), and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) was added, 0.5 ml of H_2SO_4 (2.0 M) is the optimum concentration of acid, then the solution was diluted to 7.0 ml, after 5.0 min standing time at 80 °C in water bath, the solution was cooled for about 3.0 min, dye was added, then complete to 10 ml total volume as shown in Fig. 26.

III.5.3. Effect of time and temperature

The effect of time on the oxidation process of LIS was investigated by measuring the absorbance of a solution containing 5.0 μg ml^{-1} the drug, oxidant and acid solution (H_2SO_4) against blank solution prepared by the same way without drug at λ_{max} 665, 610, 509, 521 and 485 nm using MB, AB, AR, AM and AO, respectively, at various time intervals. Also the effect of temperature was studied by heating the sample solution and the blank at different temperature (30-100 °C) in water bath. The reaction took place completely after 5.0 min; and at 80 °C temperature in water bath. Raising the temperature than 80 °C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals (1.0-5.0 min). The absorbance indicated that shaken for 1.0 min is sufficient to give reliable results. The produced color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

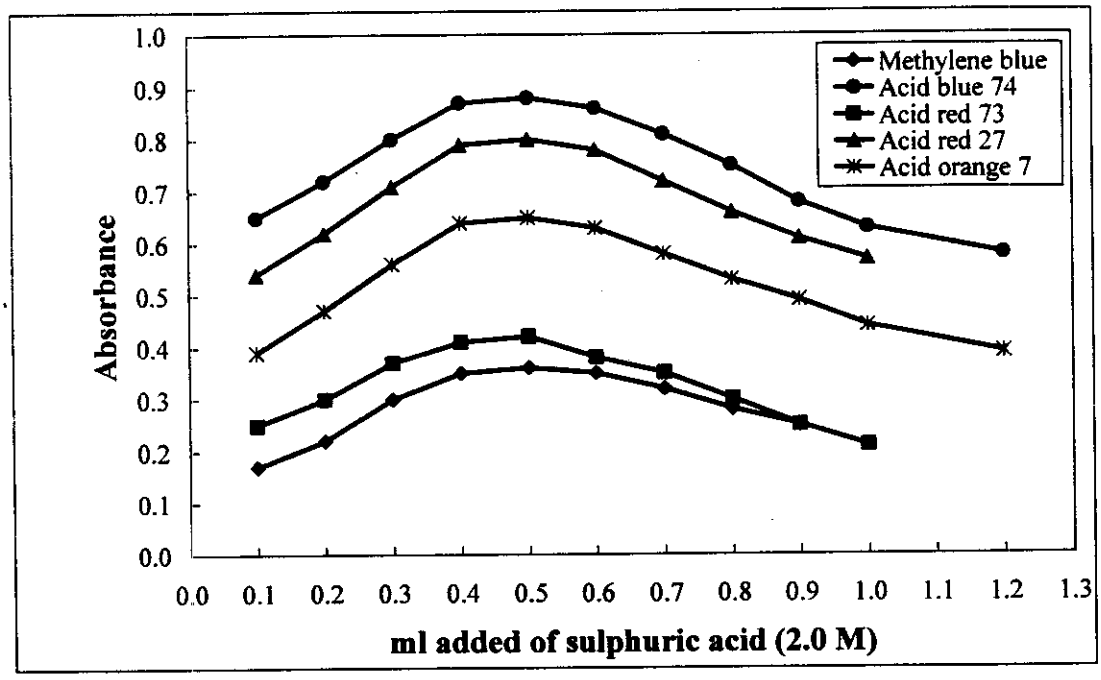


Fig. (26): Effect of ml added of sulphuric acid (2.0 M) on absorbance of 5.0 µg ml^{-1} of lisinopril dihydrate with $KMnO_4$ (5.0 x 10^{-4} M) and dyes (1.0 x 10^{-3} M) except on using methylene blue (1.0 x 10^{-4} M)

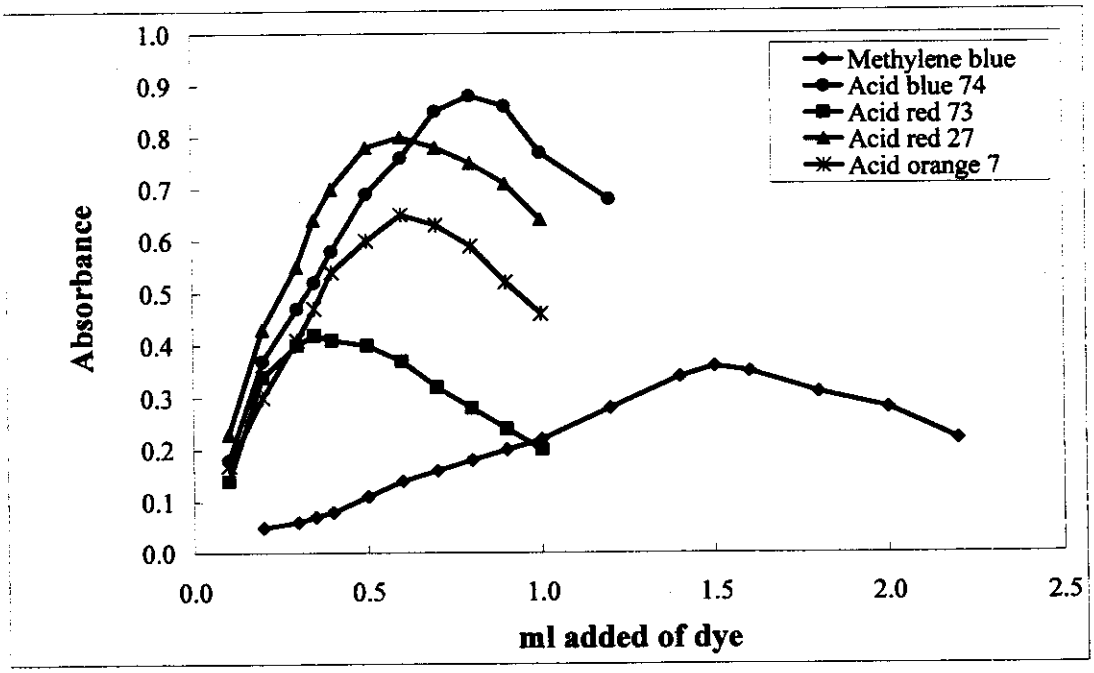


Fig. (27): Effect of ml added of dyes (1.0 x 10^{-3} M) except on using methylene blue (1.0 x 10^{-4} M) on absorbance of 5.0 µg ml^{-1} of lisinopril dihydrate with $KMnO_4$ (5.0 x 10^{-4} M)

III.5.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process of LIS was studied by measuring the absorbance of solution prepared by different sequence of additions at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.5.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of the five different dyes were added to the proposed concentration of LIS 5.0 μg ml^{-1}. The optimum volumes used for production of maximum and reproducible color intensity are 1.5 ml (1.0×10^{-4} M) MB, whereas 0.8, 0.35, 0.6 and 0.6 ml (1.0×10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum dye volume as shown in Fig. 26.

III.5.6. Molar ratio method

The molar ratio between oxidant and dye [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable concentrations of dye (1×10^{-4} M MB, 1×10^{-3} M for AB, AR, AM and AO) were added and 5.0 μg ml^{-1} of LIS. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 28. Experimental results showed that the inflection of the two straight lines at 0.33, 0.63, 1.25, 0.83 and 0.83 in case of MB, AB, AR, AM and AO, respectively. Thus the stoichiometric ratio of oxidant to dye are 1.0 : 0.3, 1.0 : 1.59, 1.0 : 0.8, 1.0 : 1.2 and 1.0 : 1.2 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 15.

