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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF TERBINAFINE HCL, TELMISARTAN ANDRAMIPRIL THROUGH REDOX REACTIONS USING BROMATE-BROMIDEMIXTURE

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ABSTRACT

Two visible spectrophotometric methods (A,B) were developed for the analysis of some drugs, namely Terbinafine HCl, Telmisartan and Ramipril based on their reactivity with bromine, generated in situ by the action of the acid on bromate–bromide mixturefollowed by the determination of unreacted bromine by reacting with a fixed amount of either methylene blue (MB) and measuring the absorbance at 678 nm for Terbinafine HCl and Ramipril and at 668 nm for Telmisartan(method A) or methyl red (MR) and measuring the absorbance at 517 nm (method B). Beer's law is valid within the concentration ranges of 1-3, 2-18 and 28-68µg/ml for Terbinafine HCl, Telmisartan and Ramipril, respectively (method A) and the concentration ranges of 0.5-2.5 and0.3-3.6 µg/ml for Terbinafine HCl and Telmisartan, respectively(method B). Correlationcoefficients, limits of detection and quantification are also reported. Recovery results were statistically compared with those of areference method by applying Student's t- and *F*-test.

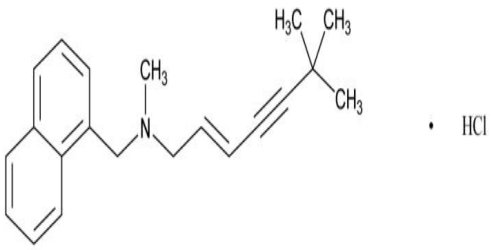
Keywords: Spectrophotometry, redox reactions, bromate–bromide mixture, anti-fungal.

INTRODUCTION

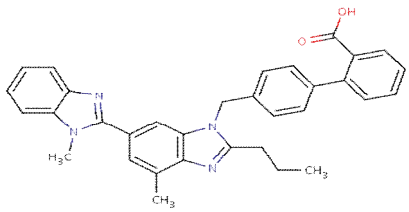
Terbinafine hydrochloride, (TH) is 1-naphthalenemethanamine, n-(6, 6-dimethyl-2-hepten-4-ynyl)-n methyl-, (E)-, hydrochloride (Figure 1). TH is a new potent antifungal agent. It belongs to an allyl amine class and has broad-spectrum activity against yeasts, dimorphic fungi, molds and dermatophytes[1-3].Literature survey shows several HPTLC[4-6], non-aqueous voltametric[7], spectrometric methods[8-12] and ion-pair RP chromatography[13] have been used for assay of TH in raw material and dosage forms. Only stability-indicating HPTLC[12, 13] method is reported for determination of the drug. Spectrophotometric[9] and chromatographic[14, 15] methods estimates TH in presence of its degradant or metabolites. Also TH has been determined in biological fluids (plasma, urine) tissues, nails and cat hair by HPLC[16-18] and in tablets and creams by HPLC[19, 20].

Telmisartan, (TEL) is 4'-[(1, 4'-dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1, 1'-biphenyl]-2-carboxylic acid (Figure 1). It is indicated in the treatment of essential hypertension. Several methods were reported for determination of Telmisartan either alone or in combination with other drugs. These methods include,Spectrophotometry[21-27] involving UV first order derivative Spectrophotometry[21] and chromatographic methods[28-34] have been reported for determination of Telmisartan alone and in combinationwith other drugs, involving RP-HPLC for determination of Telmisartan in combination with other drugs[28-30], determination of Telmisartan and forced degradation behaviour by RP-HPLC[31],in human plasma using liquid chromatography tandem mass spectrometry[32], simultaneous estimation of Telmisartan using HPTLC method[33] and first-derivative, ratio derivative spectrophotometry, TLC-densitometry and spectrofluorimetry[34] methods.

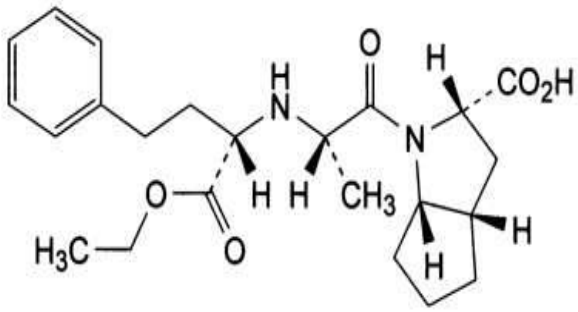
Ramipril, (RP)is [(2S, 3aS, 6aS)-1-[(S)-2- [[(S)-1-(ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl] octa- hydrocyclopenta [b] pyrrole-2-carboxylicacid (Figure 1). RP isan angiotensin-converting enzyme inhibitor(ACEI),which is widely used in the treatment of hypertension and congestive heart failure. RP plays an important role in inhibiting the conversion of the inactive angiotensin I to the active angiotensin II[35-37]. Several methods were reported for determination of Ramipril either alone or in combination with other drugs.These methods include,Spectrophotometric methods[38-63], involving voltammetry[58, 59] potentiometry[60], polarigraphic method[61], Flow-injection analysis[62] and conductometry[63] and chromatographic methods havebeen reported for determination ofRP in pharmaceutical preparations or in biological fluids by HPLC either applying UV detection[64-70] or mass spectrometric detection[71-73]. Also UHPLC method has been reported[74] and capillary electrophoresis methods have been applied for determination of ramipril[75,76]. In addition, HPTLC methods[77,78] and also a stability-indicating HPLC methods have been applied for determination of ramipril in the prescence of its degradation products [79-81].



Terbinafine hydrochloride, TH



Telmisartan, TEL



Ramipril, RP

Fig. 1: Chemical structure of TH, TEL and RP

Experimental

I. Apparatus

Spectrophotometer: SHIMADZU UV-1800 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm min^{-1}.

II. MATERIALS AND REAGENTS

All reagents were of analytical grade and distilled water was used.

1. Terbinafine HCl (Novartis, Egypt).
2. Telmisartan (Boehringer, Egypt).
3. Ramipril (Aventis, Egypt).
4. Bromate-bromide mixture

A stock standard solution of bromated-bromide solution equivalent to 100 µg/ml $KBrO_3$ and 10-foldexcess of KBr was prepared by dissolving accurately weighed 10 mg of $KBrO_3$ and 100 mg of KBr indistilled water and diluting to 100 ml in a calibrated flask.

5. Methylene blue (0.02%)

The solution was prepared by dissolving 0.01 gm of dye (Fluka, Switzerland) in distilled water then diluted by distilled water in a50ml calibrated flask.

6. Methyl red (100 µg/ml)

0.1gm of methyl red dye (Fluka, Switzerland) dissolved in 1 ml 4.5 M NaOH then diluted to 100 ml by distilled water to prepare (0.1%) solution, then take 10 ml from this solution acidify with 1 ml 4.5 M H_2SO_4 then diluted by distilled water in a100ml calibrated flask to prepare (0.01%) dye solution.

7. Hydrochloric Acid (1M, 1.5M and 2M).

The solutions were prepared by appropriate dilution of concentrated hydrochloric acid (sp. gr. 1.18, 37%) with water.

III. Standard drug solutions:

1. Terbinafine HCl stock solution 100 μgml^{-1}was prepared by dissolving 0.01 gm in 100ml distilled water. This stock solution used as working standard solution for method (A), for method (B)make dilution with distilled water to obtain working standard solution of concentration 50 μgml^{-1}.
2. Telmisartan stock solution 200 μgml^{-1}wasprepared by dissolving 0.01 gm in 0.5 ml 1M HCl then complete to 50 ml with distilled water. This stock solution used as working standard solution for method (A),for method (B)make dilution with distilled water to obtain working standard solution of concentration 30μg ml^{-1}.
3. Ramipril working standard solution 400 μgml^{-1} wasprepared by dissolving 0.02 gm in 0.5ml 2M HCl then complete to 100 ml with distilled water for method (A).

