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Application of certain ion – pairing reagents for extractive spectrophotometric determination of flunarizine hydrochloride, Ramipril and Terbinafine hydrochloride

MARWA S. ELAZAZY[1]*, MAGDA Y. EL-MAMMLI[2], ABDALLA SHALABY[2] and MAGDA M. AYAD[2]

[1]Chemistry Department, School of Humanities and Science, Virginia Commonwealth University, Richmond 23284 (USA)
[2]Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig (Egypt)

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ABSTRACT

Three simple, rapid and extractive spectrophotometric methods have been developed for the determination of flunarizine hydrochloride (F.HCl), ramipril (RAM) and terbinafine hydrochloride (T.HCl). The proposed methods depend on the formation of colored ion-pair complexes between the basic nitrogen of the drug and the inorganic complex; molybdenum (V) thiocyanate, Mo(V) (SCN) or the acid dyes; orange G (OR.G) and alizarin red S (ARS). The formed complexes were extracted with the suitable organic solvent and measured at 469 -471 nm for Mo(V) (SCN) method; 498-500 nm using (OR.G) method and at 425 -426 nm in case of (ARS). The analytical parameters and their effects on the reported systems were investigated. The methods permit the determination of the studied drugs over a concentration range of 5-75 µg ml^{-1}, 10-80 µg ml^{-1} and 5-55 µg ml^{-1} using Mo(V), (OR.G) and (ARS) respectively. The composition of the ion-pairs was found by Job's method. The proposed methods were applied for the analysis of drug substances in their dosage forms and the results obtained were in good agreement with those obtained by the official and reference methods.

Key words: Flunarizine HCl, Ramipril, Terbinafine HCl, Molybdenum (V) thiocyanate, Orange G, Alizarin red S.

INTRODUCTION

The studied compounds belong to different pharmacological classes.[1] Flunarizine HCl is a Ca^{2+} entry-blocker which is used in treatment of many diseases as vertigo, ramipril is an ACE inhibitor which is used as antihypertensive, while terbinafine HCl is recommended for treatment of fungal infections[1].

Despite of the therapeutic importance of these compounds, the literature shows only few methods for their determination either perse or formulations and biological fluids. These two factors created a need for new analytical methods for their determination. Methods reported in literature include, spectrophotometry[2-11], voltammetry[12,13], potentiometry[14, 15], capillary electrophoresis[16, 17] and HPLC[18-21].

The aim of the present work is to develop simple, reliable, reproducible and accurate spectrophotometric methods for the quality control of the pharmaceutical formulations containing the cited drugs, especially when modern and expensive apparatus as HPLC and GLC are not available. The procedure is based simply on the ability of the cited drugs to form ion-pair complexes with Mo (V) SCN, (OR.G) and (ARS).

EXPERIMENTAL

Apparatus

Measurements were carried out using Shimadzu 260 UV – Vis Spectrophotometer, double beam with 10 mm matched quartz cell. *A* Chemocadet pH meter with combined glass-calomel electrode was used for pH measurements.

Materials

Pure Samples

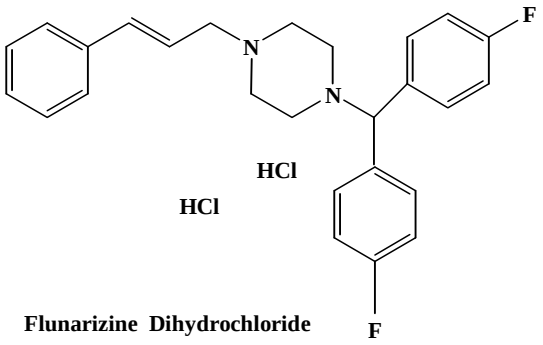
Chemicals used were of the highest purity available from their sources:

1. Flunarizine hydrochloride was kindly supplied by Janssen – Cilag, Cairo, Egypt. The purity was found to be 100.20 % according to reference method[3].
2. Ramipril was kindly supplied by Aventis-Pharma, Cairo, Egypt. The purity was found to be 100.13 % according to B.P. 2002 method[22].
3. Terbinafine hydrochloride was kindly supplied by Novartis Pharma, Cairo, Egypt. The purity was found to be 100.20 % according to reference method[23].