In order to investigate the molar ratio between LIS and oxidant at the selected conditions, the molar ratio method described by *Yoe* and *Jones*

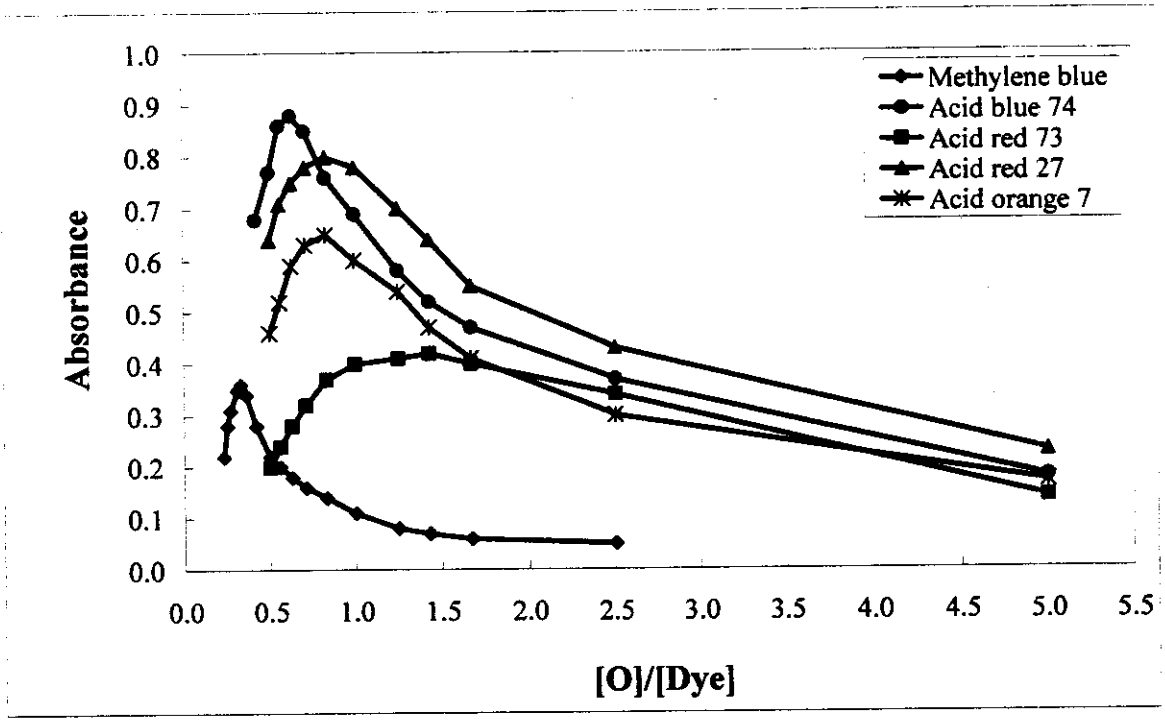


Fig. (28): Molar ratio method [O]/[Dye] for 5.0 μg ml^{-1} of lisinopril dihydrate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

[169] was carried out. In this method 1.0 ml of 5.0 $x10^{-4}$ M $KMnO_4$ is kept constant and variable concentrations (0.1-2.5 ml) of LIS 5.0 x 10^{-4} M were added. The absorbance was measured at λ_{max} against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 29. Experimental results showed that the inflection of the two straight lines at 0.65, 0.44, 0.43, 0.32 and 0.41 in case of MB, AB, AR, AM and AO, respectively. Thus molar ratios of LIS to oxidant are 1.0 : 1.54, 1.0 : 2.27, 1.0 : 2.33, 1.0 : 3.13 and 1.0 : 2.44 in case of MB, AB, AR, AM and AO, respectively.

III.5.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of LIS. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration as listed in Table 15. The correlation coefficient, slopes, intercepts, are calculated using the former equations. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1}, against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 15, while representative curves on the validity of Beer's law for LIS with different dyes are shown in Fig. 30. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

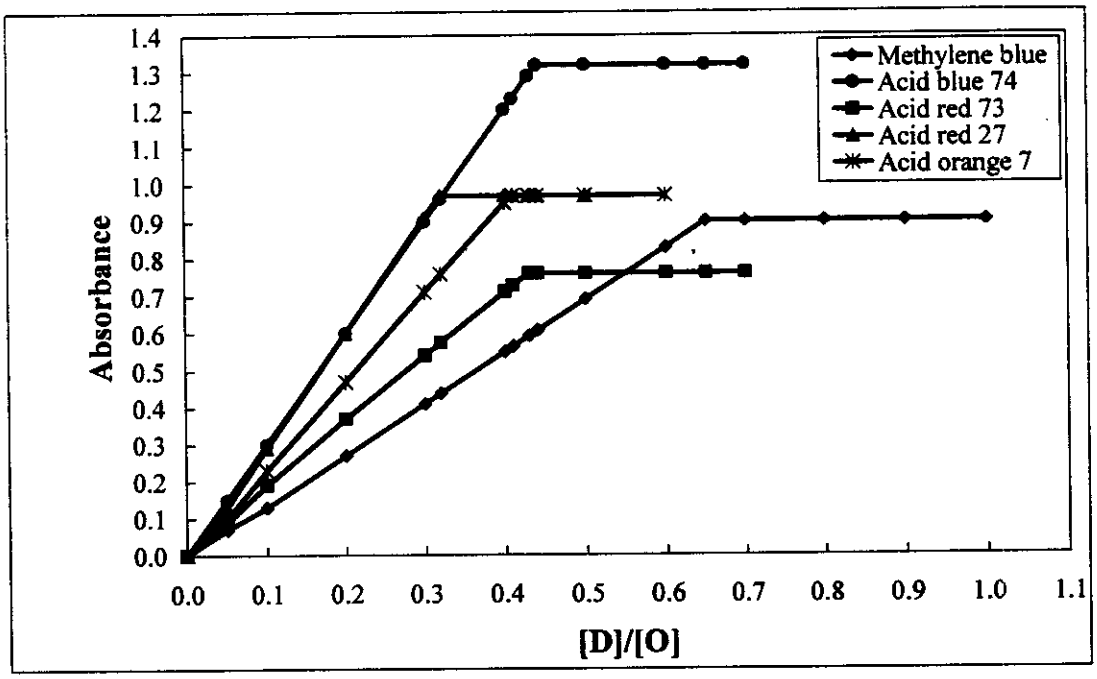


Fig. (29): Molar ratio method for lisinopril dihydrate (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

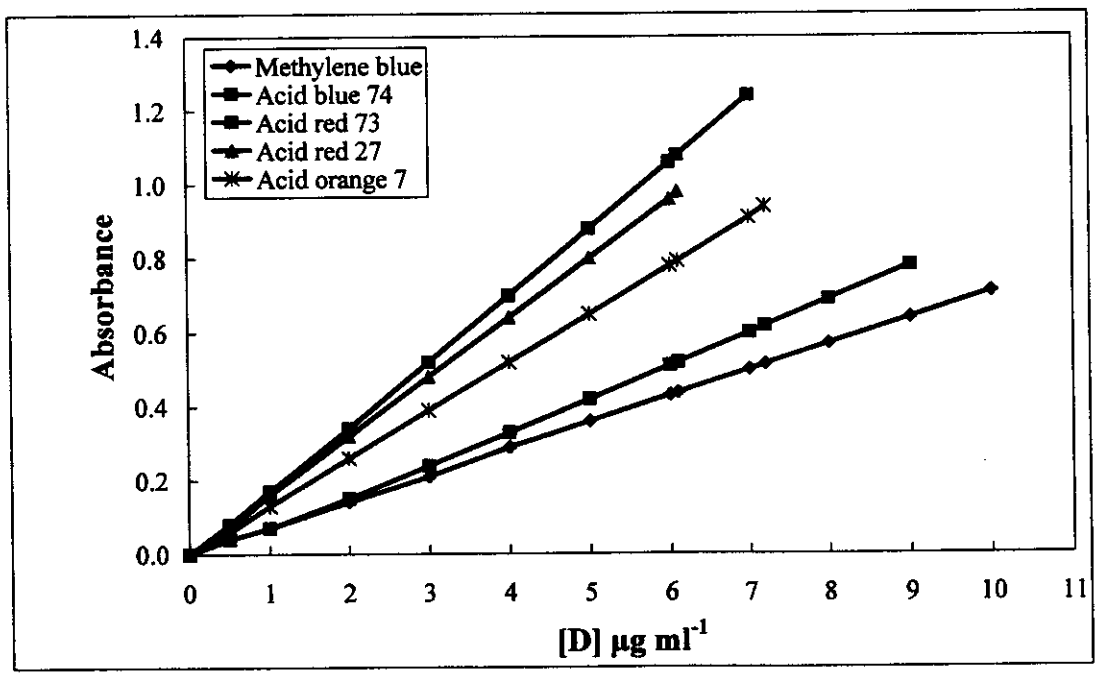


Fig. (30): Validity of Beer's law for reaction product of lisinopril dihydrate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.5.8. Accuracy and precision [182]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of LIS were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 16. The % standard deviations and the % error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

III.5.9. Interferences

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 5.0 μg ml^{-1} of LIS with varying concentration of the additives and excipients such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose 2-fold, and there are not interference for other excipients.