IV. Pharmaceutical preparations:

1. Lamisil tablets (Novartis, Egypt), labelled to contain 25mg Terbinafine hydrochloride per tablet.
2. Micardis tablets (Boehringer, Egypt), labelled to contain 8 mg Telmisartan per tablet.
3. Tritace protect tablets (Sanofiaventis, Egypt), labelled to contain 10 mg Ramipril per tablet.

V. General procedures

1- Construction of calibration curves

a. Spectrophotometric procedure using Methylene blue

To different aliquots of standard solutions containing (1-3), (2-18) and (28-68) μgml^{-1} of Terbinafine HCl, Telmisartan and Ramipril, respectively.For Terbinafine HCl,acidify using 1.9 ml 1 M HCl, add0.7 ml of 0.07 % w/v bromate working solution, stand for 5 minutes, add 1 ml 0.005M dye then stand for 35 minutes. For Telmisartan, acidify using 1.6 ml 1.5 M HCl, add 3 ml of 0.04 % w/v bromate working solution, the mixture heatedin a boiling water bath for 5 minutes, add 1 ml 0.01M dye then stand for 10 minutes.For Ramipril, 1.3 ml of 0.06 % w/v bromate working solution is added directly without addition of HCl because the drug dissolved in 0.5 ml 2M HCl and this volume is enough for the reaction, stand for 15 minutes, add 1 ml 0.01M dye then stand for 25 minutes then the volumes were made up to 10 ml mark with distilled water then measure absorbance against reagent blanksimilarly preparedat λ_{max} 678 nm for Terbinafine HCl and Ramipril and at 668nm for Telmisartan.

b. Spectrophotometric procedure using Methyl red

To different aliquots of standard solutions containing (0.5-2.5), (0.3-3.6) μg/ml for Terbinafine HCl and Telmisartan, respectively. Terbinafine HCl,1.2ml of 0.01 % w/v bromate working solution,acidify using 1 ml 1.5 M HCl, stand for 20 minutes, add 1 ml 0.015M dye. For Telmisartan,1ml of 0.04 % w/v bromate working solution,acidify using 1 ml 0.3 M HCl, the mixture heated in a boiling water bath for 5 minutes, add 2.3 ml 0.01M dye, stand for 5 minutes then the volumes were made up to 10 ml mark with distilled water then measure absorbance against reagent blanksimilarly prepared at λ_{max} 517 nm for Terbinafine HCl and Telmisartan.

In the stated methods: calibration graphswere prepared byplotting absorbance versus concentration of drug and theconcentration of the unknown was computed from theregression equation derived from the Beer's law data.

2. Pharmaceutical preparations

1. Lamisil® tablets

Ten tablets weighed and powdered. A quantity of powdered tablets equivalent to 10 mg Terbinafine HCl was shaken with distilled water then filtered and diluted to 100 ml with distilled water to obtain working solution of concentration 100 μgml^{-1}.

2. Micardis® tablets

Ten tablets weighed and powdered. A quantity of powdered tablets equivalent to10 mg of Telmisartan was shaken with cold water for 2 min, to dissolve sorbitol, filtered,washed with 20 ml distilled water and the precipitate was transferred from the filter paper into 50 ml volumetric flask with 0.5 ml of1M HCl then filtered and completed to the mark with distilled waterto obtain working solution of concentration 200 μgml^{-1}, method (A), Further dilution was made to obtain working solution of concentration 30 μgml^{-1}, method (B).

3. Tritace Protect® tablets

Ten tablets were powdered and an accurately weighed amount equivalent to 10mg Ramipril was shaken with 0.4 ml of 1 M HCl and10 ml distilled water then filtered and diluted to 25ml with distilled water to obtain working solution of concentration 400 μgml^{-1}.

Standard addition technique was used for analysis of the selected drugs in their commercial tablets.

VI. RESULTS AND DISCUSSION

The proposed spectrophotometric methods based on the determination of the unreacted bromine(insitu generated) after allowing the reaction between eachdrug and a measured amount of bromine to be complete.The unreacted bromine was determined by measuring the decrease in absorbance of methylene blue or methyl red dyes at the suitable λ_{max} 517 nm. The methods depend on the bleaching action of bromineon the dyes, the discoloration being caused by the oxidativedestruction of the dyes. Terbinafine HCl, Telmisartan or Ramipril when added in increasing amounts to a fixed amount ofinsitu generated bromine, consumes the latterproportionately and there occurs fall in theconcentaration of bromine. When a fixed amount of dye isadded to the decreasing amounts of bromine, anincrease in the concentration of dye results. Consequently,a proportional increase in the absorbance at the respectiveλmaxis observed with increasing concentration of eachdrug.The insitu generation of bromine is carried out using a mixture of potassium bromide and potassium bromate inpresence of HCl according to the following equation:

$$5Br^- + BrO_3^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

VI.1. Absorption spectra

The resulting absorption spectra are due to the blue colour of residual unoxidized methylene blue at 678 nm for Terbinafine HCl and Ramipril and at 668 nm for Telmisartan (Fig.2) or red colour of residual unoxidized methyl redat 517 nm (Fig.3).

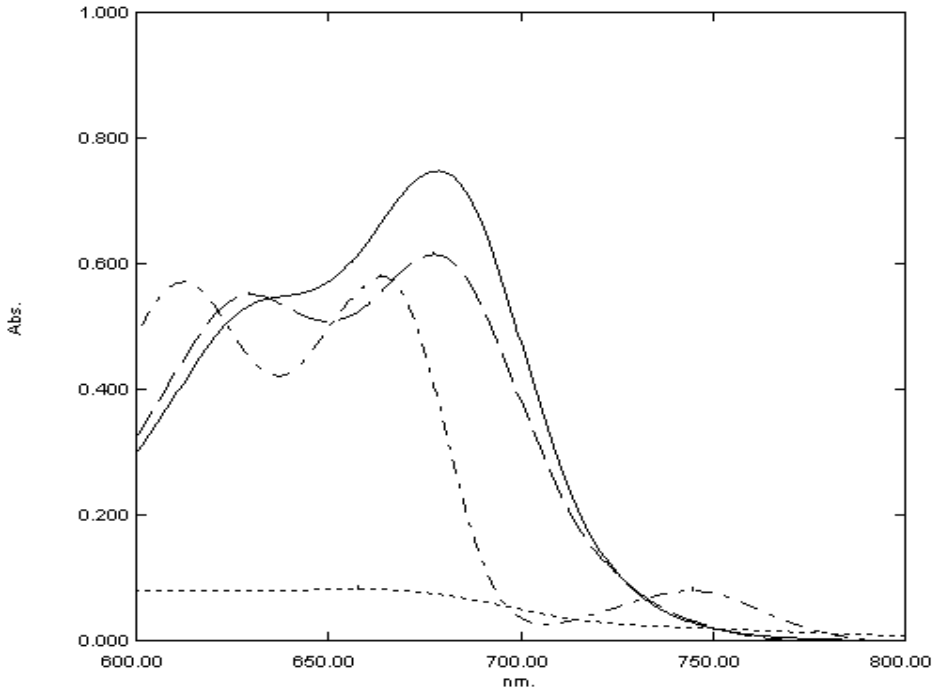


Fig. 2: Absorption spectra of the reaction products of Bromated and methylene blue dye with:

- **2.6µg/ml Terbinafine HCl (—)**
- **4.6µg/ml Ramipril (— —),**
- **8µg/ml Telmisartan (– - –) and blank (.....).**