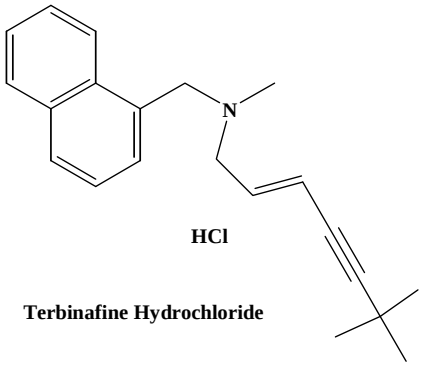
Market samples

Formulations used were collected from local pharmacy stores:

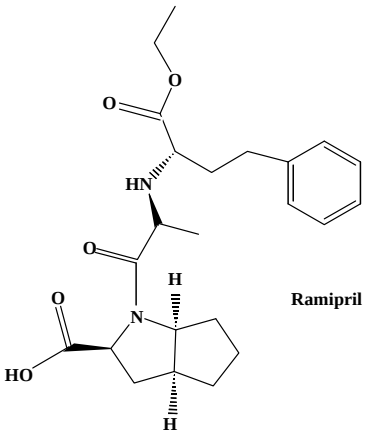
1. Sibelium® capsules; 5 mg flunarizine/capsule Janssen-Cilag, Egypt.
2. Tritace® tablets; 5 mg ramipril / tablet, Aventis -Pharma, Egypt.
3. Ramipril® capsules; 5 mg ramipril / capsule, El-Pharonia, Egypt.
4. Terbine® tablets; 250 mg terbinafine hydrochloride / tablet, Global Napi, Egypt.



Flunarizine Dihydrochloride



Terbinafine Hydrochloride



Ramipril

Table 1: Analytical parameters for the reaction of the studied drugs with mo (v) scn

Compound	Volume of ammonium molybdate	Volume of ammonium thiocyanate	Volume of ascorbic acid (ml)	Volume of HCl (ml)	Time for Mo(V) SCN complex formation (min).
Flunarizine HCl	0.5	1.5	1	2	10
Ramipril	1.5	1.5	0.5	2.5	5
Terbinafine HCl	1	2	1	1.5	1.5

Reagents

1. Molybdenum (VI) solution; $1x10^{-3}$ M, was prepared from ammonium molybdate (BDH chemicals Ltd., Poole, England) in distilled water containing a few drops of ammonia and standardized as prescribed. [24]
2. Ammonium thiocyanate (Merck, Germany) and ascorbic acid (BDH chemicals Ltd., Poole, England) aqueous solutions (10% w/v each) were prepared in distilled water.
3. Hydrochloric acid, 3 M and 10% aqueous solutions.
4. Acetic acid, 10% aqueous solution.
5. Orange G (Fluka, Germany), 0.1% w/v aqueous solution.
6. Alizarin Red S (Sigma – Aldrich, USA), 0.1% w/v aqueous solution.

Standard drug solutions

Stock solutions of 1mg ml^{-1} and 2 mg ml^{-1} were freshly prepared by dissolving the appropriate drug amounts in methanol (F.HCl), and in distilled water (RAM, T.HCl). Working solutions were prepared by further dilutions.

Procedures

Bulk Powder

Molybdenum Thiocyanate Method

Into a series of 125 ml separating funnels, 0.5-1.5 ml of ammonium molybdate, 1.5-2.5 ml of 3 M HCl, 0.5-1 ml of ascorbic acid and 1.5-2 ml of ammonium thiocyanate were placed and the mixture was left for 5-15 min. at room temperature, Table 1. Aliquots of the standard drug solutions ranging from 50-750 μgml^{-1} were added to each funnel and the volume was completed to 10 ml with distilled water. Accurately measured 10 ml of dichloromethane were added and the solutions were shaken vigorously for 2 min., and then allowed to stand for clear separation of the two phases. The organic layer was collected, dried over anhydrous sodium sulphate and its absorbance was measured at 469- 471 nm against a reagent blank similarly prepared.

