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ANALYTICAL STUDY OF SOME ANTIHISTAMINES WITH HAEMATOXYLIN REAGENT

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في هذا البحث تم دراسة التفاعل بين مادة الهيماتوكسيلين وثمانية مركبات مضادة للهستامين. هذا وقد تم الإستفادة من هذه الدراسة لاستنباط طريقة طيفية جديدة وبسيطة وحساسة لتقدير هذه المركبات. وتعتمد هذه الطريقة على التفاعل بين محلول هذه المركبات فى الميثانول ومحلول الهيماتوكسيلين فى ٣×١٠$^{-٣}$ حامض اليوريك لتعطى لون أحمر بنفسجى عند ٥٥٥ ن.م. هذا وقد تم الحصول على علاقة خطية بين تركيز هذه المركبات والامتصاص عند طول الموجة المذكور سلفا بتركيز عام ٥-٢٠٠ ميكروجرام لكل ملليتر من التركيز النهائى للمحلول القاعدى.

كذلك لوحظ عدم وجود تداخل نتيجة المواد الإضافية المستعملة فى تصنيع الأقراص أو بعض المركبات الإضافية مثل الفينازون ، الفينازون سلسيلات ، الباراسيتامول ، البروبيفينازون والكافيين عند تطبيق الطريقة المقترحة لتحليل الأقراص.

The reaction between haematoxylin reagent and eight antihistaminic drugs (chlorpheniramine maleate, pheniramine maleate, cyproheptadine hydrochloride, astemizole, terfenadine, ranitidine hydrochloride, famotidine and cimitidine) was investigated and utilized as the basis for a new, simple and sensitive spectrophotometric procedure for their determinations. The procedure is based on the reaction of the methanolic solution of the antihistaminic compounds with haematoxylin reagent in $3x10^{-3}$ M boric acid to give a reddish-violet colour (λ_{max} 555 nm). The absorbance was linear with all compounds over the 5-200 μg/ml general concentration range of the final measured antihistamine base solution. A good correlation was found between log A (1%, 1 cm) and pk_a values for six of the studied drugs. The method can be applied successfully to the analysis of commercially available tablet formulations. No interference was observed due to additives which are commonly present in tablet formulations or coformulated compounds such as phenazone, phenazone salicylate, sodium salicylate paracetamol, propyphenazone and caffeine.

INTRODUCTION

There are three histamine receptors: H_1, H_2 and H_3. The present available H_1-antihistamines (chlorpheniramine maleate, pheniramine maleate, astemizole and cyproheptadine hydrochloride) competitively antagonize the effects of histamine at receptor sites but they do not block the release of histamine. Most of these agents are effective in allergic reactions to blood and plasma, seasonal allergic rhinits and as adjuncts to conventional therapy in anaphylactic reactions. H_2-antihistamines (cimetidine, famotidine and ranitidine hydrochlorides) inhibit both acid and gastrin stimulated secretion[1]. In fact most of antihistamines are amine derivatives belong to different chemical classes.

Several methods have been reported for the the determination of the investigated compounds, either alone or in combination with others. These methods include: chromatographic[2-9], titrimetric[10-11], colourimetric[12-14], fluorimetric[15-17], UV-spectrophotometric methods[18-20] and derivative spectrophotometric methods[21-23]. In addition; comprehensive reviews were also published for some of the chosen compounds[24-29].

Haematoxylin is considered as catechol derivative; naturally obtained from logwood

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(Haematoxylin Campeachianm)[30]. It has been used as metallochromic indicator[31], and for the determination of phosphorus[32] and fluorine[33]. It is mainly used in manufacturing of ink and as a stain in microscopy[34] as well as for the detection and analysis of several metal ions[35,36]. Oxidized haematoxylin has been used for the analysis of some pharmaceuticals such as some penicillins and cephlosporins[30], benzthiazide and hydrofluoromethiazide[37]. Recently haematoxylin-permangate reagent has been reported for the spectrophotometric determination of some thiols of pharmaceutical interest[38].

The objective of the present work is to study the possible chemical reaction between some H_1-antagonists such as astemizole, chlorpheniramine maleate, pheniramine maleate, cyproheptadine hydrochloride and terfenadine,in addition to some H_2-antagonist such as cimetidine, famotidine and rantidine hydrochloride (Scheme 1) with haematoxylin reagent in order to develop a suitable method for the quantitative analysis of these drugs.

EXPERIMENTAL

Apparatus

A uvidec-320 (Tokyo, Japan) spectrophotometer with 1 cm quartz cells was used. All volumetric measurements were made with standard glassware.

Reagents and materials

All solvents and reagents were of analytical reagent grade. Double distilled water was used throughout this work.