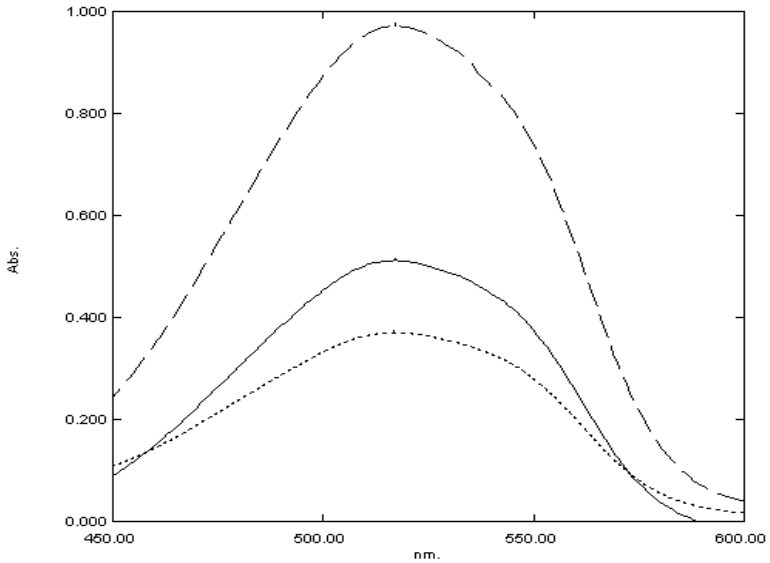


Fig. 3: Absorption spectra of the reaction products of bromated and methyl red dye with:

- **1.5µg/ml Terbinafine HCl (__)**
- **3.6 µg/ml Telmisartan (__ __) and blank (.....).**

Methylene blue exhibited two types of absorption spectraaccording to different volumes of hydrochloric acid used. Inlow HCl concentration (0.5 ml 5M HCl and complete to 10ml) it exhibits peak maximum at 666 nm and in high HClconcentration (2 ml 5M HCl and complete to 10 ml) itexhibits peak maximum at 745 nm. Detailed investigation regarding different forms of methylene blue was carried out[82] explaining the reasons for variation in absorptionmaximum at different acidities. Fig4.

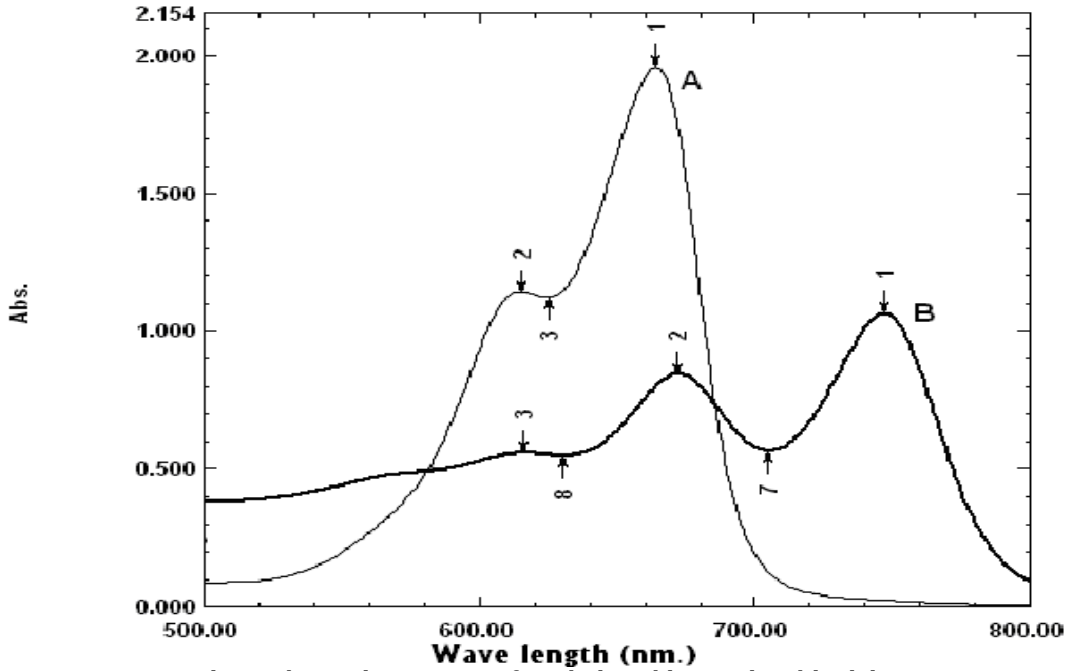


Fig. 4: Absorption spectra of methylene blue against blank in weak acidic medium (A) and in strong acidic medium (B)[82]

VI.2. Effect of dye concentration

Experiments were performed to found appropriate dyeconcentrations by stabilizing other experimental conditionsand using different volumes of dyes and results illustratedthat increasing dye volume above 1ml (0.005 M) in case ofTerbinafine HCl does not result in change ofabsorption while 1 ml (0.01 M) were sufficient in case of Telmisartan and Ramipril. (Fig. 5a, 5band 6a, 6b)

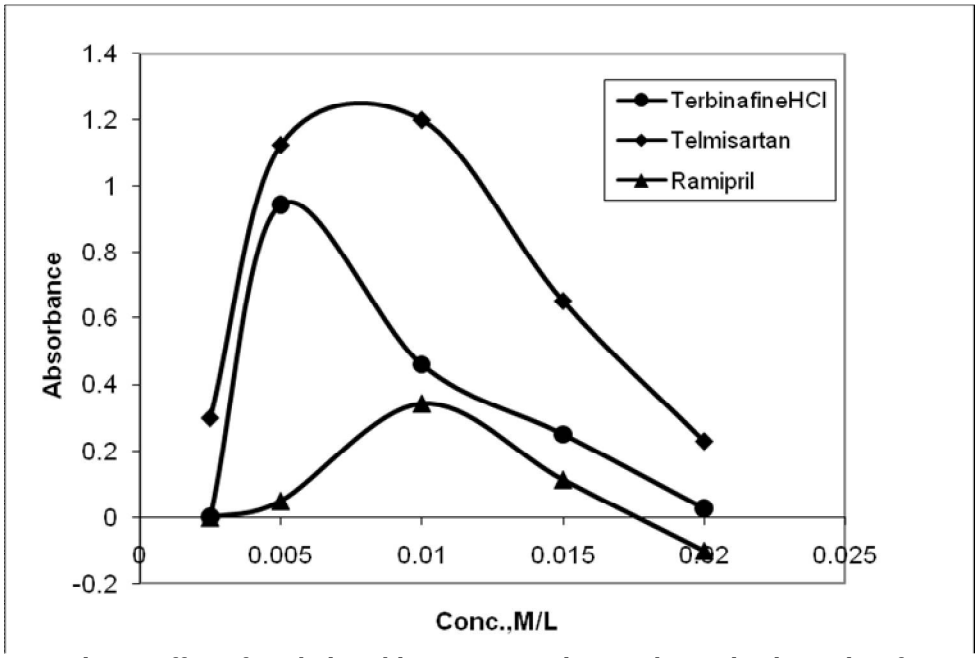


Fig. 5a: Effect of methylene blue concentration on absorption intensity of:

- **2 µgml^{-1}TerbinafineHCl**
- **18 µgml^{-1}Telmisartan**
- **36 µgml^{-1}Ramipril**