III.5.10. Analytical applications

The validity of the proposed procedures is tested by determining LIS in tablets obtained from local manufacturing companies as mentioned before. The concentration of LIS in dosage forms were calculated from the appropriate calibration graphs. There was no shift in the absorption maximum or absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [174]. The results obtained were compared statistically by the Student's t-test and variance ratio F-test with those obtained by official methods on the sample of the same batch. The Student's t-test

values obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as shown in Table 17.

Table (15): Optical and regression characteristics of lisinopril dihydrate with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	665	610	509	521	485
Bear's law limits ($\mu g\ ml^{-1}$)	0.5-10	0.3-7.0	0.5-9.0	0.4-6.1	0.4-7.2
Ringbom limits ($\mu g\ ml^{-1}$)	0.7-9.4	0.5-6.7	0.6-8.7	0.6-5.8	0.5-7.0
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	3.15×10^4	7.85×10^4	3.82×10^4	7.10×10^4	5.76×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	14.01	5.62	11.55	6.22	7.67
Detection limits ($\mu g\ ml^{-1}$)	0.137	0.083	0.116	0.092	0.101
Quantitation limits ($\mu g\ ml^{-1}$)	0.455	0.276	0.386	0.308	0.336
Regression equation* : Slope (b)	0.0714	0.1779	0.0866	0.1609	0.1304
Intercept (a)	0.0011	- 0.0086	- 0.0137	- 0.0031	- 0.0017
Correlation coefficient (r)	0.9999	0.9998	0.9996	0.9997	0.9996
RSD** %	0.57	0.68	0.49	0.28	0.72
Stoichiometric ratio [O]/[Dye]	1.0 : 0.3	1.0 : 1.59	1.0 : 0.8	1.0 : 1.2	1.0 : 1.2
Stoichiometric ratio [D]/[O]	1.0 : 1.54	1.0 : 2.27	1.0 : 2.33	1.0 : 3.13	1.0 : 2.44

* With respect to $A = a + b\ C$ where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.

** Relative standard deviation for six determinations

Table (16): Evaluation of the accuracy and precision of the proposed procedure of lisinopril dihydrate.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	4.0	100.5	0.53	0.56	4.02±0.0224
	5.0	99.6	0.93	0.97	4.98±0.0485
	6.0	97.8	0.64	0.67	5.87±0.0392
Acid blue 74	4.0	98.5	0.46	0.49	3.94±0.0192
	5.0	99.4	0.87	0.91	4.97±0.0453
	6.0	100.2	0.68	0.71	6.01±0.0429
Acid red 73	4.0	100.3	1.00	1.05	4.01±0.0421
	5.0	99.6	0.99	1.04	4.98±0.0518
	6.0	99.0	0.93	0.97	5.94±0.0577
Acid red 27	4.0	100.8	0.82	0.86	4.03±0.0348
	5.0	97.2	0.80	0.84	4.86±0.0410
	6.0	100.3	0.70	0.73	6.02±0.0441
Acid orange 7	4.0	99.5	0.72	0.75	3.98±0.0300
	5.0	98.2	0.70	0.74	4.91±0.0362
	6.0	98.3	0.64	0.67	5.90±0.0396

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

Table (17): Evaluation of the accuracy and precision of the proposed and official procedures for lisinopril dihydrate in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A1	4.0	99.3	100.4	0.38	2.37	99.6	0.68	1.99	99.8	0.40	1.48	99.9	0.28	1.58	99.7	0.77	1.85
	5.0	98.9	99.5	0.68	2.69	99.4	0.27	1.65	99.5	0.87	2.38	100.6	1.29	2.58	100.1	0.89	1.97
	6.0	99.3	100.3	0.15	1.64	100.1	1.31	2.15	99.7	0.68	1.68	99.8	1.22	2.98	99.7	0.74	1.80
A2	4.0	99.1	99.8	0.39	2.06	100.4	0.38	1.99	99.7	1.11	2.64	99.8	0.87	3.42	99.8	0.92	2.24
B2	4.0	99.2	99.7	0.49	2.42	99.6	0.26	2.37	100.1	0.38	2.31	100.2	0.63	3.08	100.4	0.47	2.27
A2	5.0	98.8	99.1	0.28	1.58	99.9	0.81	3.14	100.2	0.60	2.27	99.4	0.85	2.24	99.9	0.19	1.89
B2	5.0	99.2	100.3	0.83	1.87	100.2	0.18	2.18	99.8	0.81	1.67	99.7	0.55	1.87	99.7	0.44	3.16
A2	6.0	98.6	99.3	0.48	1.88	99.1	0.89	1.72	100.3	0.21	1.20	99.3	0.49	1.57	99.6	0.37	1.47
B2	6.0	99.2	99.5	0.84	2.36	99.8	0.78	1.57	100.2	0.33	2.34	99.6	0.37	2.24	99.2	1.21	2.39
A3	4.0	98.5	99.8	0.19	1.36	100.2	0.49	1.98	99.7	0.91	2.34	100.3	0.28	1.20	99.8	1.81	2.24
B3	4.0	98.8	99.6	0.49	2.42	99.6	0.28	2.38	100.1	0.42	2.11	99.7	0.63	3.08	100.5	0.48	2.17
A3	5.0	99.1	99.5	0.36	2.94	99.9	0.73	3.14	100.2	0.38	2.28	99.4	0.81	2.24	99.8	0.19	1.89
B3	5.0	99.2	100.3	0.82	1.88	99.6	0.18	2.18	99.8	0.78	1.67	100.3	0.55	1.87	99.7	0.47	2.16
A3	6.0	99.0	99.3	0.49	1.88	99.2	0.21	1.12	100.3	1.61	3.07	99.7	0.49	1.57	99.6	0.57	2.47
B3	6.0	98.9	99.5	0.84	2.36	99.8	0.84	1.57	100.2	0.75	2.14	99.6	0.37	2.04	100.3	0.24	1.39

* Dosage forms

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

A1 Lisopril 10 mg/tablet

A2 Sinopril 10 mg/tablet B2 Sinopril 20 mg/tablet

A3 Zestril 5 mg/tablet B3 Zestril 10 mg/tablet

III.6. Absorption spectra of the enalapril maleate with $KMnO_4$ and different dyes

No attempts have been made to develop a spectrophotometric method for determination of enalapril maleate (ENM) by oxidation with potassium permanganate, using five dyes under studies. $KMnO_4$ solution is remarkably stable over prolonged periods, need not be protected from light, and may even be boiled for a short time without appreciable change in concentration; because of its high oxidation potential and excellent solution stability. These methods involve two stages, oxidation of drug by $KMnO_4$ then estimation of unconsumed $KMnO_4$ with the five different dyes (MB, AB, AR, AM, AO) which are preferable because they react with $KMnO_4$ simultaneously however they are used as indicator to estimate ENM. $KMnO_4$ reacts with ENM, resulting in oxidation depending upon the functional group (-NH, -N-) present in ENM, probably a mixture of products, with reproducible data under specified experimental condition. The remaining $KMnO_4$ react with dye to form an oxidative dye (colorless product). The remaining dye is measured spectrophotometrically at their corresponding λ_{max}. The absorption spectra of the reaction products of the method show characteristic λ_{max} value (665, 609, 510, 521 and 484 nm using MB, AB, AR, AM and AO, respectively), as shown in Fig. 31.