Haematoxylin (Aldrich Chemical Co., Poole, Dorset, Uk); $6.6x10^{-3}$ M solution in $3x10^{-3}$ M aqueous boric acid is prepared fresh daily. Boric acid solution, $0.3x10^{-2}$ M is prepared in distilled water. Chlorpheniramine maleate, pheniramine maleate (Hoechst Orient S.A.A. Cairo, Egypt; under licence of Hoechst, AG. Frankfurt, Germany), astemizole (Glaxo Egypt; Janssen Pharmaceutica beerse-Belgium), terfenadine (Amoun Pharm. Ind. Co., El Salam city , Cairo, Egypt),cyproheptadine hydrochloride and cimetidine (Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt), ranitidine hydrochloride (Medical Union Pharm. Co., Ismailia, Egypt), and famotidine (Memphis Co. for Pharm. & Chem. Ind. Cairo, Egypt) were used as working standards.

Tablets

The following ingredients represent the label claim contents per one tablet. Avil retard tablets (Hoechst Orint-Egypt) labeled to contain 75 mg pheniramine maleate. Allergyl tablets (Arab Drug Co., Cairo, Egypt) labeled to contain 4 mg chlorpheniramine maleate. Cosavil tablets (Hoechst Orient-Egypt) labeled to contain 11.25 mg pheniramine maleate, 250 mg phenazone salicylate, 15 mg caffeine. Perafene caffeine tablets (Memphis Chemical Co., Cairo, Egypt) labeled to contain 2 mg chlorpheniramine maleate, 20 mg caffeine. Trictin tablets (Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt) labeled to contain 4 mg cyproheptadine hydrochloride. Histadin tablets (Amoun Pharmaceutical Ind. Co., El-Salam city, Cairo) labeled to contain 60 mg terfenadine. Hismanal tablets (Glaxo Egypt; Janssen Pharmaceutica Beerse-Belgium) labeled to contain 10 mg astemizole. Ranitidine tablets (Medical Union Pharmaceuticals Co., Ismailia Egypt) labeled to contain 150 mg ranitidine. Tagamet tablets (Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt) labeled to contain 200 mg cimetidine. Famotin tablets (Memphis Co. for Pharm. & Chem. Ind. Cairo, Egypt), labeled to contain 20 mg famotidine.

Preparation of standard solutions

Weigh accurately an amount of drug salt equivalent to 50 mg base, dissolve in about 20 ml distilled water and transfer to a 100 ml separating funnel. Render alkaline with about 5 ml of 33% ammonia solution and extract with five portions of 20 ml quantities of chloroform. Pass the separated organic layers through anhydrous sodium sulphate, about 0.5 gm suitably supported in a small funnel and wash the filter paper with about 5 ml chloroform. Evaporate chloroform under vacuum, dissolve the residue in about 10 ml methanol, transfer the resulting solution, quantitatively, into 25 ml

volumetric flask and complete to the mark with the same solvent. For drug base dissolve 50 mg, accurately weighed, into 25 ml methanol. In either case dilute the resulting solution quantitatively with the same solvent to obtain general concentration range 50-2000 μg/ml.

Tablets

For coloured sugar coated tablets, gently rub with water moistened filter paper and leave to dry overnight at room temperature. Weigh and finely powder 20 to 100 tablets (according to the labeled amount). Accurately weigh a portion of powdered tablets equivalent to 50 mg drug base. Transfer this amount to 25 ml volumetric flask and complete to volume with methanol. Shake for about 5 to 10 minutes, filter the resulting mixture and reject the first portion of the filtrate. Dilute the resulting solution quantitatively with the same solvent to obtain proper concentrations as specified under preparation of standard solution. For tablets containing drug salt, transfer an amount of powdered tablet equivalent to 100 mg drug base into 50 ml volumetric flask, then complete to volume with distilled water. Shake for about 5 to 10 min, filter the resultig mixture and reject the first portion of the filtrate. Transfer 25 ml (equivalent to 50 mg drug base) of this solution, accurately measured, to 100 ml separating funnel and continue as described under preparation of standard solutions starting from "render alkaline....."

General procedure

Pipet 1.0 ml of the standard or sample solutions into a dry 10 ml volumetric flask, followed by 1.0 ml of haematoxylin solution. Allow to stand for about 30 min at room temperature, then complete to volume with 3x 10^{-3} M aqueous boric acid and measure the absorbance at 555 nm against a reagent blank prepared similarly.

RESULTS AND DISCUSSION

With Haematoxylin solution, in $3x10^{-3}$ M boric acid, all the studied compounds (Scheme 1) showed a major band at 555 nm with apparent molar absorptivities of up to 4900.71 (Table 1). Typical absorption curve of this reaction is shown in Fig. 1. The reagent and any of the studied compounds have no absorption at this region.

