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INCREASING SENSITIVITY OF COLORIMETRIC DETERMINATION OF BASIC DRUGS THROUGH ION PAIR COMPLEX FORMATION

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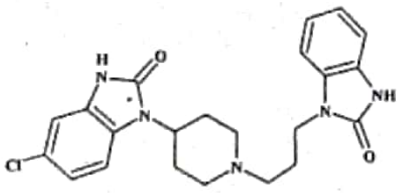
Abstract:

The method describes a colorimetric procedure for determination of some vasoacting drugs namely; domperidone, dipyridamole, and cinnarizine. In this procedure, metanil yellow and methyl orange were utilized in the determination of the concerned compounds by forming ion pair complex in aqueous solution at acidic pH's. Upon extraction of the ion pair into methylene chloride, the yellow extract showed absorption maxima at 413 and 425 nm for metanil yellow and methyl orange, respectively. Sensitivity has been increased nearly two times with metanil yellow and nearly six times with methyl orange on acidification of the methylene chloride extract with acidified ethanol which resulted in conversion of the yellow organic extract into magenta red color with absorption maxima at 541 nm and 525 nm for metanil yellow and methyl orange, respectively. The procedure was applied for the determination of these compounds in their formulations. Statistical analysis showed no significant difference between the proposed method and official or reported methods.

INTRODUCTION

Domperidone (**1**), dipyridamole (**2**), and cinnarizine (**3**) are prescribed as vasoacting drugs[1-3]. Many analytical methods have been adopted for the determination of these drugs in pharmaceutical preparations and biological systems. Reported methods are based on spectrophotometry,[4-15] spectrofluorimetry,[16-18] chromatography,[19-32] and electrochemical procedures[33-37]. In the present investigation, simple, sensitive, and rapid spectrophotometric procedure for the determination of the concerned drugs, using ion-pair complex formation with metanil yellow and methyl orange dyes has been developed.



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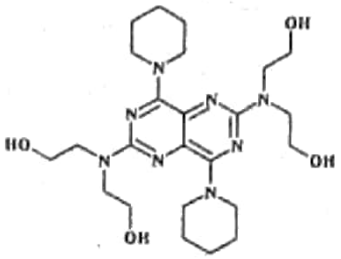

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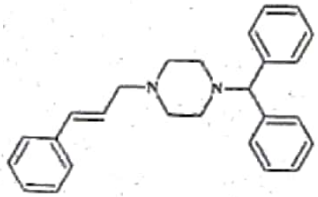

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EXPERIMENTAL

1. Apparatus:

Spectronic Genesys® UV/VIS spectrophotometer with 1 cm quartz cell (Milton Roy, USA), connected to an IBM PC computer loaded with the WINSPEC® software. Jenco® microcomputer pH-meter was used. All calculations were carried out on an IBM computer using Microsoft Excel 2003 and statistical methods in analytical chemistry (SMAC) program, designed by Meier and Zund.

2. Materials and reagents:

All solvents used were of analytical grade quality. Domperidone (Sedico, Egypt), dipyridamole (CID, Egypt), and cinnarizine (Minapharma, Egypt), were used as working standards without further purification. Metanil yellow {sodium m-[(p-anilinophenyl)azo]-benzene sulfonate} (S.D. fine chemicals, India), 0.1 g % in methanol (filtered if necessary).

Methyl orange {sodium p-dimethylaminoazobenzene sulfonate} (Winlab Chemicals, U. K.), 0.5 g % in water (dissolved in hot water and filtered).

Hydrochloric-acetate buffer; pH 2.4, 14 g sodium acetate trihydrate and 25 g sodium chloride were dissolved in 800 ml distilled water and concentrated hydrochloric acid was added dropwise till pH 3 then the pH was adjusted with 2 M hydrochloric acid till pH 2.4, the volume was completed to 1000 ml with distilled water.

Acetic-acetate buffer; pH 3.5, 14 g sodium acetate trihydrate and 25 g sodium chloride were dissolved in 800 ml distilled water and glacial acetic acid was added dropwise till pH 3.5, the volume was completed to 1000 ml with distilled water.

Acidified ethanol; 2 ml concentrated hydrochloric acid were mixed with 98 ml absolute ethanol.