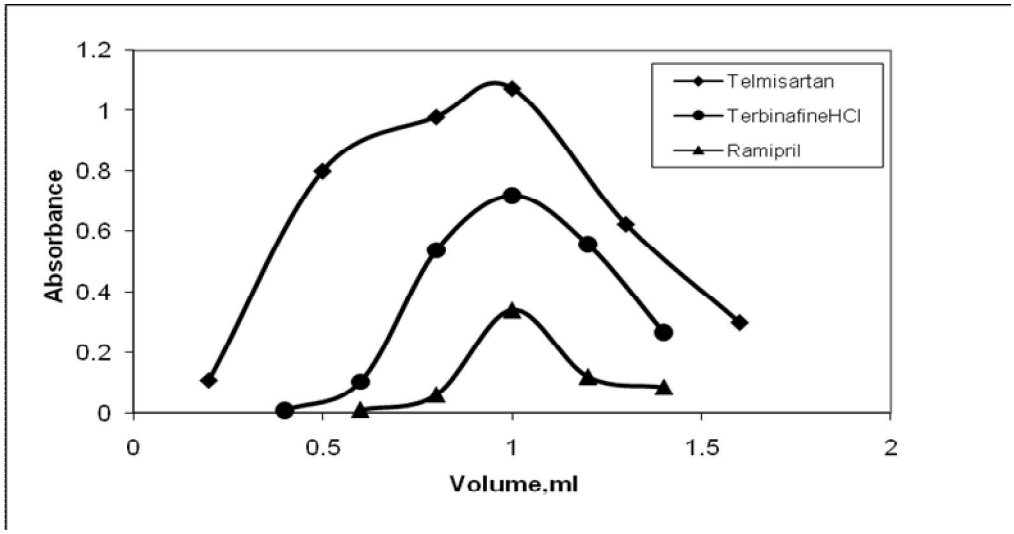


Fig. 5b: Effect of volumeof methylene blue on absorption intensity of:

- **2 µgml^{-1}Terbinafine HCl**
- **18 µgml^{-1}Telmisartan**
- **36 µgml^{-1}Ramipril**

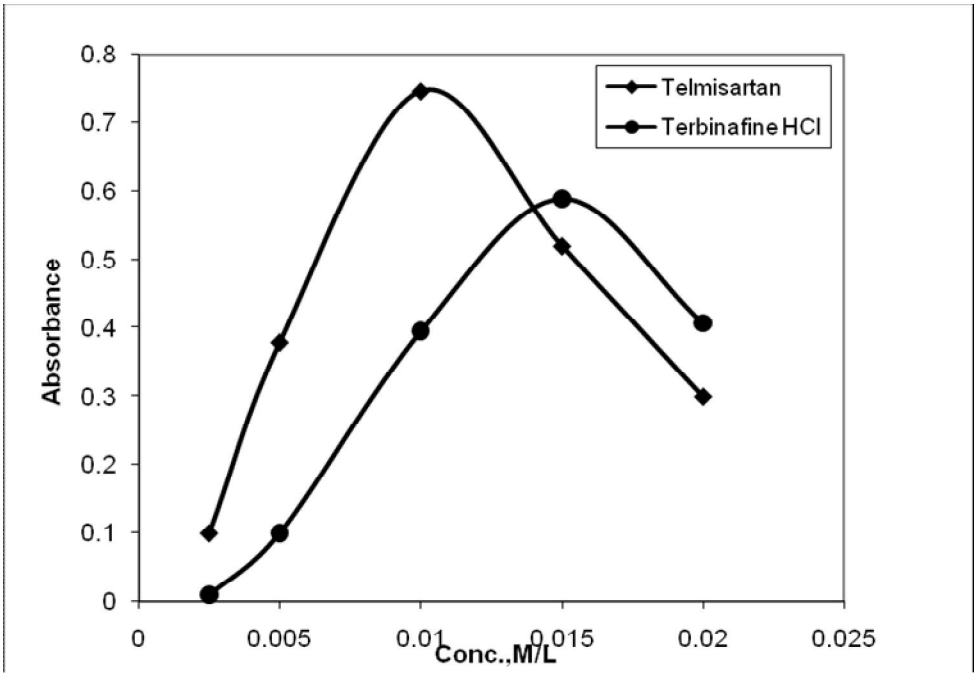


Fig. 6a: Effect of methyl red concentration on absorption intensity of:
- **1.5 µgml-1TerbinafineHCl**
- **3.6 µgml-1Telmisartan**

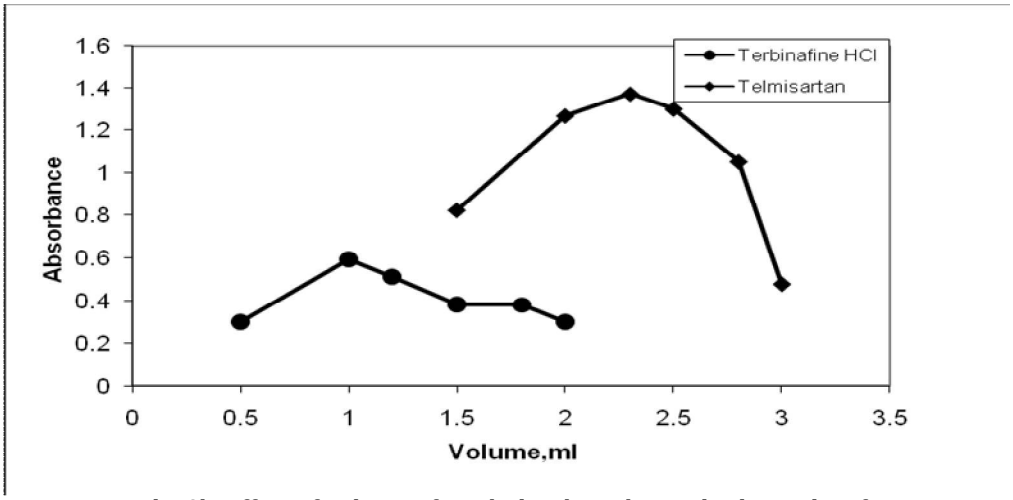


Fig. 6b: Effect of volume of methyl red on absorptionintensity of:
- **1.5 µgml-1Terbinafine HCl**
- **3.6 µgml-1Telmisartan**

VI.3. Effect of Acidity

Different acids were tested as a medium for brominegeneration including sulphuric acid, hydrochloric acid, nitric acid and phosphoric acid. Hydrochloric acid produced the most precise and accurate results. Therefore, in case of method (A), it wasfound that 1.9 ml 1 M HCl, 1.6 ml 1.5 M HCl are the appropriate acid volumesfor determination of Terbinafine HCl, Telmisartan, respectively but for Ramipril,the drug dissolved in 0.5 ml 2M HCl and this volume is enough for the reaction. Increasing or decreasing HClvolume above these volumes result in a rapid decrease in absorption.While, in case of method (B) 1 ml 1.5 M HCl and 1 ml 0.3 M HClare suitable for Terbinafine HCl and Telmisartan, respectively. (Fig.7a, 7b) for method (A) and (Fig.8a, 8b) for method (B).

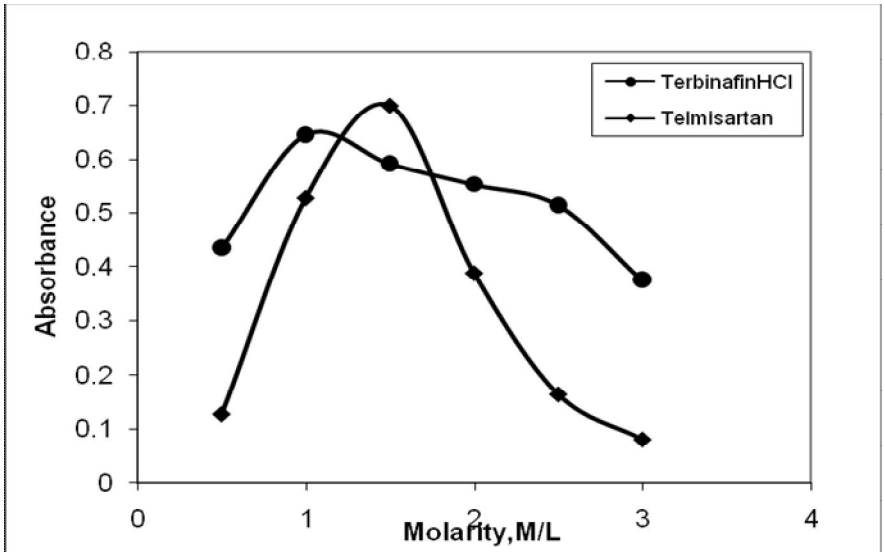


Fig. 7a: Effect of HCl molarity on absorption intensity of 2 µgml^{-1} Terbinafine HCl and 18 µgml^{-1} Telmisartan using method (A)