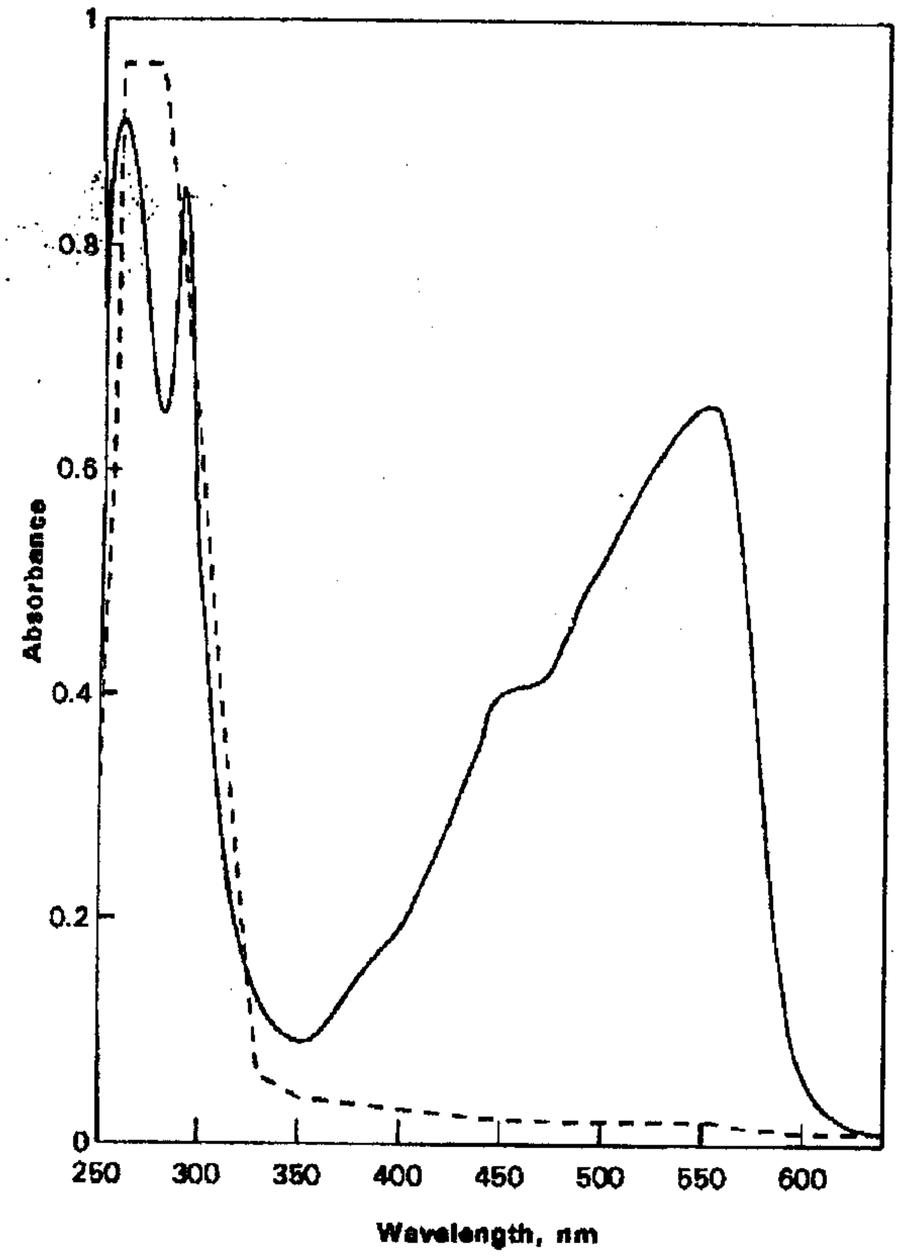
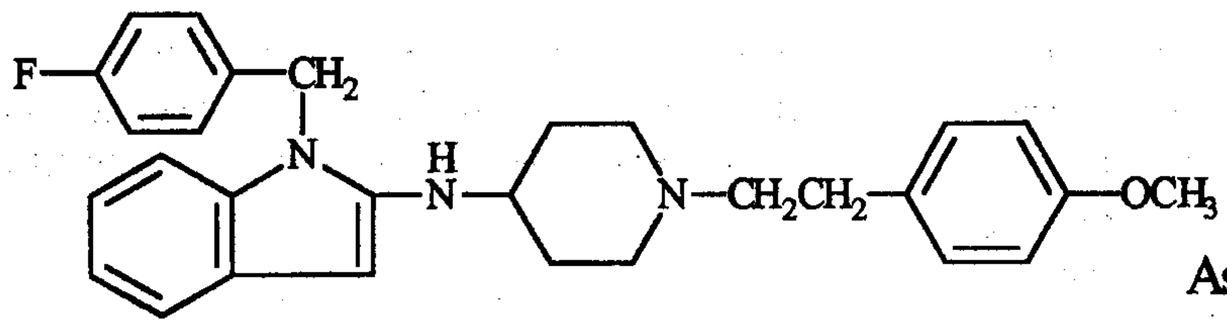


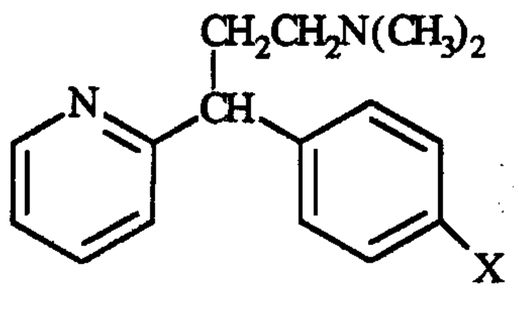
Fig. 1: Absorption spectra of haematoxylin 2 mg/ml (...) and its reaction with ranitidine, 42.4 μg/ml (-).