Methylene chloride (El Nasr Chem. Co, Egypt).

Standard solutions were prepared as follows: domperidone, dipyridamole, and cinnarizine were prepared in the concentration of 5 mg % (w/v) in a few drops of concentrated hydrochloric acid and the volume was completed with distilled water.

Other chemicals like starch, glucose, lactose, sucrose, and magnesium stearate were purchased from (El Nasr Chem. Co, Egypt).

3. Pharmaceutical formulations:

Domperidone® tablets, B.N. 505154 (labeled to contain 10 mg domperidone per tablet), SEDICO, Egypt. Persantin® tablets B.N. 305103 (labeled to contain 75 mg dipyridamole per tablet), CID, Egypt. Stugeron® tablets, B.N. B5DE0467 (labeled to contain 25 mg cinnarizine per tablet), Minapharma, Egypt.

4. Standard solutions:

Standard drug stock solutions; 2 mg % (w/v): 5 mg of each drug were dissolved in a few drops of concentrated hydrochloric acid and volumes were completed by distilled water.

5. General procedures:

5.1. Construction of calibration graphs:

5.1.1 Aliquots containing 0.05-0.40 mg of the standard solutions of the concerned drug were transferred to a separating funnel followed by 20 ml of buffer and 5 ml of the dye solution, extracted with 20 ml of methylene chloride (added in three portions). The combined extracts were mixed, transferred into 20-ml measuring flask containing 2 ml of ethanol (Method A), or to 2 ml of acidified ethanol (method B) to complete to the volume. Reagent blank was prepared by the same procedure except addition of drug. The yellow color produced in method A was measured at 413 nm, and the red color produced in method B was measured at 541 nm against reagent blank.

5.1.2. Aliquots containing 0.04-0.20 mg (method C) or 0.008-0.040 mg (method D) of the standard solutions of the concerned drugs were transferred to a separating funnel followed by 20 ml of buffer and 5 ml of the dye solution, extracted with 20 ml of methylene chloride (added in three portions). The combined extracts were mixed, transferred into 20-ml measuring flask containing 2 ml of ethanol (Method C), or to 2 ml of acidified ethanol (method D) to complete to the volume. Reagent blank was prepared by the same procedure except addition of drug. The yellow color produced in method C was measured and at 425 nm, and the red color produced in method D was measured at 525 nm against reagent blank.

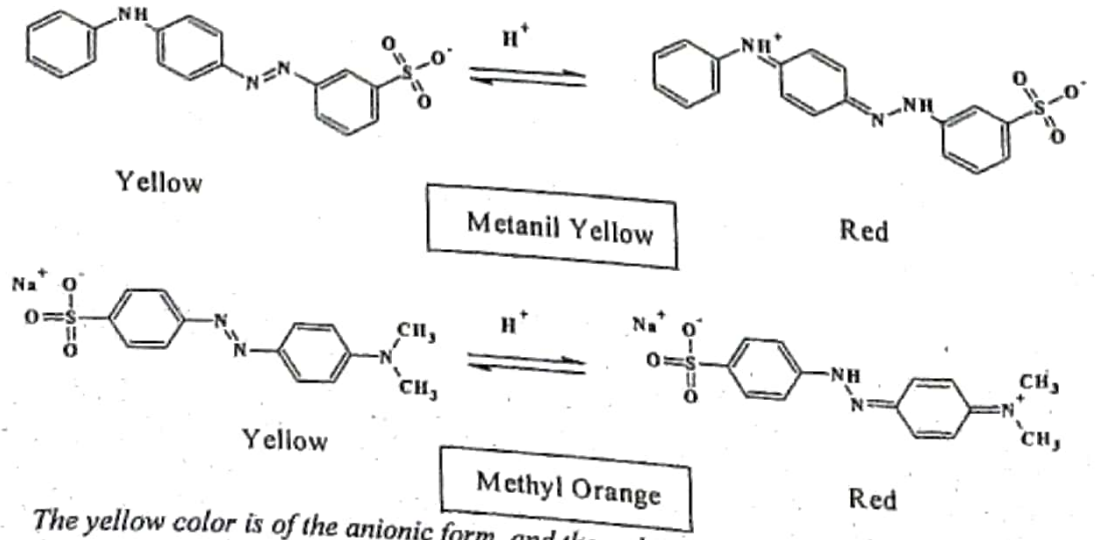
5.2. Analysis of pharmaceutical formulations:

Twenty tablets were accurately weighed and the average weight of one tablet was determined. The tablets were thoroughly powdered and an amount equivalent to 5 mg of the drug was taken and shaken with 20 ml of 2N hydrochloric acid for 5 minutes, the volume was completed to 100 ml with the buffer, filtered discarding the first 10 ml of filtrate, and the procedure was completed as under procedures 2.5.1.