Orange G method

Into a series of 125 ml separating funnels, 1.5-2 ml of dye solution and 2 ml of 10 % w/v acetic acid were placed. Suitable aliquots of the standard drug solutions (100-800 μgml^{-1}) were added to each funnel and the mixture was left for 0-5 min. at room temperature then extracted with 4, 3, 3 ml portions of dichloromethane. The absorbance of organic layer was measured at 498-500 nm against a reagent blank, Table 2.

Alizarin red S method

Into a series of separating funnels, 0.8-1

Table 2: Variables affecting the reaction of the studied drugs with acid dyes; orange G (OR.G) and alizarin red s (ARS)

Parameter	Flunarizine HCl		Ramipril		Terbinafine HCl	
	OR.G method	ARS method	OR.G method	ARS method	OR.G method	ARS method
Reaction Time (min.)	5	0	-	0	0	0
Acid Type	Acetic acid	Acetic acid	-	Hydrochloric acid	Acetic acid	Acetic acid
Volume of Acid (ml)	2	1	-	1	2	0.8
Volume of Dye (ml)	1.5	1	-	1	2	1.2
Extracting Chloroform	Methylene	Methylene	-	Methylene	Methylene	
Solvent	Chloride	Chloride		Chloride	Chloride	
λmax (nm)	498	425	-	425	500	426

ml of either 10 % w/v acetic acid or hydrochloric acid, followed by aliquots of standard drug solutions (50-550 μgml^{-1}) and 1-1.2 ml of ARS were mixed well. The mixture was extracted with 2 × 5 ml portions of the suitable organic solvent. The organic layer was collected into a series of 10 ml calibrated flasks after drying over anhydrous sodium sulphate. The absorbance was measured at 425 – 426 nm against a reagent blank, Table 2.

Pharmaceutical formulations

Tablets

Twenty tablets of both ramipril and terbinafine hydrochloride formulations after determining the average weight, were finely powdered. Powder of each drug equivalent to 100-200 mg was accurately weighed, transferred into 100 ml calibrated flasks and shaken with distilled water. The solution was filtered and the volume was made up to 100 ml with distilled water. The recommended procedures were followed.

Capsules

The contents of 20 capsules were completely emptied. Powder equivalent to 100 mg was extracted with 3 x 30 ml portions of methanol (for Sibelium® capsules) or distilled water (for Ramipril® capsules). These portions were filtered into 100 ml volumetric flasks and the volume was made up to the mark with the suitable solvent. The procedure was completed as previously described.

RESULTS AND DISCUSSION

Molybdenum thiocyanate method

A survey of literature revealed that no attempt has been made to study the color reactions of the studied drugs with Mo (VI) thiocyanate, for the spectrophotometric determination of either molybdenum or the cited drugs. Thus, we have undertaken such a study in pursuit of a new application of ion-pair associate for the determination of such drugs and their pharmaceutical preparations. In the proposed procedure, Mo (V) formed by reduction of Mo (VI) with ascorbic acid combines with ammonium thiocyanate to form a red binary complex in 0.8-3.2 M hydrochloric acid solution. [25] On adding the standard drug solutions, an orange-red ion-pair is formed. The ion-pair is soluble in dichloromethane while the binary, Mo (V)-thiocyanate complex, is not. The absorption spectra of the ion-pairs in dichloromethane show a maximum at 469-471 nm against a reagent blank, Fig.1.