An aqueous solution of haematoxylin in $0.3x10^{-2}$ M boric acid showed an absorption band at about 292 nm. The consistency of the 555 nm band, in spite of the structural variations among the studied compounds, is probably due to haematein which is considered the oxidized form of haematoxylin[39]. Since haematein could be formed rapidly under alkaline condition[39], its formation is possible in the presence of any of the studied bases, as proved by the new band which is in agreement with the reported literature value[39], Scheme 2.

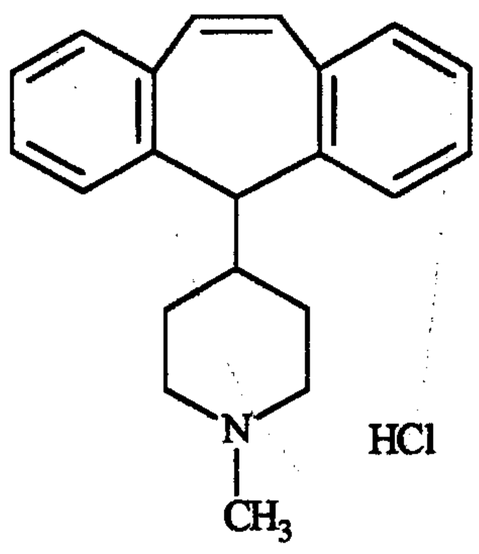
Being as indicator, the redish violet colour was reported to develop at pH 6.2[40], therefore the stability of the reagent was carefully investigated. Its aqueous solution darkens within short peroid of time upon standing at room



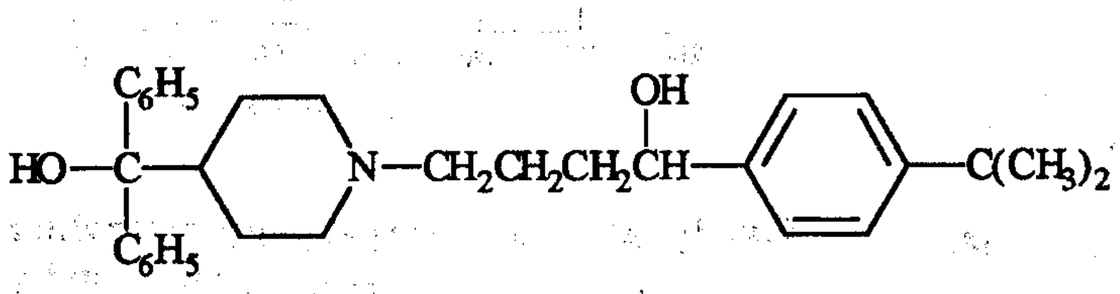
Astemizole



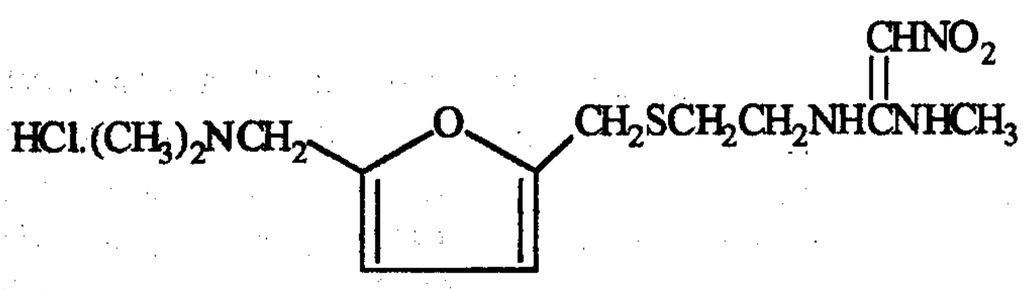
X = H Pheniramine maleate
X = Cl Chlorpheniramine maleate