RESULTS AND DISCUSSION

1. Theory:

Anionic dyes like metanil yellow and methyl orange in aqueous solution form extractable salts or ion pairs with the positively charged amino compounds at the proper acidic pH's. The yellow methylene chloride extract showed absorption maxima at 413 nm for metanil yellow and 425 nm for methyl orange. Attempts were made to increase the sensitivity through acidification of the organic extract with acidified ethanol. This modification resulted in converting these dyes into the red zwitter ionic forms of absorption maxima 531 nm with about two times enhanced sensitivity for metanil yellow and 525 nm for methyl orange with about six times greater sensitivity. In other words, acidification of the extracted ion pair resulted in both hyperchromic and bathochromic shifts (Fig. 1).



The yellow color is of the anionic form, and the red color is of the zwitter ionic form [38].

The addition of ethanol after extraction is necessary to prevent adsorption of the dye to the wall of the flask and to ensure homogeneity between the aqueous immiscible hydrochloric acid and the organic extract[39]. Anhydrous sodium sulfate was found to be unsatisfactory for drying the organic extract as it adsorbs the dye. The amount of dye in the aqueous layer should be sufficient enough as the excess did not affect the intensity of the color.

2. Optimization of variables:

2.1. Effect of pH:

Since pH is critical to the success of the method[39], we were urged to study the optimum pH's for each drug with each dye. It was found that optimum pH's were in the range of 2.2-2.6 and 3.3-3.7 for metanil yellow and methyl orange, respectively (Fig. 3). Acetate buffers were found to serve well in the mentioned ranges. Sodium chloride was included as an aid in affecting complete separation of the organic and aqueous phases.

2.2. Stability of the formed complex:

The stability of the formed ion-pair for all investigated drugs was studied. The color of the organic extract showed good stability for at least 24 hours.

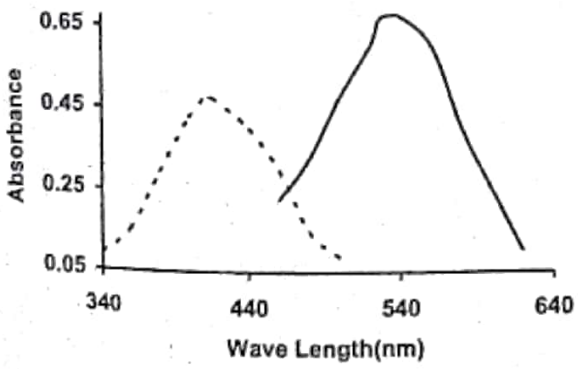


Fig. 1: Absorption curves of metanil yellow ion pair complex(....), and after acidification (—).

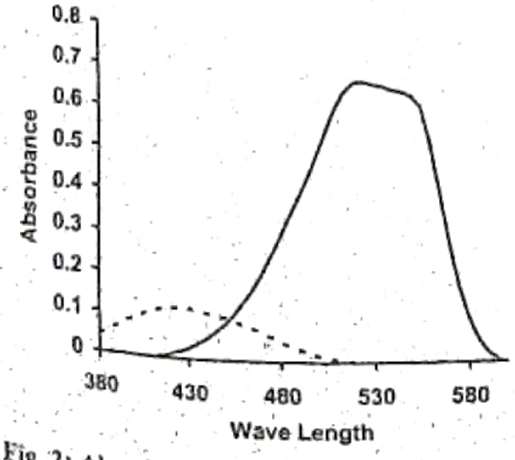


Fig. 2: Absorption curves of methyl orange ion pair complex (....), and after acidification (—).