It was found that the same color in dichloromethane was formed in the absence of ascorbic acid. The reduction of Mo (VI) to Mo (V) by SCN^- in acidic media can be considered. However, during the present study, it was observed that rapidity, the sensitivity and the stability of Mo(V) -thiocyanate ion-pair can be enhanced using ascorbic acid. Other variables were studied and stated in Table 1.

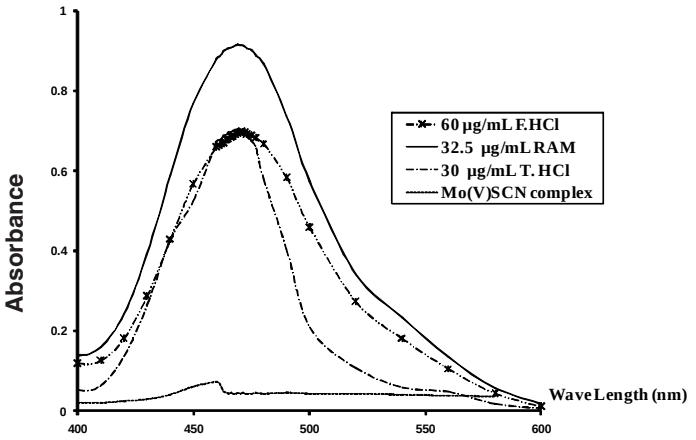


Fig. 1: Absorption spectra of Mo(V) ion-pairs in methylene chloride vs. reagent blank

Orange G method

(F.HCl), and (T.HCl) can be transferred from the aqueous phase into the organic phase as an ion-pair formed with the anionic form of the acid dye;

$$X^{+}_{(aq.)} + D^{-}_{(aq.)} \leftrightarrow X^{+}D^{-}_{(aq.)} \leftrightarrow X^{+}D^{-}_{(org.)}$$

Where X^{+} and D^{-} represent the protonated drug and the anion of the dye, respectively; and the subscript (aq.) and (org.) refer to the aqueous and

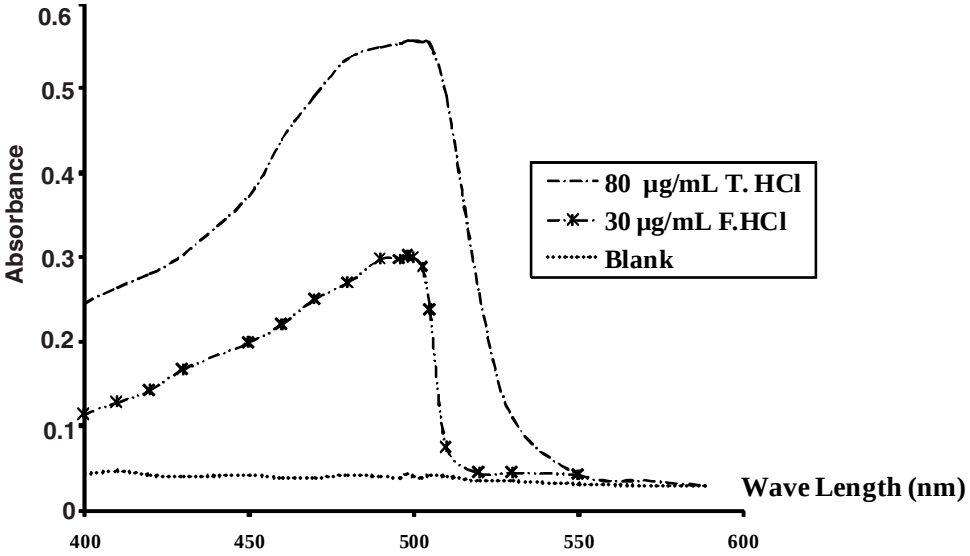


Fig. 2: Absorption spectra of orange G ion pairs in with the studied drug against a reagent blank