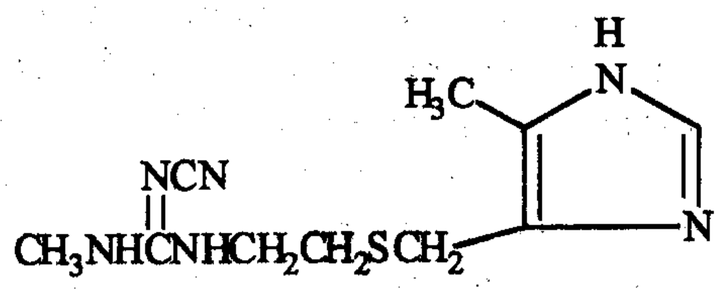
Cyproheptadine hydrochloride



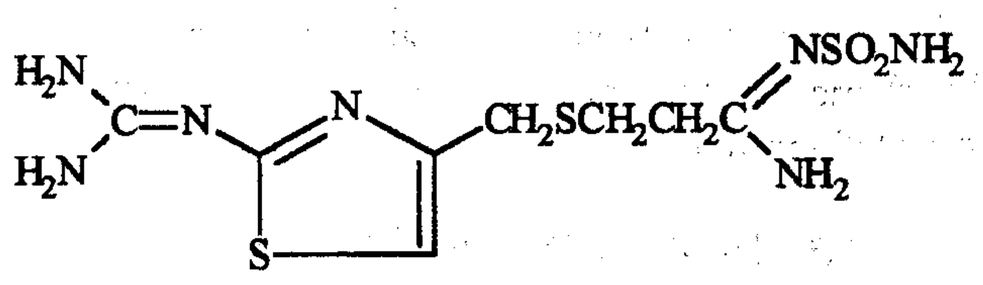
Terfenadine



Ranitidine hydrochloride

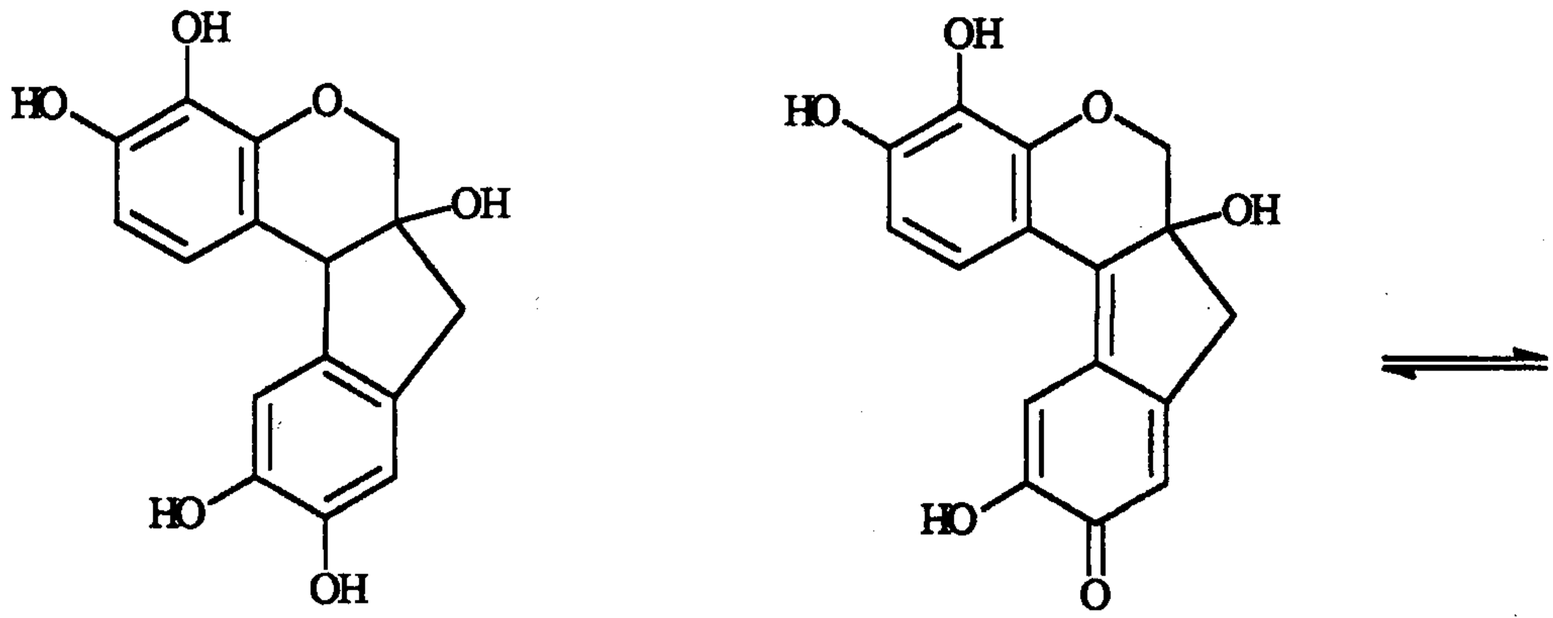


Cimetidine

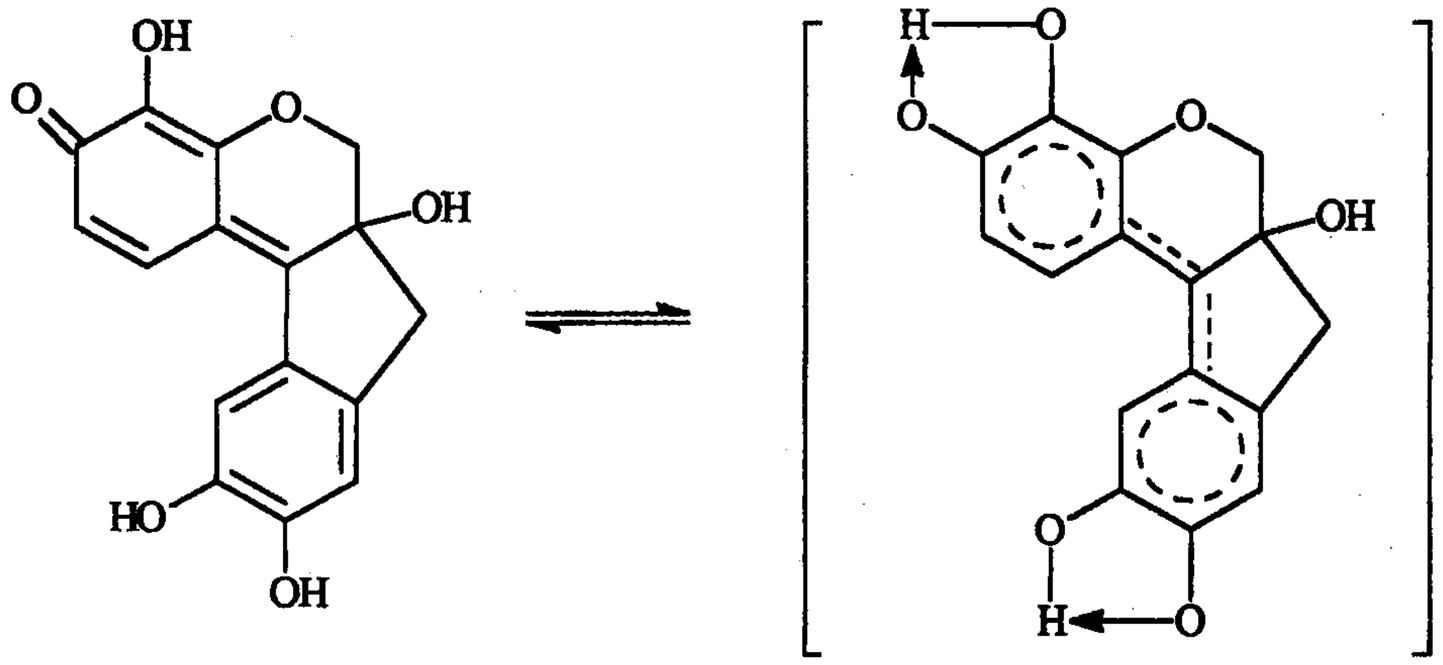


Famotidine

Scheme 1: Structure of the studied compounds.



Haematoxylin Haematein



Scheme 2: Haematoxylin reagent and Haematin coloured product.

Table 1: Some spectral characteristics of the investigated compounds.

Base	pK_a	Linear Range μg/ml	Inter-cept (a)	Slope (b)	Corr. Coeff. (r)	ϵ^*	A (1%, 1cm)	logA (1%, 1cm)	S #
Chlorphenir-amine	9.1[b]	5-80	-0.0134	0.0097	0.9996	2594	94.4	1.98	0.106
Pheniramine	9.3[b]	5-70	-0.0475	0.0116	0.9958	2566	106.8	2.03	0.094
Cyprohep-tadine	9.3[a]	5-90	-0.0029	0.0087	0.9997	2486	86.5	1.94	0.116
Astemizole	8.35[a]	10-100	-0.0294	0.0082	0.9997	3556	77.5	1.89	0.129
Terfenadine	8.58[a]	20-200	0.0018	0.0037	0.9977	1730	36.7	1.56	0.273
Ranitidine	8.3[a]	5-60	0.0429	0.0145	0.9939	4901	155.9	2.19	0.064
Famotidine	7.1[a]	80-180	0.0698	0.0021	0.9963	866	25.7	1.41	0.390
Cimetidine	6.8[b]	40-130	-0.0039	0.0034	0.9989	838	33.2	1.52	0.301

[a] Ref. 1 and [b] Ref. 46.

* Apparent Molar Absorptivity (l $mole^{-1}$ cm^{-1}).