III.1.1. Effect of potassium permanganate concentration

The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 1.0×10^{-5} - 1.0×10^{-4} M. The highest result was obtained with 5.0×10^{-4} M; higher concentrations of $KMnO_4$ caused the color to fade. The effect of different experimental variable were studied and recorded below

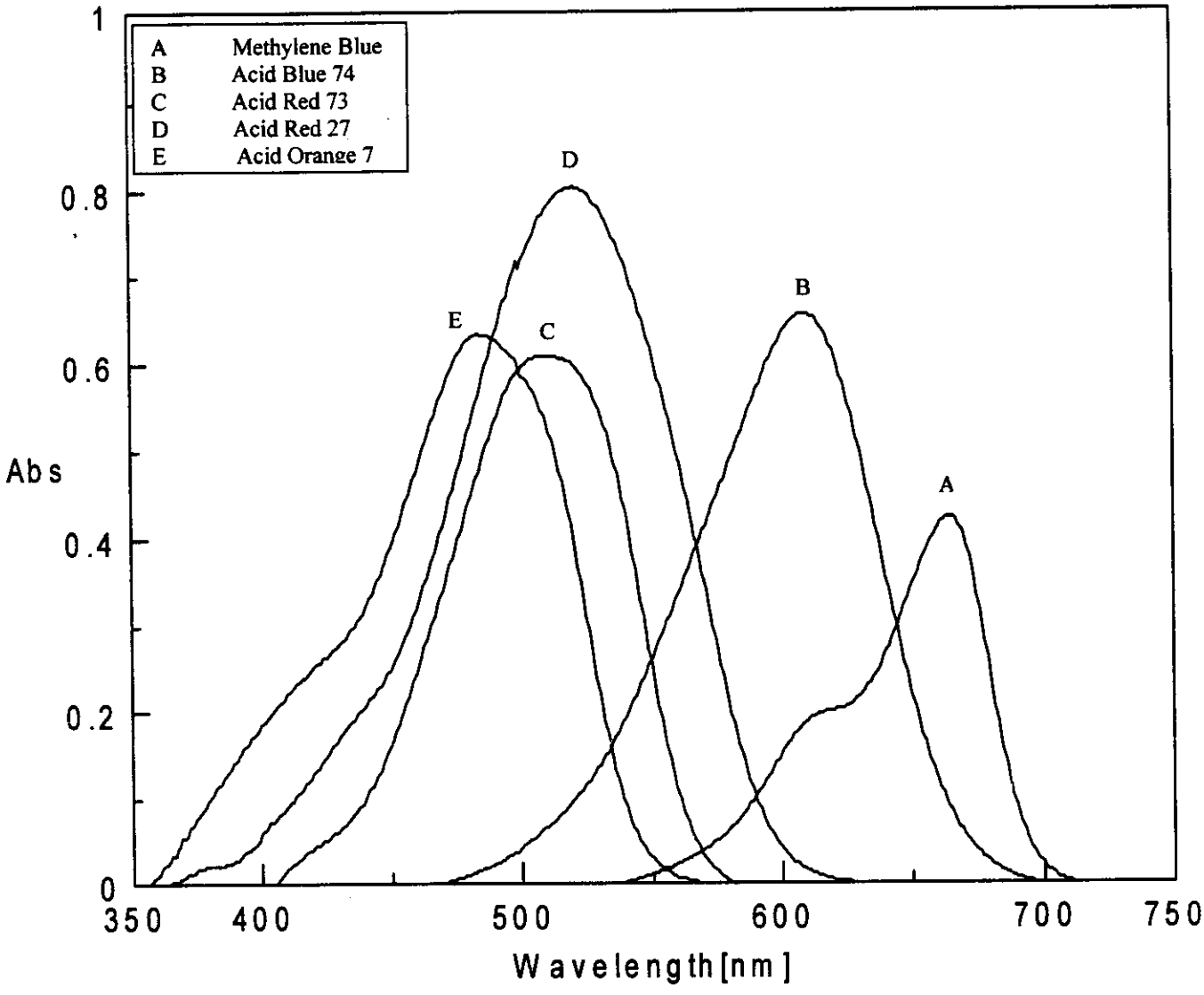


Fig. (31): Absorption spectra for the reaction product of 6.0 μg ml^{-1} of enalapril maleate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.6.2. Effect of acid concentration

Different types of acids were examined (H_2SO_4, H_3PO_4 and CH_3COOH) to achieve maximum yield of redox reaction. The most suitable acid to be used was found H_2SO_4. To each 10 ml measuring flask, 0.6 ml of the ENM (100 μg ml^{-1}) and 1.0 ml of $KMnO_4$ (5.0×10^{-4} M) was added, 1.0 ml of H_2SO_4 (0.2 M) is the optimum concentration of acid, then the solution was diluted to 7.0 ml, after 5.0 min standing time at 60 °C in water bath, the solution was cooled for about 3.0 min, dye was added; then complete to 10 ml total volume as shown in Fig. 32.

III.6.3. Effect of time and temperature

The effect of time on the oxidation process of ENM was investigated by measuring the absorbance of a solution containing 6.0 μg ml^{-1} of the drug, oxidant and acid solution against blank solution prepared by the same way without drug at λ_{max} 665, 609, 510, 521 and 484 nm using MB, AB, AR, AM and AO, respectively, at various time intervals. Also the effect of temperature was studied by heating the sample solution and the blank at different temperature (30-100 °C) in water bath. The reaction took place completely after 5.0 min, and at 40 °C temperature in water bath. Raising the temperature than 40°C did not accelerate the oxidation process. The effect of time after addition of dye was studied by measuring the absorbance at various time intervals (1.0-5.0 min). The absorbance indicated that shaken for 1.0 min, is sufficient to give reliable results. The color remains constant for at least 48 hours and stable until 90 °C in case of all dyes.

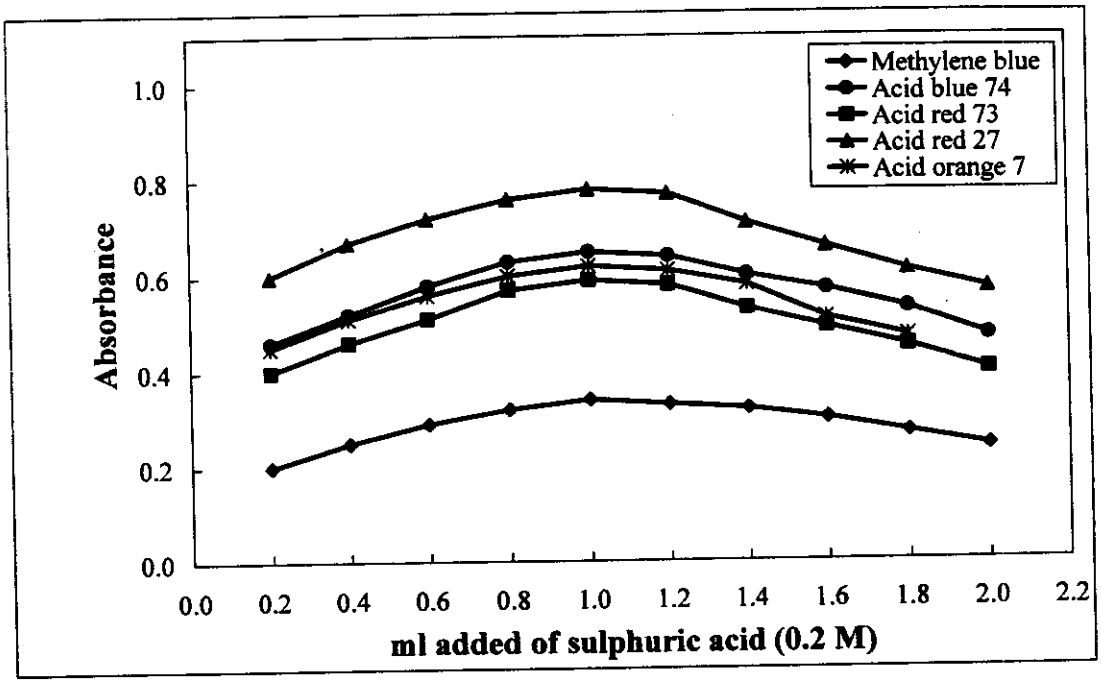


Fig. (32): Effect of ml added of sulphuric acid (0.2 M) on absorbance of 6.0 μg ml^{-1} of enalapril maleate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