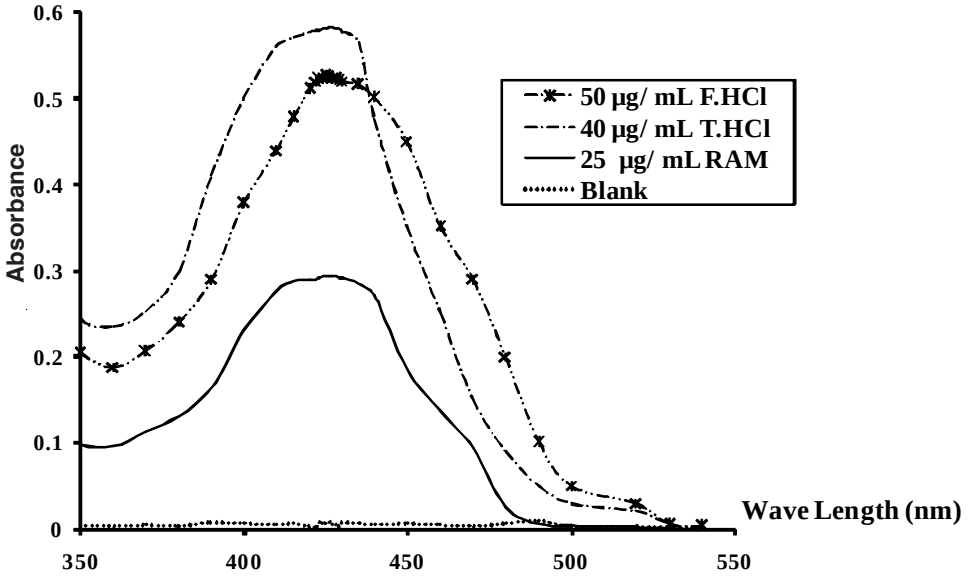


Fig. 3: Absorption spectra of alizarin red S ion pairs with the studied drug atainst a reagent blank

Table 3: Quantitative Parameters for the Determinations of the studied Drugs with Mo(V)SCN (A), OR.G (B) and ARS (C) methods

Parameter	Flunarizine HCl			Ramipril			Terbinafine HCl		
	A	B	C	A	B	C	A	B	C
Linearity Range (µg ml-1)	12 -75	10 - 55	20 - 55	5 - 32.5	-	10 - 50	7.5 - 30	20 - 80	5 - 55
Intercept (a)	- 0.0562	- 6.2 x 10-3	- 0.144	0.0109	-	8.57 x 10-3	- 0.081	0.0232	0.0393
Slope (b)	0.0127	0.0103	0.0134	0.0279	-	0.0117	0.0261	6.68 x 10-3	0.0138
Limit of detection (µg ml-1)	0.936	1.391	0.682	0.278	-	1.376	0.381	1.932	0.835
Limit of quantation (µg ml-1)	3.12	4.636	2.28	0.928	-	4.587	1.269	6.439	2.78
RSD %	0.682	0.372	0.426	0.484	-	0.690	0.292	0.536	0.446
Correlation Coefficient (r)	0.99997	0.99998	0.99997	0.99998	-	0.99995	0.99999	0.99994	0.99999

Table 4: Quantitative Parameters for the Determinations of the studied Drugs with Mo(V)SCN (A), OR.G (B) and ARS (C) methods

Parameter	Flunarizine HCl				Ramipril			Terbinafine HCl			
	A	B	C	Reference method[3]	A	C	Official method[22]	A	B	C	Reference Method[23]
Recovery* (%)	100.00	100.06	100.02	100.2	99.9	100.00	100.13	99.98	99.95	99.98	100.2
S.D.	0.682	0.372	0.426	0.411	0.484	0.690	0.395	0.292	0.536	0.446	0.650
Variance	0.465	0.138	0.181	0.169	0.234	0.476	0.156	0.085	0.287	0.199	0.423
t - value	0.659	0.587	0.67		0.853	0.377		0.69	0.663	0.673	
	(2.201)**	(2.262)**	9 (2.306)**		(2.262)**	(2.262)**			9 (2.262)**	(2.306)**	(2.179)**
F - value	2.751	1.225	1.07		1.50	3.05		4.98	1.474	2.13	
	(4.12)**	(5.19)**	(6.39)**		(4.76)**	(5.19)**		(5.19)**	(6.39)**	(3.84)**	
n a	8	6	5	5	7	6	4	6	5	9	5