Sandell's sensitivities (μg cm^{-2}/ 0.001 absorbance).

temperature even on dark. On the other hand, its solution in methanol was stable for about 2 days even if exposed to day light at room temperature, however no colour is observed upon mixing its solution with methanolic solution of any of the free base under investigation. In the last case colour is developed only upon dilution with water or water/methanol mixture. Thus several trials have been carried out to stablize the reagent in water such as using water/methanol mixture in different proportions, some aqueous buffers of pH 6-6.2 such as acetate, phosphate buffers and lastely sodium hydrogen sulphite as an anti-oxidant. With all cases either unstable colour or retardation of the chromogen development were observed. Boric acid, as 2 ml of 0.5 % (w/v), was reported as stabilizer for haematoxylin used for determination of some local anaesthetics[41] and alkaloids[42] of pharmaceutical interest. Thus the optimum concentration of aqueous boric acid was studied, where an about $3x10^{-3}$ M was found to be suitable solvent for the reagent. It was observed that higher concentrations of boric acid result in retardation of the colour development. Lower concentrations result in unstable, nonreproducible readings. Interestingly the chosen concentration of boric acid is equivalent to half of the reagent concentration which support the suggested haematoxylin borate complex[41,42]. The best amount of reagent for colour development was found to be within 0.6 to 1.4 ml of $6.6x10^{-3}$ M solution. Therefore 1 ml was selected throughout this work. Maximum absorbance readings at 555 nm were observed after mixing the reagent solution with methanolic solution of the free base for about 25-35 min duration at room temperature (25 ± 5°C), followed by dilution with $3x10^{-3}$ M boric acid and the colour developed was stable for further 20-25 min. The effect of diluting solvent was also investigated using $3x10^{-3}$ M aqueous boric acid, water, acetone ethanol, methanol, propan-1-ol and water/methanol mixture in different proporations. Maximum stability and sensitivity was observed with $3x10^{-3}$ M aqueous boric acid, others gave either lower or unstable readings. The colour formed was also found to be non-extractable by other solvent such as chloroform, methylene chloride, or dichloroethane which reflect the formation of highly ionizable chromogen extractable only by highly polar solvents.

Concentration ranges of the final assayed solution, slopes, intercepts and correlation cofficients as well as Sandell's sensitivities are given in Table 1. Under the specified reaction conditions, Beer's plots were obeyed with all the studied compounds. The detection limit of the proposed procedure was calculated according to a reported method and was found to be 0.058[43].

It was clear that good correlation exists between pK_a for six of the studied compounds and their corresponding log A (1 %, 1 cm). Regression analysis using least square line equation result in the following equation.

$$\log A = -0.056 + 0.22\ pK_a \quad (r = 0.9546) \quad . \text{ Eq 1}$$

Terfenadene and ranitidine were not included in this correlation, although their pka values: 8.3, 8.58 respectively[1], indicating that the pk_a may not be the only factor that determine the sensitivity of the colour development but there are others which should be taken into consideration. In case of ranitidine; good sensitivity can be explained based on the presence of furan ring's oxygen which may facilitate the approach of the reagent, through hydrogen bonding, toward the basic nitrogen. From Eq. 1, log A (1 %, 1 cm) for the coloured product resulted from the interaction of any antihistamine(s), structurally correlated to the investigated compound(s), with the reagent could be predicted from its corresponding pKa value(s).

Applying Job's method of continous variation[44], using equimolar concentration of either the drug base or haematoxylin ($5x10^{-4}$ M for all cases except $1x10^{-3}$ in case of cimetidine and famotidine), the reaction stoichiometry was proved to be 1:1 reagent : base respectively with all the studied compounds. This ratio is in aggreement with the presence of only one highly basic center (scheme 1), where some of these compounds are available as monoacid salt.

The reproducibility of the proposed method was checked through analyses of 5 replicate samples of the different investigated compounds, each containing suitable concentration within Beer's law limits of the free base/ml in the final test solution. At the chosen concentration level the Coefficient of Variation of 1.13 % - 1.40 % were obtained which is good enough for quality control analysis of the cited drugs either in bulk forms or in tablet preparations.

The results of Table 2 represent the recovery studies performed on two different amounts of drug salt or base each was analysed by the proposed and a compendial methods[10,11] and the obtained data are in good aggreement which indicate the accuracy and further confirm the suitability of the proposed method for control analysis and unit dose assay of the cited drugs. The applicability of the proposed procedure to commerical tablets and content uniformity was checked (Tables 3,4). Since placebos of commerical products were not available, the method of standard addition was adopted. Common tablet excipents such as lactose, starch, micro-crystalline cellulose, polyvidone, magnesium stearate, silica and sodium lauryl sulphate were found not to interfere in the method when following the described extraction procedure.

Table 2: Assay of antihistaminc Drugs in bulk form by proposed and official methods.