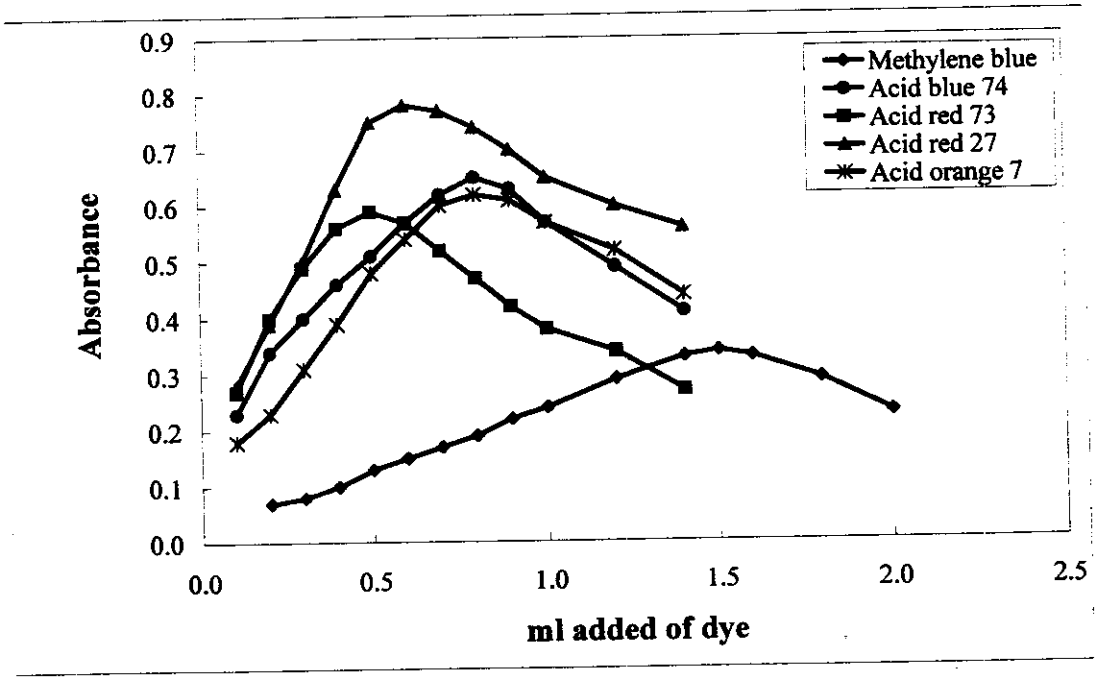


Fig. (33): Effect of ml added of dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M) on absorbance of 6.0 μg ml^{-1} of enalapril maleate with $KMnO_4$ (5.0×10^{-4} M)

III.6.4. Effect of sequence of additions

The effect of sequence of additions on the oxidation process of ENM was studied by measuring the absorbance of solution prepared by different sequence of additions at λ_{max} against a blank solution prepared in the same manner. Experiments showed that (oxidant-acid-drug) gave the best results.

III.6.5. Effect of dye concentration

To establish the optimum concentration of dye, different volumes of the five different dyes were added to the proposed concentration of ENM (6.0 μg ml^{-1}). The optimum volumes used for production of maximum and reproducible color intensity are 1.5 ml (1.0 x 10^{-4} M) MB as shown in Fig. 40, whereas 0.8, 0.5, 0.6 and 0.8 ml (1.0 x 10^{-3} M) for AB, AR, AM and AO, respectively were used as optimum volume as shown in Fig. 33.

III.6.6. Molar ratio method

The molar ratio between oxidant and dye [O]/[Dye] at the selected conditions was carried out, the oxidant was kept constant and variable concentrations of dye (1 x 10^{-4} M MB, 1 x 10^{-3} M for AB, AR, AM and AO) were added and 6.0 μg ml^{-1} of ENM. The absorbance values were then plotted against the molar ratio [O]/[Dye] as shown in Fig. 34. Experimental results showed that the inflection of the two straight lines at 0.33, 0.63, 1.0, 0.83 and 0.63 in case of MB, AB, AR, AM and AO, respectively. Thus the stoichiometric ratio of oxidant to dye are 1.0 : 0.3, 1.0 : 1.59, 1.0 : 1.0, 1.0 : 1.2 and 1.0 : 1.59 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 18.

In order to investigate the molar ratio between ENM and oxidant at the selected conditions, the molar ratio method described by *Yoe* and *Jones*

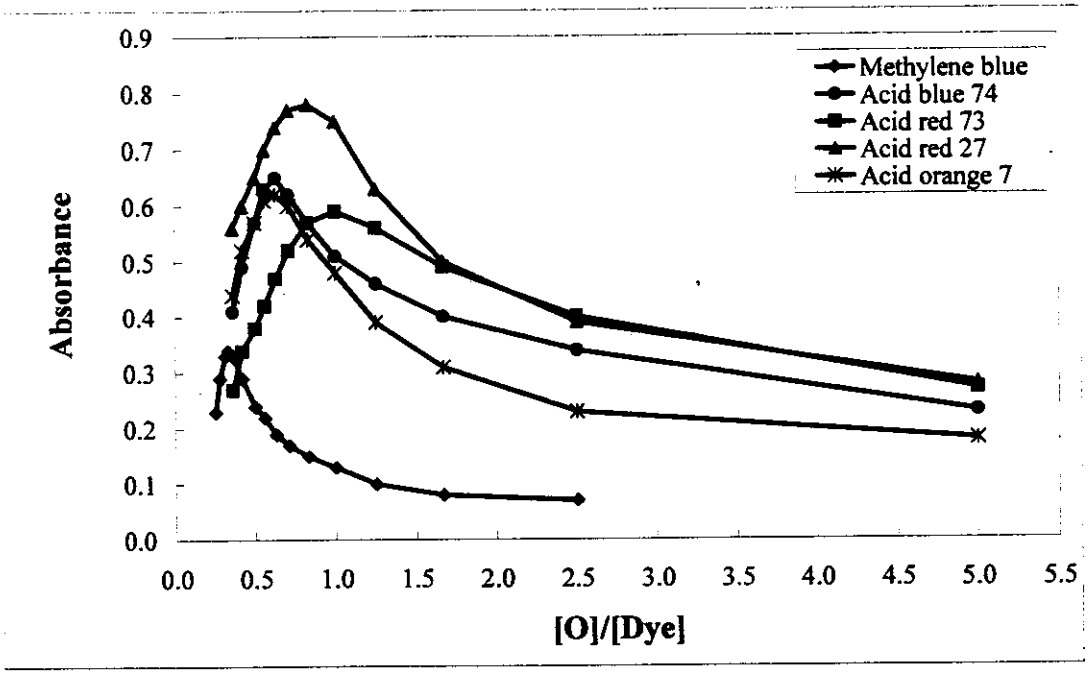


Fig. (34): Molar ratio method [O]/[Dye] for 6.0 μg ml^{-1} of enalapril maleate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

[169] was carried out. In this method 1.0 ml of 5.0 x10^{-4} M $KMnO_4$ is kept constant and variable concentrations (0.1-2.5 ml) of ENM 5.0 x 10^{-4} M were added. The absorbance was measured at λ_{max} (665, 609, 510, 521 and 484 nm using MB, AB, AR, AM and AO, respectively) against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O] as shown in Fig. 35. Experimental results showed that the inflection of the two straight lines at 0.60, 0.60, 0.77, 0.37 and 0.47 in case of MB, AB, AR, AM and AO, respectively. Thus molar ratios of ENM to oxidant are 1.0 : 1.67, 1.0 : 1.67, 1.0 : 1.3, 1.0 : 2.7 and 1.0 : 2.13 in case of MB, AB, AR, AM and AO, respectively as recorded in Table 18.