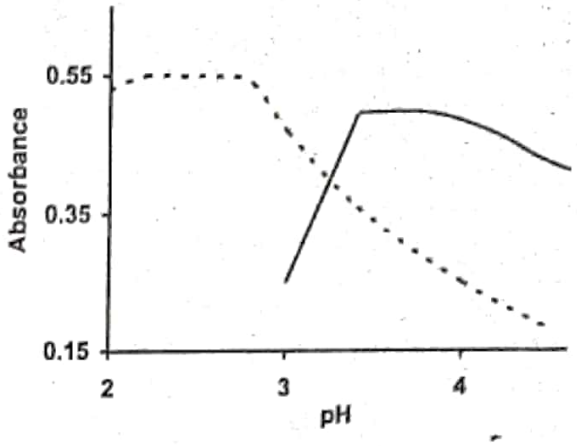


Fig. 3: Effect of pH on ion pair complex formation with metanil yellow (....), and methyl orange (—).

2.3. Effect of metanil yellow concentration:

The amount of metanil yellow should be sufficient enough as the excess does not affect the intensity of the color.

3. Quantifications:

Linear correlations were obtained between absorbance of extracted dye-drug (ion-pair) complex and concentration of the studied drugs over the ranges stated in table 1, with good correlations and small intercepts. The regression equations were derived using the least-squares method.[40] The intercepts (a), slopes (b), correlation coefficients (r), limits of detection (LOD) and limits of quantitation (LOQ) were calculated. The slopes were used as a measure of the sensitivity of the proposed method, while the intercepts are used as a measure of the interfering background (Table 1). Results indicated higher sensitivity and lower background effect of the proposed methods B and D (after acidification) than methods A, and C.

Table 1: Optical characteristics, Beer's law data and statistical analysis for the proposed methods:

Drug	Method	Beer's law (μg ml^{-1})	a	b	r	LOD μg ml^{-1}	LOQ μg ml^{-1}
Domperidone	A	2.5-50	0.012	0.165	0.9987	0.732	1.91
	B	1.25-25	0.014	0.187	0.9991	0.3879	1.155
	C	2-20	0.011	0.188	0.9997	0.432	1.54
	D	0.4-4	0.009	0.179	0.9996	0.152	0.351
Dipyridamole	A	2.5-50	-0.0168	0.177	0.9994	0.821	1.86
	B	1.25-25	-0.018	0.182	0.9993	0.259	1.151
	C	2-20	0.01	0.178	0.9995	0.432	1.52
	D	0.4-4	-0.013	0.183	0.9996	0.163	0.356
Cinnarizine	A	2.5-50	-0.0137	0.148	0.9991	0.809	1.81
	B	1.25-25	0.0168	0.165	0.9994	0.324	1.154
	C	2-20	0.018	0.183	0.9997	0.432	1.53
	D	0.4-4	0.021	0.163	0.9999	0.169	0.355

a: Intercept, b : Slope, r : Correlation coefficient, LOD: Limit of detection, LOQ : Limit of quantitation.

4. Validation of the proposed procedure:

4.1. Accuracy and precision:

In order to study the accuracy and precision[41], of the proposed methods, standard solutions containing three different concentrations of the concerned drug and eight measurements were made on each reaction product according to the general procedure. The overall standard deviations and the overall relative standard deviations are summarized in Table 2. The small relative standard deviations indicate high precision and accuracy.

4.2 Selectivity:

In the present study, the procedure was carried out for analysis of the concerned drug in the presence of some common excipients and additives such as starch, glucose, lactose, sucrose, and magnesium stearate to explore their effects on the results. Samples were prepared by mixing known amount of the concerned drug with various amounts of the common excipients. It was shown that there was no interference from the frequently encountered excipients and additives reflecting the selectivity of the proposed method.

Table 2: The accuracy and precision of the proposed methods at three concentration levels.

		A	B	C	D
Domperidone	X	99.0	99.45	99.59	99.76
	± S.D.	0.785	0.821	0.985	0.968
	R.S.D.	0.793	1.01	1.03	1.04
Dipyridamole	X	99.4	99.48	99.66	99.57
	± S.D.	0.851	0.914	0.954	0.935
	R.S.D.	0.861	0.9142	0.998	0.977
Cinnarizine	X	99.38	99.85	99.55	99.34
	± S.D.	0.892	0.88	0.671	0.774
	R.S.D.	0.899	0.886	0.661	0.599

X: The mean, **SD:** The standard deviation, **RSD:** The relative standard deviation.