Compounds	Amount taken mg	% Found* Proposed	% Found* Official**	F#	t#
Chlorpheniramine maleate	100	99.18 ± 1.42	99.86 ± 1.33	1.14	0.37
	120	99.58 ± 1.18	98.68 ± 1.20	1.03	1.19
Pheniramine maleate	100	98.91 ± 1.15	99.00 ± 1.18	1.05	0.12
	120	99.38 ± 1.75	98.28 ± 1.06	2.73	1.20
Cyproheptadine	200	98.74 ± 1.12	98.38 ± 1.25	1.25	0.48
	250	98.58 ± 1.36	98.28 ± 1.39	1.03	0.35
Astemizole	50	99.12 ± 0.80	—	—	—
	100	99.34 ± 1.35	—	—	—
Terfenadine	250	99.22 ± 1.09	98.98 ± 1.49	1.87	0.29
	300	99.08 ± 1.62	98.78 ± 1.25	1.68	0.33
Ranitidine	50	99.46 ± 1.84	—	—	—
	100	98.74 ± 1.75	—	—	—
Cimetidine	200	99.22 ± 1.12	99.07 ± 1.11	1.02	0.22
	250	99.74 ± 1.78	98.87 ± 1.35	1.74	0.88
Famotidine	250	99.02 ± 1.88	98.66 ± 1.54	1.49	0.33
	300	99.99 ± 1.85	100.08 ± 1.53	1.46	0.07

* Average of five determinations.
** BP 1993 except USP 1995 for Terfenadine and Famotidine.
# at p = 0.05, F-test is 6.39 and t-test is 3.83.

Table 3: Analysis of certain antihistamines in presence of some other ingredients commonly present in tablet formulations.

Ingredients	Amountadded mg	% Recovery of antihistamine, ± SD*
<u>With chlorpheniramine maleate**</u>		
1- Caffeine	32	99.21 ± 0.83
2- Paracetamol	400	100.14 ± 1.41
3- Propyphenazone	200	98.48 ± 0.69
4- Phenylpropanolamine hydrochloride	24	701.40 ± 2.41
<u>With Pheniramine maleate**</u>		
1- Caffeine	15	98.54 ± 0.61
2- Phenazone	125	99.16 ± 1.11
3- Phenazone salicylate	250	98.84 ± 1.21
4- Sodium salicylate	125	99.94 ± 1.71

* Average of five determinations

** Ingredients added per 3 mg chlorpheniramine maleate or 15 mg pheniramine maleate.

Table 4: Analysis of some tablet formulations containing the studied drugs.

Tablets	label claim mg	% Found ± SD*	Added, mg	% Recovery ± SD*
Avil Retard[a]	75.00	98.96 ± 1.80	75.00	98.08 ± 1.24
Cosavil[a]	11.25	99.58 ± 0.93	11.25	99.28 ± 1.22
Allergyl[b]	4.00	99.86 ± 1.74	4.00	98.28 ± 1.86
Pirafine[b]	2.00	98.72 ± 1.61	2.00	99.52 ± 0.89
Trictin[c]	4.00	99.83 ± 1.96	4.00	100.60 ± 1.79
Hismanal[d]	10.00	99.32 ± 1.34	10.00	99.70 ± 1.72
Histadin[e]	60.00	99.92 ± 2.16	60.00	100.76 ± 1.75
Ranitidine[f]	150.00	100.42 ± 1.84	150.00	98.25 ± 1.27
Famotin[g]	20.00	99.86 ± 1.66	20.00	99.56 ± 1.23
Tagamet[h]	200.00	98.60 ± 0.66	200.00	99.60 ± 1.45

[a]: pheniramine maleate, [b]: chlorpheniramine maleate, [c]: cyproheptadine, [d]: astemizole, [e]: terfenadine, [f]: ranitidine, [g]: famotidine and [h]: cimetidine.

* n = 5 in all cases.

The proposed method was unaffected by the presence of caffeine, phenazone, phenazone salicylate, propyphenazone, paracetamol and sodium salicylate which are commonly present with chlorpheniramine or pheniramine maleates in tablet formulations. The single partition at alkaline pH of the procedure effectively separated the acidic compounds relative to drug base such as salicylate anion and paracetamol which are retained in the aqueous layer. Phenazone salicylate being insoluble in cold water[45], therefore most of it is expected to be

removed during the filtration step recommended during tablet sample preparation. Thus, any liberated phenazone from its salt, at alkaline pH, should not interfere with the base under investigation as indicated in Table 3. This observation can be accounted on the basis of weak basicity of phenazone (pK_a 1.2[46]) relative to pheniramine or chlorpheniramine base. By the same way negative interference of caffeine may be accounted based on the fact that this compound has very low pK_a values which in fact was considered as weak acid[46]. Table 3 shows the results obtained from the analysis of pheniramine or chlorpheniramine in presence of some other ingredients commonly co-formulated with either one of them. However, as clear from Table 3, the suggested method must be considered nonspecific with regard to differentation between them or when present together with other basic amine(s) such as phenylpropanolamine hydrochloride which is commonly co-formulated with chlorpheniramine maleate for treatment of common cold. These shortcommings do not affect the utility of the proposed method in routine analysis and content uniformity determination of singly prescribed antihistamines. The drawbacks may also be overcome by coupling the suggested method to a suitable separation procedure techniques such as paper chromatography, partition column chromatography or TLC.

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