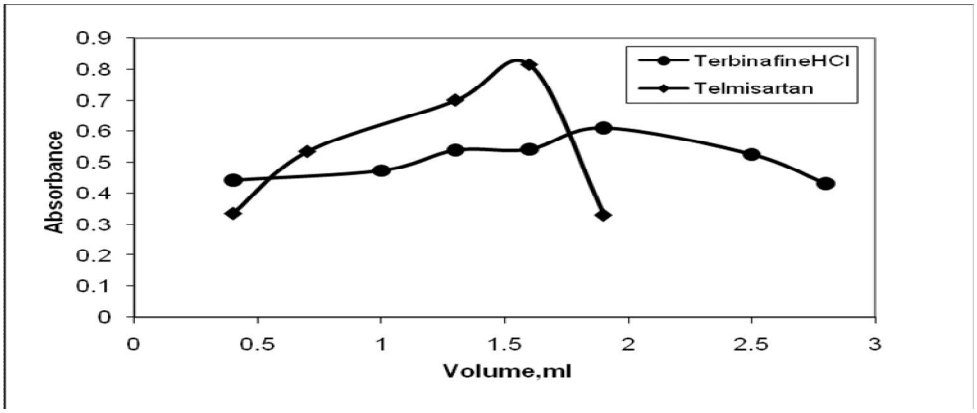


Fig. 7b: Effect of HCl volume on absorption intensity using method (A)

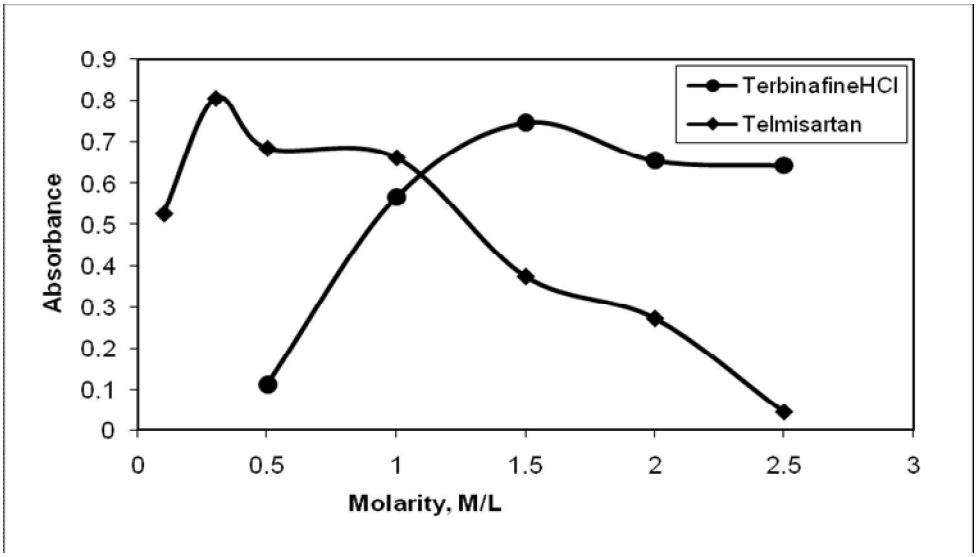


Fig. 8a: Effect of HCl molarity on absorption intensity 2 µgml^{-1} Terbinafine HCl and 3µgml^{-1}Telmisartan using method (B)

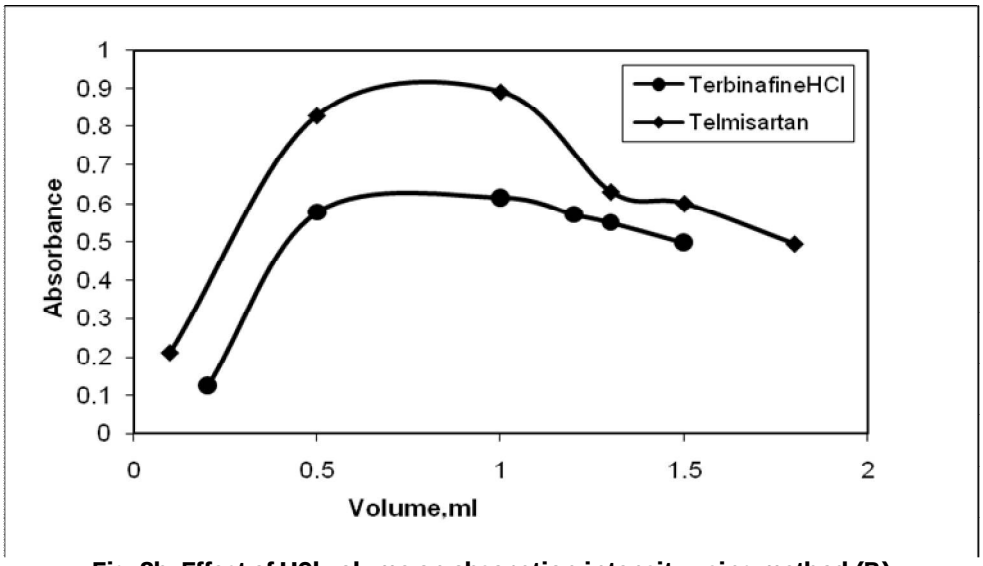


Fig. 8b: Effect of HCl volume on absorption intensity using method (B)

VI.4. Effect of time

Time required to brominate and oxidize the drug beforeaddition of dye and time required to irreversibly oxidizedye after its addition was studied throughout differentexperiments using both dyes. In case of method (A), it was found that thebromination reaction was found to be complete in 5minutes for Terbinafine HCl, in 15 minutes for Ramiprilfig. (9) andin boiling water bath for 5 minutes incase of Telmisartan, fig.(10, 11). A contact time of 35, 10 and 25 minutes were necessaryfor the bleaching of the dye colour by the residual bromine for Terbinafine HCl, Telmisartan and Ramipril respectively, fig.(12).In case of method (B), it was found that thebromination reaction was found to be complete in 20 minutes for Terbinafine HCl, fig. (13) and for 5 minutesin boiling water bath incase of Telmisartan, fig. (14, 15). A contact time of 5 minutes was necessaryfor bleaching the dye colour by the residual bromine for both drugs, fig. (16, 17).

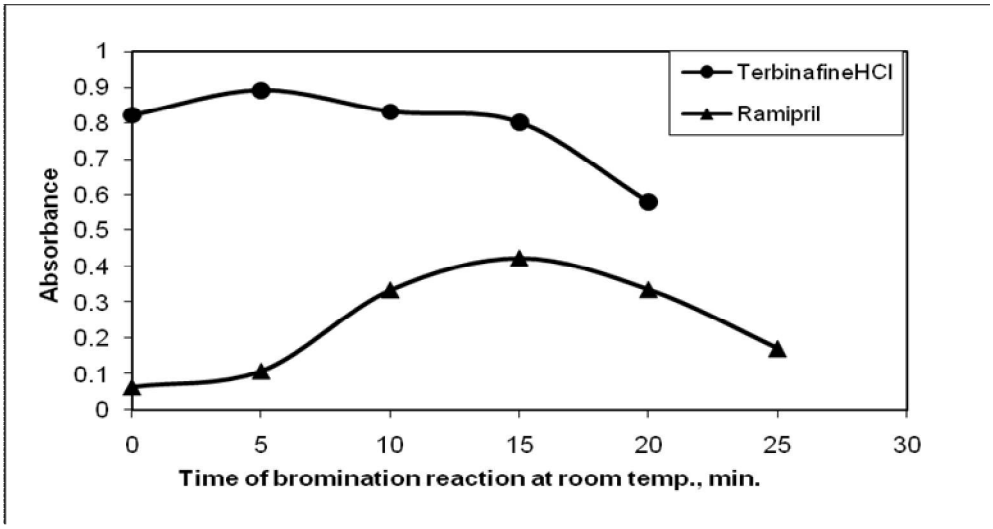


Fig. 9: Effect of time of bromination reaction at room temperature (time required before methylene blue addition)on absorption intensity of:

- **3 µgml^{-1} Terbinafine HCl**
- **36 µgml^{-1} Ramipril**

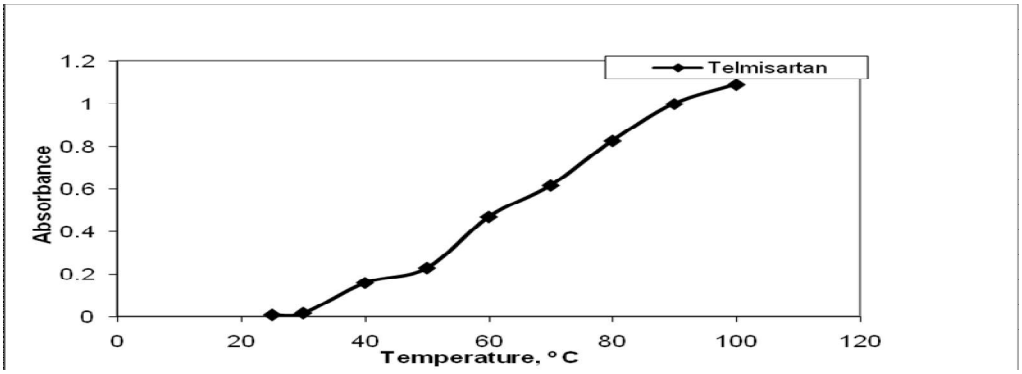


Fig. 10: Effect of bromination reaction temperature on absorption intensity of 18µgml^{-1}Telmisartan using method (A)

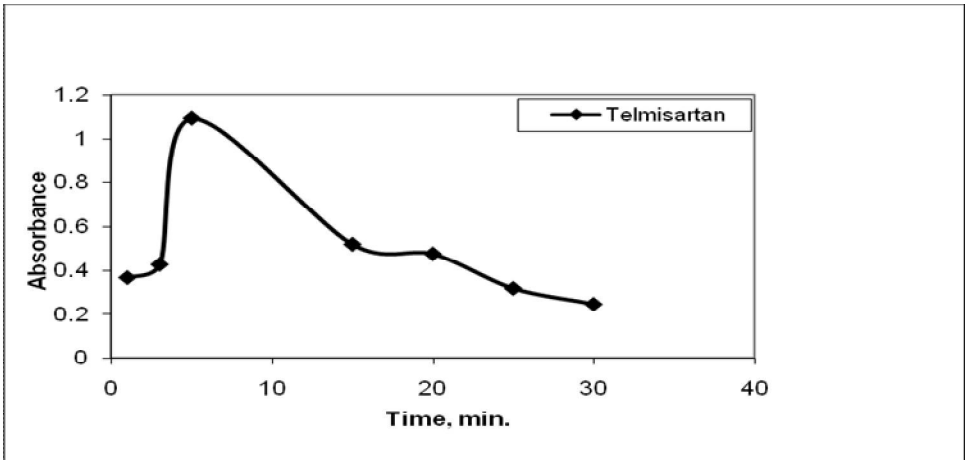


Fig. 11: Effect of bromination reaction time (time of boiling required before methylene blue addition)on absorption intensity of 18µgml^{-1}Telmisartan

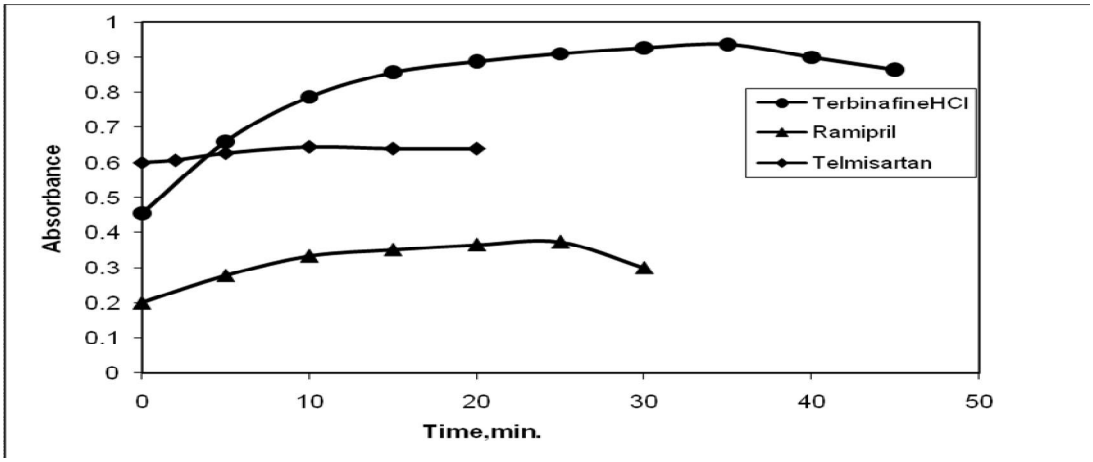


Fig. 12: Effect of time required for bleaching of methylene bluecolour (time after dye addition) on absorption intensity of:

- **3 µgml^{-1}TerbinafineHCl**
- **10 µgml^{-1}Telmisartan**
- **36 µgml^{-1} Ramipril**

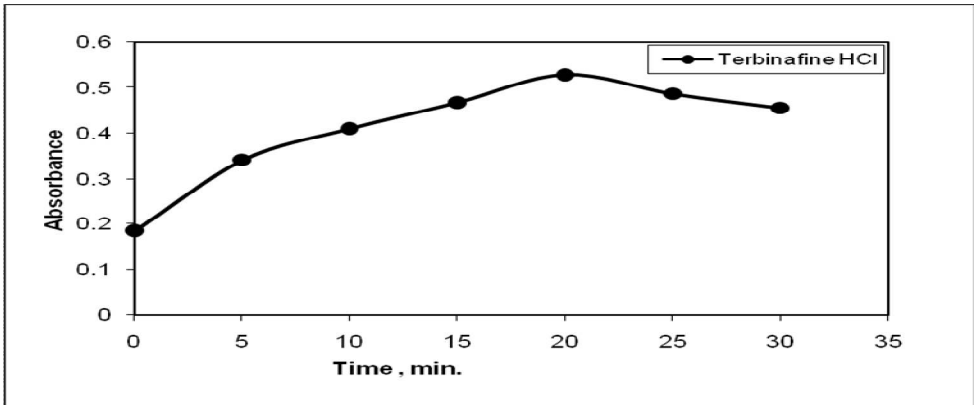


Fig. 13: Effect of bromination reaction time at room temperature (time required before methyl red addition) on absorption intensity of 1.5 µgml^{-1}Terbinafine HCl

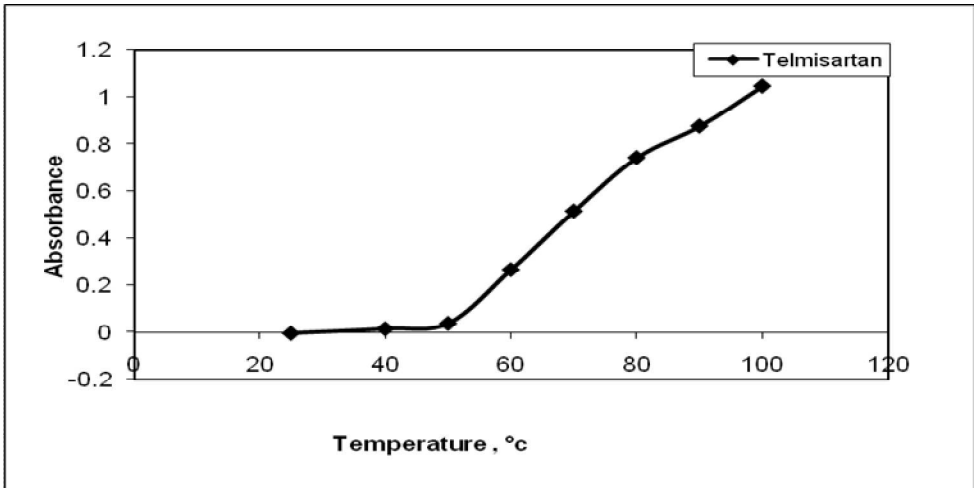


Fig. 14: Effect of bromination reaction temperature on absorption intensity of 3.6µgml^{-1}Telmisartan using method (B)