*Mean of five determinations ** "t" and F- values at P = 0.05 a Number of Experiments

Table 5: Determination of the studied drugs in pharmaceutical formulations by the proposed, official and reference methods

Formulation	Recovery* % ± S.D.						
	Molybdenum thiocyanate Method		Orange G method		Alizarin red method		Reference and official method
	Direct method	Standard addition method	Direct method	Standard addition method	Direct method	Standard addition method	
Sibelium® Capsules 3	99.87 ± 0.794	99.71±0.797	100.46±0.786	99.91±0.738	100.36±0.688	100.99±0.630	100.5±0.350
Tritace® Tablets 22	99.94±0.856	100.4±0.560	-	-	99.71±0.524	99.76±0.915	99.6±0.833
Ramipril® Capsules 22	99.98±0.659	99.75±0.966	-	-	99.94±1.185	99.78±0.769	100.01±0.898
Terbine® Tablets 23	97.88±0.597	99.15±0.567	97.94±0.772	99.15±0.791	100.04±0.906	100.02±1.121	99.00±0.930

*Mean of five determination

organic phases respectively. The effect of pH on drug-reagent system was studied using acetate buffer pH 2-5 and different types of acids (acetic, hydrochloric, phosphoric and sulphuric). Acetic acid 10% w/v was found to give the highest absorbance values in addition to the stability of the formed color. Other variables affecting the reaction were studied and the results are listed in Table 2.

Alizarin red S method

In this study, ARS was used as a very suitable anionic dye, to form an intense yellow color ion- pair complex with the studied drugs, in an acidic pH (10% HCl or acetic acid). This ion-pair is soluble in either chloroform or dichloromethane and can be measured at 425-426 nm. It should be mentioned that ARS wasn't extracted in the organic solvent in the absence of the drug even from high acidic solutions. This observation confirmed that ARS as an anionic reagent can be only extracted from chloroform or dichloromethane as an ion associated complex in the presence of lipophilic protonated drug.

In preliminary experiments, a number of immiscible organic solvents were examined in order to provide an applicable extractive procedure. Complexes had higher molar absorpitivities and stabilities in dichloromethane in case of both (F.HCl) and (RAM) while in case of (T.HCl), chloroform was used as it achieved higher stability than dichloromethane despite of the slightly higher molar absorpitivity of T.HCl-ARS ion-pair in dichloromethane Table 3.

Determination of the stochiometry of the reaction

The composition of the ion-pairs was established by Job's method of continuous variation[26] using both variable reagent and drug concentrations. The results indicate that 1: 2 (metal: drug) ion-pairs are formed using Mo (VI) SCN. On using (OR.G) and (ARS), the molar ratio was 1:1 (drug: dye) indicating that ion-pairs are formed through the electrostatic attraction between the positive protonated drug X^+ and the negative dye ARS^- or $OR.G^-$.

Calibration graphs and statistical parameters

The regression equations calculated with

the methods described before are assembled in Table 4, together with RSD. The limits of detection (LOD) and limits of quantification (LOQ) were calculated by means of statistical treatment of data [27-28] and the results are represented in Table 3.

The proposed methods were applied for the analysis of pure drug samples and pharmaceutical preparations Tables 3-5. The data shows that there is no significant difference between the proposed and official [22] or reference [3, 23] methods.

The validity of the proposed methods was assessed by applying either the direct or the standard addition techniques. The results are shown in Table 5.

CONCLUSION

No substantial differences among the three proposed methods arose from an analysis of experimental results. Molybdenum method is more sensitive with a wider concentration range, while OR.G. and ARS methods have nearly the same sensitivity.

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