III.6.7. Validity of Beer's law

Calibration graphs were constructed using standard solution of ENM. Under the optimum conditions, a linear relationship existed between the absorbance and drug concentration. The correlation coefficient, slopes, intercepts, are calculated. For more accurate results, Ringbom optimum concentration ranges were determined by plotting log [D], concentration of the drug in μg ml^{-1}, against percent transmittance. The linear portion of the S-shaped curve gave the accurate range of analysis. The mean molar absorptivities and Sandell sensitivities are calculated from Beer's law as recorded in Table 18, while representative curves on the validity of Beer's law for ENM with the different dyes are shown in Fig. 36. The detection and quantitation limits were calculated from the standard deviation of absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and of quantitation (K =10) were established according to IUPAC definitions [181]

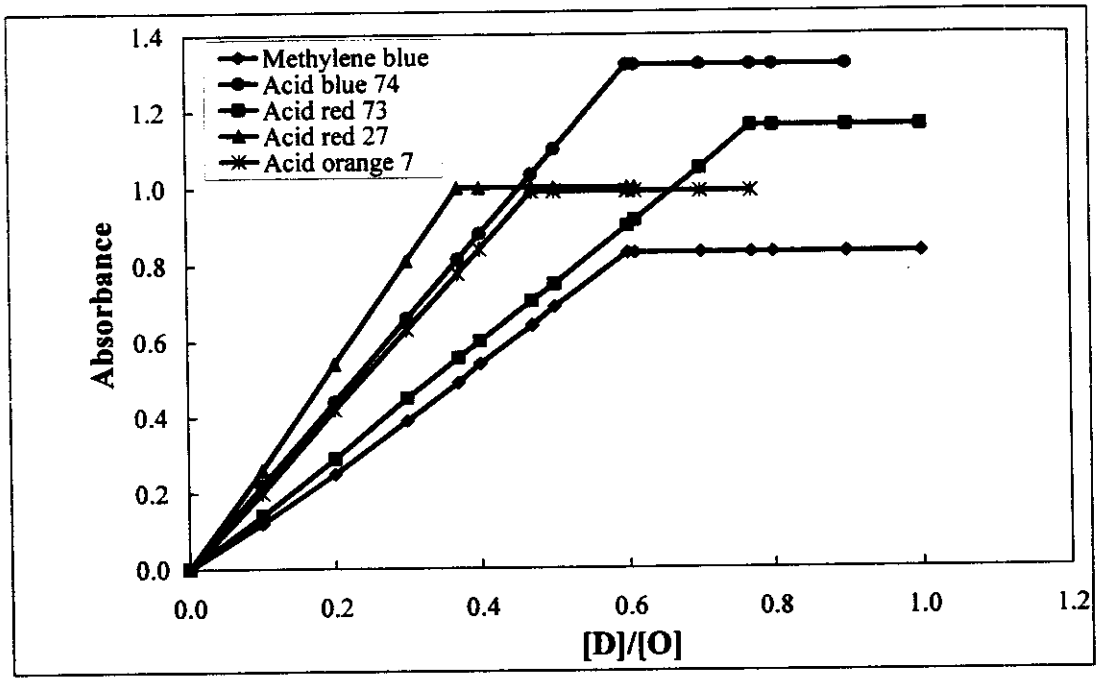


Fig. (35): Molar ratio method for enalapril maleate (5.0×10^{-4} M) using $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

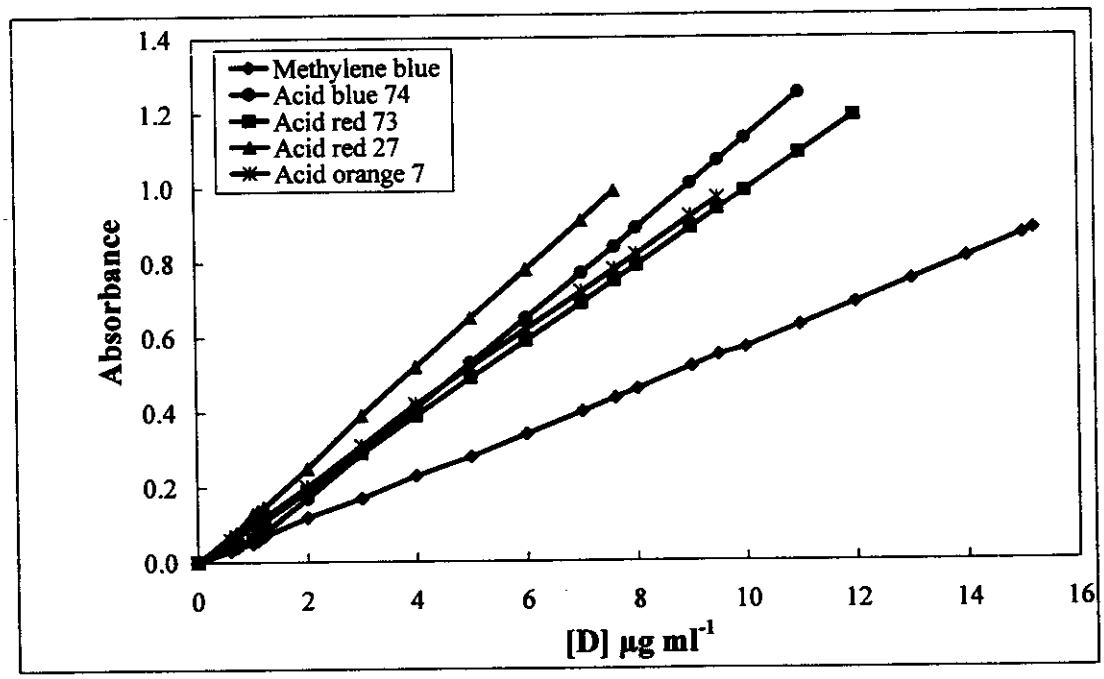


Fig. (36): Validity of Beer's law for reaction product of enalapril maleate with $KMnO_4$ (5.0×10^{-4} M) and dyes (1.0×10^{-3} M) except on using methylene blue (1.0×10^{-4} M)

III.6.8. Accuracy and precision [182]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of ENM were prepared and analyzed in six replicates. The results are summarized in Table 19. The % standard deviations and the % error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

III.6.9. Interferences

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 6.0 μg ml^{-1} of ENM with varying concentration of the additives and excipients such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate and starch. There are interference for glucose, fructose and lactose 2-fold, and there are not interference for other excipients.

III.6.10. Analytical applications

The validity of the proposed procedures is tested by determining ENM in tablets obtained from local manufacturing companies as mentioned before. The concentration of ENM in dosage forms were calculated from the appropriate calibration graphs. There was no shift in absorbance due to the presence of other constituent on the dosage forms. The results are compared with those obtained by applying the official methods [175]. The results obtained were compared statistically by the Student's t-test and variance ratio F-test with those obtained by official methods on the sample of the same batch. The Student's t-test values obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated

value indicating no significant difference between accuracy of the proposed and the official method. Also, the variance ratio F-test obtained at 95% confidence level and five degree of freedom did not exceed the theoretical tabulated value indicating no significant difference between precision of the proposed and the official methods, as shown in Table 20.