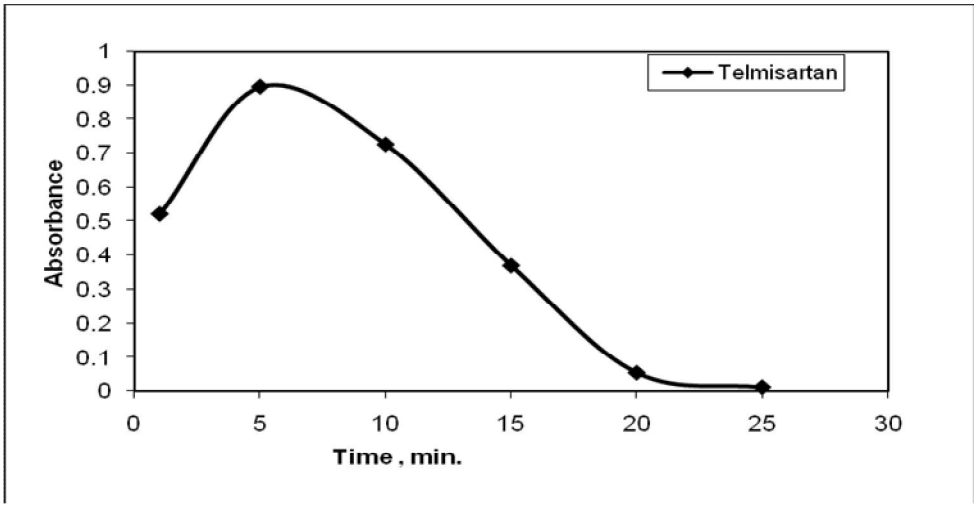


Fig. 15: Effect of bromination reaction time (time of boiling required before methyl red addition)on absorption intensity of 3.6µgml^{-1}Telmisartan

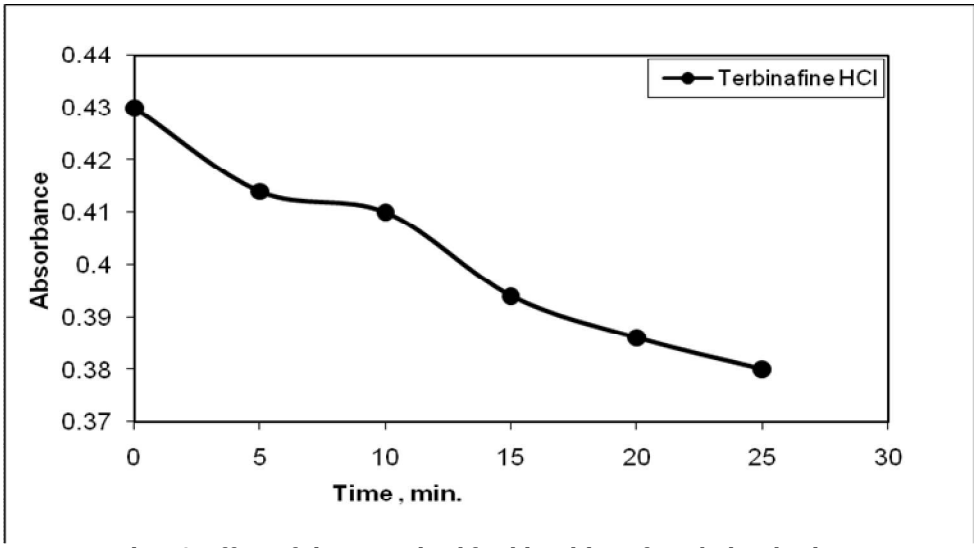


Fig. 16: Effect of time required for bleaching of methyl red colour (time after dye addition)on absorption intensity of Terbinafine HCl

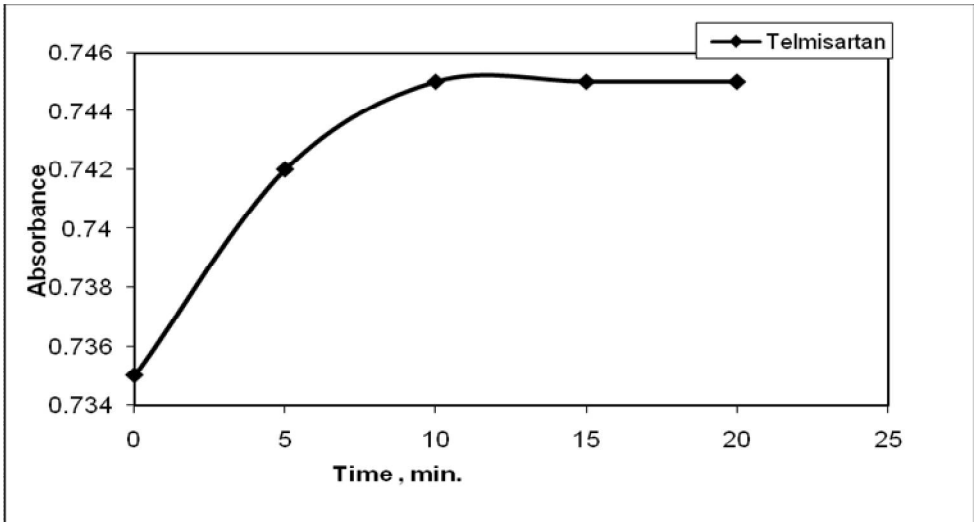


Fig. 17: Effect of time required for bleaching of methyl red colour on absorption intensity Telmisartan

Method validation

VII. Validation of the proposed methods

Linearity

Using the proposed procedures, the regression plots show a linear dependence of the absorbance over Beer's law concentration range given in (Table 1), linear regression equations were obtained. The table also shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear least-squares treatment of the results and Molar absorptivity. Results of recovery studies with pure drugs by proposed methods (Table 2) show small values of standard deviation and variance that indicates low scattering of the points around the calibration line and high precision.

Limit of quantitation and limit of detection

The limits of quantitation (LOQ) were determined by establishing the lowest concentration that can be measured according to ICH recommendation[83] below which the calibration graph is non linear. The results are shown in Table 1. The limits of detection (LOD)

were determined by evaluating the lowest concentration of the analyte that can be readily detected. The results are also summarized in Table 1.

LOQ and LOD were calculated according to the following equations[84]:

LOQ = 10 Sa/b

LOD = 3.3 Sa/b

Where Sa is the standard deviation of the blank, and b is the slope of the regression line.

Precision

The precisions of the assays (intra-day and inter-day) were determined for the studied drugs concentrations cited in Table 3. The assays, gave satisfactory results (Table 3). This level of precision of the proposed methods was adequate for the quality control analysis of TER, TEL and RP.

Accuracy

Theobtained results were in good agreement with those obtained using the reference methods (85, 86 and 87). Statistical analysis of the result obtained using student t-test and the variance ratio F-test revealed no significance differences between the proposed and references methods regarding the accuracy and precision, respectively (Table 4).