Table 3: Determination of the studied drugs in their pharmaceutical preparations by the proposed and reported methods

Drug	Preparation	Parameter	Proposed method				Reported methods[4,6,31]
			A	B	C	D	
Domperidone	Domperidone tablets	X	98.64	98.67	99.01	99.211	99.66
		± S.D.	1.0058	0.8333	1.0821	1.022	0.6037
		t	1.956	2.164	2.01	1.951	
		F	2.776	4.571	3.213	5.608	
Dipyridamole	Persantin tablets®	X	99.13	99.34	99.11	99.54	99.83
		± S.D.	0.9741	0.9659	0.881	0.921	0.772
		t	1.296	0.8939	1.97	1.821	
		F	1.627	1.251	1.989	1.824	
Cinnarizine	Stugeron tablets®	X	99.46	99.95	99.34	99.76	99.66
		± S.D.	0.751	0.5056	0.44	0.632	0.717
		t	0.4781	0.9424	1.051	1.752	
		F	2.918	2.01	2.655	3.28	

Five determinations were used for the reported and proposed methods. Tabulated values at 95 % confidence limit, t = 2.31 and F = 6.3.

4.3. Analysis of pharmaceutical preparations:

The proposed methods were applied to the determination of the cited drugs in commercial tablets. Five replicate determinations were made. Satisfactory results were obtained and were in a good agreement with the label claims (Table 3). The results obtained were compared with those of the reported methods namely, colorimetric through ion pair formation with bromophenol blue[4], second-derivative spectrophotometric determination[6], and reversed phase HPLC[31]. Statistical analysis of the results was performed with regard to accuracy and precision using Student's *t*-test and *F*-ratio at 95% confidence (Table 3). There is no significant difference between the proposed methods and official or reported methods with regard to accuracy and precision.

CONCLUSION

The proposed method is economical with reasonable precision and accuracy. In addition, it has greater sensitivity compared to the ordinary ion pair method. The validity of the proposed method was well demonstrated by analyzing dosage forms of domperidone, dipyridamole, and cinnarizine. Moreover, the method was free from interference by common additives and excipients. These merits, in addition to the use of simple reagents, suggest their utility for routine quality control.

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زيادة حساسية طريقة الإزدواج الأيوني للتقدير اللوني للعقاقير القاعدية

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يصف هذا البحث طريقة التقدير اللوني لبعض العقاقير الفاعلة على الأوعية الدموية وهم الدومبيريدون والسيناريرين والديبيريدامول حيث أنه تم استخدام صبغات أصفر الميتانيل وبرتقالي الميثيل في تقدير هذه المركبات بتكوين متراكب المزدوج الأيوني في وسط مائي حامضي الأس الهيدروجيني وبعدها تم استخلاص متراكب المزدوج الأيوني بمذيب الميثيلين كلورايد. الخلاصة الناتجة أظهرت قصوى إمتصاصا للضوء عند الأطوال الموجية ٤١٣, ٤٢٥ ن م مع أصفر الميتانيل وبرتقالي الميثيل بالتوالي. ولكنه بعد اضافة الإيثانول المحمض للخلاصة العضوية تغير لون الخلاصة من اللون الأصفر إلى اللون الأحمر بقصوى امتصاص للضوء عند الأطوال الموجية ٥٤١, ٥٢٥ ن م مع أصفر الميتانيل و برتقالي الميثيل بالتوالي مع زيادة قدرة الإمتصاص إلي الضعف تقريبا مع أصفر الميتانيل وإلى الست أضعاف تقريبا مع برتقالي الميثيل. كذلك تم تطبيق هذه الطرق لتقدير المركبات موضوع الدراسة في صورها النقية وأشكالها الصيدلية, كما تم إجراء تحاليل إحصائية لكافة النتائج التي تم الحصول عليها ومقارنتها بنتائج الطرق المنشورة, الأمر الذي اتضح منه عدم وجود اختلاف معنوي مع نتائج الطرق المنشورة.