Table (18): Optical and regression characteristics of enalapril maleate with different dyes.

Parameters	Methylene blue (Basic blue 9)	Acid blue 74 (Indigo carmine)	Acid red 73 (Brilliant crocein MOO)	Acid red 27 (Amaranth dye)	Acid orange 7 (Orange II)
λ_{max} (nm)	665	609	510	521	484
Bear's law limits ($\mu g\ ml^{-1}$)	0.8-15.2	0.7-11	0.7-12	0.5-7.6	0.6-9.5
Ringbom limits ($\mu g\ ml^{-1}$)	1.0-15	0.9-10.5	0.9-11.2	0.6-7.2	0.8-9.1
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2.82×10^4	5.57×10^4	4.96×10^4	6.49×10^4	5.09×10^4
Sandell sensitivity ($ng\ cm^{-2}$)	17.48	8.85	9.93	7.59	9.68
Detection limits ($\mu g\ ml^{-1}$)	0.215	0.186	0.197	0.138	0.147
Quantitation limits ($\mu g\ ml^{-1}$)	0.717	0.621	0.658	0.460	0.489
Regression equation* : . Slope (b)	0.0572	0.113	0.1007	0.1317	0.1033
Intercept (a)	- 0.0082	- 0.0454	- 0.0166	- 0.0098	- 0.0006
Correlation coefficient (r)	0.9999	0.9994	0.9998	0.9998	0.9995
RSD** %	0.88	0.71	0.58	0.44	0.61
Stoichiometric ratio [O]/[Dye]	1.0 : 0.3	1.0 : 1.59	1.0 : 1.0	1.0 : 1.2	1.0 : 1.59
Stoichiometric ratio [D]/[O]	1.0 : 1.67	1.0 : 1.67	1.0 : 1.3	1.0 : 2.7	1.0 : 2.13

* With respect to A = a + b C where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.
** Relative standard deviation for six determinations

Table (19): Evaluation of the accuracy and precision of the proposed procedure of enalapril maleate.

Dye	Taken µg ml^{-1}	Recovery %	RSD[a] %	RE[b] %	Confidence limits[c]
Methylene blue	5.0	99.2	0.91	0.95	4.96±0.0472
	6.0	100.3	0.98	1.03	6.02±0.0619
	7.0	98.7	0.58	0.61	6.91±0.0420
Acid blue 74	5.0	100.2	0.82	0.86	5.01±0.0430
	6.0	100.8	0.80	0.83	6.05±0.0505
	7.0	97.4	0.75	0.78	6.82±0.0535
Acid red 73	5.0	98.6	0.45	0.47	4.93±0.0230
	6.0	99.7	0.52	0.54	5.98±0.0325
	7.0	100.1	0.57	0.60	7.01±0.0420
Acid red 27	5.0	100.6	0.38	0.40	5.03±0.0200
	6.0	99.2	0.49	0.51	5.95±0.0304
	7.0	97.1	0.54	0.57	6.80±0.0390
Acid orange 7	5.0	97.6	0.59	0.62	4.88±0.0304
	6.0	98.8	0.69	0.73	5.93±0.0430
	7.0	100.4	0.43	0.45	7.03±0.0315

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

Table (20): Evaluation of the accuracy and precision of the proposed and official procedures for enalapril maleate in dosage forms.

Official method			Proposed methods														
			Methylene blue			Acid blue 74			Acid red 73			Acid red 27			Acid orange 7		
*	Taken µg ml^{-1}	Recovery %	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio	Recovery %	t-* value	F-* ratio
A1	5.0	99.3	99.8	0.29	1.36	100.5	0.59	1.99	99.7	0.81	2.34	100.3	0.28	1.20	99.8	1.85	2.19
B1	5.0	98.7	100.2	0.48	2.43	99.6	0.28	2.48	99.8	0.42	2.11	101.0	0.63	3.08	100.4	0.48	1.17
A1	6.0	98.9	99.6	0.37	2.94	99.9	0.73	2.14	100.3	0.38	2.28	99.4	0.81	2.24	99.8	0.19	1.89
B1	6.0	99.2	100.3	0.72	1.78	99.6	0.18	1.18	99.8	0.78	1.67	100.3	0.55	1.87	99.7	0.47	2.16
A1	7.0	99.3	99.7	0.48	1.88	99.2	0.21	1.12	100.3	1.65	2.07	99.8	0.49	1.57	99.6	0.57	2.47
B1	7.0	99.2	99.5	0.84	2.36	99.8	0.84	1.57	99.6	0.75	2.14	99.6	0.37	2.04	100.3	0.34	1.49
A2	5.0	98.9	99.5	0.78	1.59	99.8	0.49	1.71	99.4	0.97	1.89	99.8	0.49	1.77	100.1	0.64	1.58
	6.0	99.4	99.6	0.29	1.65	100.4	0.54	1.97	99.3	0.28	1.71	99.7	0.54	1.94	99.5	0.84	2.39
	7.0	99.1	100.1	0.92	2.15	100.2	0.89	2.84	100.4	1.31	2.28	100.2	0.87	2.74	100.2	0.59	1.37
A3	5.0	99.1	100.7	0.75	1.85	99.8	0.55	1.54	99.5	0.45	1.33	99.8	0.64	1.88	100.2	0.92	1.79
	6.0	98.7	99.2	0.86	2.34	100.4	0.34	1.46	99.4	0.64	2.47	99.7	0.24	1.64	99.3	0.19	1.71
	7.0	99.2	100.1	0.68	2.14	99.5	0.84	2.65	100.2	0.52	1.64	100.5	0.47	1.35	100.4	1.31	3.18

* Dosage forms
* Theoretical values for t- and F- values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.
A1 Enalapril maleate 10 mg/tablet B1 Enalapril maleate 20 mg/tablet
A2 Acapril 5 mg/tablet
A3 Ezapril 10 mg/tablet

III.7. Potentiometric titrations

The potentiometric techniques have been extensively used in many branches of solution chemistry. Potentiometry is by far the most accurate and widely applicable technique currently available for the study of ionic equilibrium. The potential can arises from two types of phenomena:

i) Oxidation-reduction equilibrium.

ii) The formation of ionic concentration gradients across membranes.

If the reversible electron-transfer reaction:

$$x\,X + y\,Y + \cdots\cdots + Ze \rightleftharpoons pP + q\,Q + \cdots\cdots$$

can occur, the potential acquired by contact with an equilibrium mixture of P, Q, ..., X, Y ..... is given by Nernest equation:

$$E = E^{o} + \frac{RT}{ZF} \ln \frac{[X]^{x}[Y]^{y}}{[P]^{p}[Q]^{q}}$$

Where the standard potential, E^{o}, is the potential acquired when all species are at unit activity. The electrode may be either, as in the so-called redox system or itself composed of one of the participating species.

The electrode used in pH-metric titration is the glass electrode, which can be used to determine the hydrogen ion concentration (h) in solutions of constant ionic medium, which contain an excess of sodium ions. Also many types of glass electrodes are available commercially; the active membrane is in form of a fixed acidity, in contact with a reference electrode, usually calomel electrode, if the titrations are carried out in aqueous solutions. The whole electrode system may be represented as reference electrode, **[RE/H^{+} fixed/glass membrane (GE)]**.

The potential of the half-cell H^+/GE at a constant ionic medium is given by:

$$E = E^{o} + \frac{RT}{F} \ln a_{H^+} \quad \text{----------} \quad (1)$$

Where the standard electrode potential, E^{o}, is the potential acquired when all species are at unit activity. It depends on the pH of the internal solution and on the potential of the reference half-cell. The range of hydrogen ion concentration over which equation (1) is valid for particular electrode depends on the types of the used electrode and the state of hydration.