Analytical applications

The results obtained by applying the proposed methods for the determination of drugs in there pharmaceutical formulations (Lamisil, Micardis and Tritace protect tablets) (Table5, 6) suggest satisfactory recovery. Further, standard addition technique followed to check the validity of the method has given good recoveries of the drugs. Hence, these methods can be recommended for adoption in routine analysis of TEL, TER and RP.

Table 1: Spectral data for determination of Terbinafine HCl, Telmisartan and Ramipril using the proposed methods

Parameters	Method (A)			Method (B)	
	Terbinafine HCl	Telmisartan	Ramipril	Terbinafine HCl	Telmisartan
Linearity range (µg ml^{-1})	1-3**	2-18	28-68	0.5-2.5**	0.3-3.6
Wavelength (nm)	678	668	678	517	517
Limit of detection (µg ml^{-1})	0.3	0.5	9	0.15	0.09
Limit of quantification(µg ml^{-1})	0.95	1.7	27	0.46	0.28
Regression equation*:					
Slope (b)	0.4112	0.0434	0.0186	0.3950	0.1931
Intercept (a)	-0.2935	0.1893	-0.2722	-0.0268	0.2411
Correlation coefficient (r)	0.9999	0.9996	0.9998	0.9995	0.9998
SE	0.25	0.47	0.2	0.44	0.48
Reproducibility (R.S.D%)	0.67	0.97	0.4	0.92	0.98
Repeatability(R.S.D%)	0.98	0.83	0.57	0.67	0.72
Molar absorptivity (L mol-1cm-1)	$7.9 \cdot 10^4$	$3.9 \cdot 10^4$	$5.09 \cdot 10^3$	1.210^5	$2 \cdot 10^5$

* A=a + bc

** Low narrow range is due to high sensitivity of the method confirmed by high molar absorptivity.

Table 2: Determination of Terbinafine HCl, Telmisartan and Ramipril using the proposed methods

	Method (A)						Method (B)			
	Terbinafine HCl		Telmisartan		Ramipril		Terbinafine HCl		Telmisartan	
	Taken µg ml^{-1}	Recovery* %	Taken µg ml^{-1}	Recovery* %	Taken µg ml^{-1}	Recovery* %	Taken µg ml^{-1}	Recovery* %	Taken µg ml^{-1}	Recovery* %
	1	101.05	2	101.04	28	99.31	0.5	100.66	0.3	101.67
	1.4	99.10	4	100.63	36	100.39	0.75	101.54	1.2	98.35
	1.8	100.32	6	98.96	40	100.97	1	98.68	1.35	100.85
	2	99.53	10	101.08	44	100.22	1.25	99.20	1.8	100.38
	2.8	100.19	14	98.37	50	100.24	1.5	100.89	3	100.28
	3	99.99	16	99.90	52	100.52	2	99.59	3.3	99.16
			18	100.70	68	100.03	2.25	98.99	3.6	100.54
							2.5	100.94		
Mean±S.D.	100.03±0.61		100.1±1.07		100.24±0.51		100.06±1.07		100.18±1.10	
N	6		7		7		8		7	
S.D.	0.61		1.07		0.51		1.07		1.10	
R.S.D.	0.61		1.06		0.50		1.07		1.10	
V	0.45		1.14		0.26		1.22		1.21	
S.E.	0.25		0.47		0.2		0.44		0.48	

*Average of three experiments

Table 3: Precision of the proposed methods for analysis of Terbinafine HCl, Telmisartan and Ramipril

	Drug	Terbinafine HCl	Telmisartan	Ramipril	Terbinafine HCl	Telmisartan
	Method	Method (A)			Method (B)	
	Wavelength(nm)	678 nm	668 nm	678 nm	517 nm	517 nm
	Weight taken (µg/ml)	2	16	52	1	3.3
	Validation Parameters / %Recovery / Experiment					
Intra-day	1	99.4	99.47	100.31	101.47	99.16
	2	100.13	98.03	99.48	101.47	100.26
	3	101.35	100.19	100.31	100.46	98.69
	4	99.4	99.47	100	99.95	99.16
	5	101.35	98.75	100.52	100.46	100.26
	Mean	100.33	99.18	100.12	100.76	99.51
	S.D.	0.98	0.82	0.40	0.68	0.71
	R.S.D.	0.98	0.82	0.40	0.67	0.72
Inter-day	%Recovery / Experiment					
	1	98.92	100.91	101.55	98.94	100.26
	2	99.16	99.47	100.31	101.47	99.16
	3	100.13	100.91	100.52	100.46	97.59
	4	100.5	98.75	100.1	99.95	99.48
	5	99.4	99.47	100.31	100.46	99.16
	Mean	99.62	99.9	100.56	100.25	99.13
	S.D.	0.67	0.97	0.57	0.92	0.97
	R.S.D.	0.67	0.97	0.57	0.92	0.98

Table 4: Statistical data for determination of Terbinafine HCl, Telmisartan and Ramipril using proposed methods compared with reference one

Drug	Parameters	Method (A)	Method (B)	Reference method
Terbinafine HCl	Mean ±S.D	100.03±0.61	100.06±1.07	100.60±0.96[85]
	N	6	8	5
	Variance	0.45	1.22	0.92
	Student-t-test	1.06(2.262)*	0.89 (2.201)*	
	F-test	2.04 (5.19)*	1.33 (4.12)*	
Telmisartan	Mean ±S.D	100.1±1.07	100.18±1.10	99.99±1.02[86]
	N	7	7	4
	Variance	1.14	1.21	1.03
	Student-t-test	0.168 (2.262)*	0.284 (2.262)*	
	F-test	1.11(4.76)*	1.17 (4.76)*	
Ramipril	Mean ±S.D	100.24±0.51		100.17±0.86[87]
	N	7		5
	Variance	0.26		0.74
	Student-t-test	0.178 (2.228)*		
	F-test	2.85 (4.63)*		

*Tabulated values of t and F at p = 0.05

Table 5: Application of standard addition technique for determination of Terbinafine HCl, Telmisartan and Ramipril in their pharmaceutical formulations using method (A)

	Terbinafine HCl (Lamisil tablets)			Telmisartan (Micardis tablets)			Ramipril (Tritace Protect tablets)		
	Taken	Added	Recovery*	Taken	Added	Recovery*	Taken	Added	Recovery*
	µg ml^{-1}		%	µg ml^{-1}		%	µg ml^{-1}		%
	1.4	--	99.27	4	--	100.63	28	--	101.42
		1	100.15		4	101.79		28	100.61
		1.1	100.55		6	102.42		30	101.08
		1.2	100.89		8	102.74		32	101.48
		1.5	100.65					36	100.66
Mean±S.D.	100.56±0.31			101.89±0.80			101.05±0.41		
N	4			3			4		
V	0.09			0.86			0.17		
S.D.	0.31			0.80			0.41		
S.E.	0.14			0.40			0.18		

* Mean of three different experiments

Table 6: Application of standard addition technique for determination of Terbinafine HCl and Telmisartan in their pharmaceutical formulations using method (B)

	Terbinafine HCl (Lamisil tablets)			Telmisartan (Micardis tablets)		
	Taken	Added	Recovery*	Taken	Added	Recovery*
	μg ml^{-1}		%	μg ml^{-1}		%
	0.5	--	100.66	0.3	--	98.22
		0.5	99.65		0.3	98.22
		0.75	98.84		0.6	100.03
		1	98.94		0.9	98.34
		1.5	98.36		1.5	100.43
		1.75	99.07			
Mean±S.D.	99.25±0.80			99.05±1.09		
N	5			4		
V	0.65			1.19		
S.D.	0.80			1.09		
S.E.	0.33			0.55		

* Mean of three different experiments

CONCLUSION

Two new spectrophotometric methods for determination ofTerbinafine HCl, Telmisartan and Ramipril have been developedbased on insitu generation of bromine and using methylene blue or methyl red as chromogenic agents. Theyproved to be rapid, accurate and reproducible.

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