Measurements of the free hydrogen ion concentration of solutions of different analytical composition may be used for studying a number of types of equilibria involving protons, thus for the system H, L the average number of hydrogen ions bounded to the free L can be expressed by the following equation:

$$n_H = \frac{H\text{-}h + [OH]}{L}$$

Where H is the total ion concentration of dissociable hydrogen, and OH; may usually be neglected in solution of pH < 7.

The proton-drug stability constant K^H was evaluated by *Calvin and Willson* [183].

The average number of protons associated with the drug molecule, $\bar{n}_A$, was determined at different pH values using the following equation:

$$\bar{n}_A = Y + \frac{(v_1 - v_2)(N^{o} + E^{o})}{(v^{o} + v_1) TC_L^{\,o}} \quad \text{----------} \quad (2)$$

where v_1 and v_2 are the volumes of alkali required to reach the same pH in the titration curves of hydrochloric acid and the drug, respectively, v^o is the initial volume (50 ml) of the mixture, TC_L^o is the total concentration of the drug, Y is the total number of dissociable protons attached with the drug, N^o is the normality of sodium hydroxide solution and E^o is the initial concentration of the free acid. The values of $\bar{n}_A$ are plotted *vs.* pH and the values of proton-drug stability constant K^H was calculated by interpolation at half $\bar{n}_A$ values.

The pH-metric titration is performed firstly with the acid mixture which consisting of 5.0 ml of 0.01 M hydrochloric acid and 5.0 ml of 1.0 M potassium chloride, then completed to 50 ml with the appropriate volume of bidistilled water and ethanol to achieve 40% (v/v) ethanolic aqueous solution (mixture A)

The second mixture contains the volume of hydrochloric acid and potassium chloride used in mixture (A) and 5.0 ml of 1.0×10^{-2} M of drug solution, then completed to 50 ml with the appropriate volume of bidistilled water and ethanol to achieve 40% (v/v) ethanolic aqueous solution (mixture B)

Each mixture was separately titrated against 0.05 M of 40% (v/v) ethanolic sodium hydroxide free-carbonate and the potentiometric titration curves obtained are shown in Fig. 35. These curves are S-shaped with a sharp jump. Potentiometric titration showed that, the curves of the titration of drugs, appears to have a lower pH value (lower potential) than that of the acid mixture.

The average number of the protons associated with drug, $\bar{n}_A$ is calculated using equation (2). The plots of $\bar{n}_A$ against pH of solution give the proton-drug dissociation shown in Fig. 37. The proton dissociation constants are obtained by the interpolation at half $\bar{n}_A$ values for the

ionization -COOH, as in CAP, LIS and ENM, $-SO_3H$, as in ADB, -OH, as in ATL and –NH, as in LIS and ENM. The obtained results indicated that four dissociation constants appeared for LIS $pK_1 = 2.5$, $pK_2 = 4.1$, $pK_3 = 6.67$, $pK_4 = 10.11$ (2.5, 4.0, 6.7 and 10.1) [184], two dissociation constants appeared for ENM $pK_1 = 3.0$, $pK_2 = 5.34$ (3.0 and 5.4) [185] and one dissociation constants appeared for CAP $pK_1 = 3.66$ (3.7) [7], ADB $pK_1 = 4.1$ and ATL $pK_1 = 9.55$ (9.6) [121].

The free energy change ΔG for such dissociation was calculated using the following relationship.

$$\Delta G = - 2.303\ RT \log K^H = 2.303\ RT\ pK^H$$

Where T is the absolute temperature in Kelvin, $R=8.3 \times 10^{-3}$ k J mol^{-1} deg^{-1} and ΔG in k J mol^{-1}.

The free energy change ΔG for LIS is 19.02, 31.19, 50.75 and 76.92 k J mol^{-1}, 22.82 and 40.63 for ENM, 27.61 for CAP, 31.19 for ADB and 72.66 k J mol^{-1} for ATL.

The positive values of ΔG reveal that the dissociation of these drugs is not spontaneous.

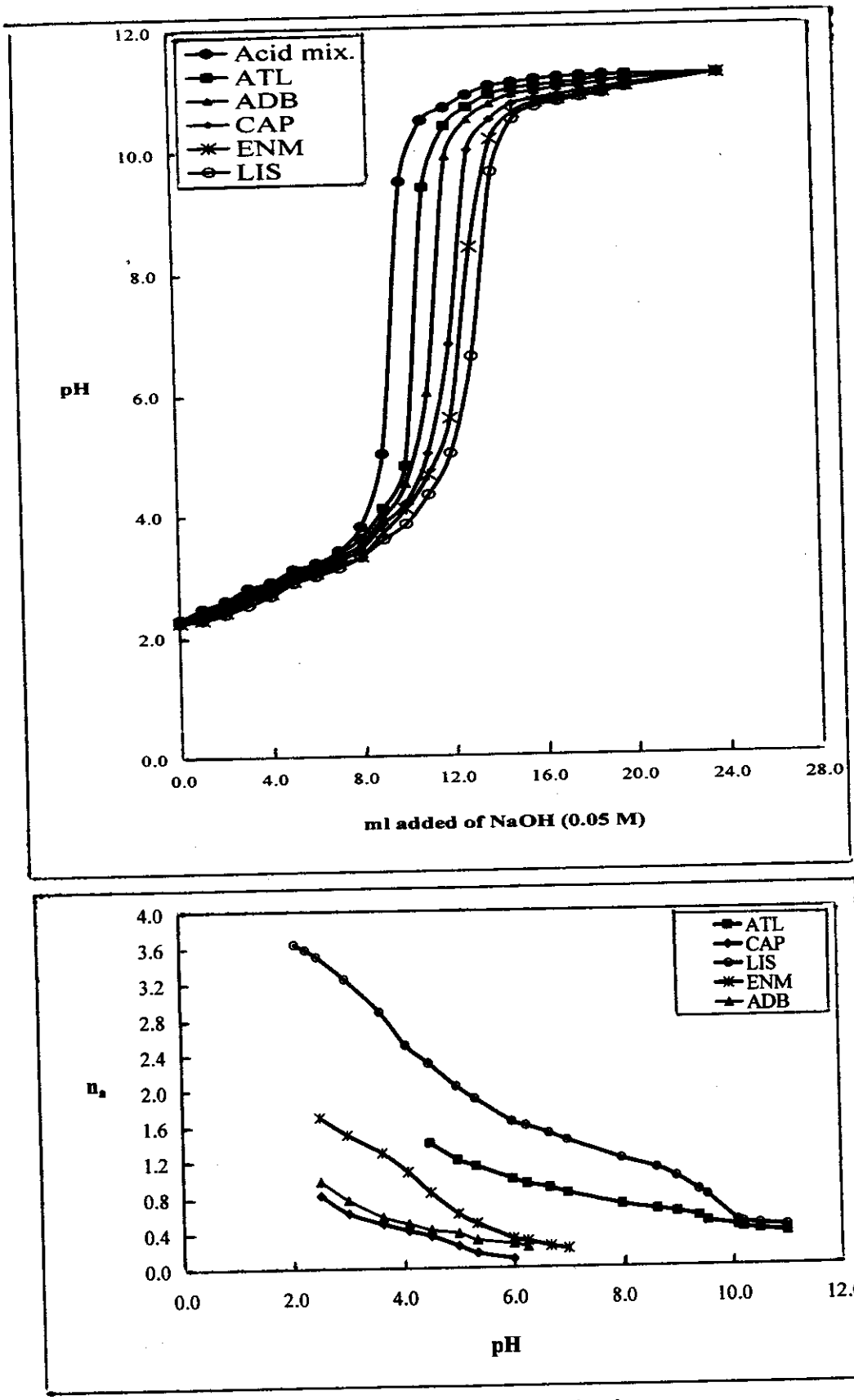


Fig. (37): Potentiometric titration of drugs under investigation