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Spectrophotometric Determination of Ornidazole, Secnidazole and Tinidazole in Pharmaceutical Preparations Based on Formation of Dyes

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SIMPLE and accurate spectrophotometric method was developed for the assay of ornidazole (I), secnidazole (II) and tinidazole (III) in tablet dosage form. The method depends on the reduction of the drugs with zinc dust and hydrochloric acid followed by condensation with p-hydroxy benzaldehyede at 50°C to produce colored chromogens having absorbance at 402 nm for drugs I, II and III. The optimum experimental conditions for the proposed method were optimized and Beer's law was obeyed in the concentration ranges of 20-300 μg ml^{-1} for drugs I and II 20-250 μg ml^{-1} for drug III. The apparent molar absorptivities of the resulting colored products were found to be $1.00x10^2$, $1.24x10^2$ and $1.70x10^2$ L mol^{-1} cm^{-1}, whereas Sandell sensitivities are $4.5x10^{-6}$, $3.9x10^{-6}$ and $6.7x10^{-6}$ μg cm^{-2} for I, II and III, respectively. The proposed method was applied successfully for the analysis of the investigated drugs in tablet form. The results obtained were statistically compared with a reported method using the student's t -test and F-variance ratio tests.

Keywords: Ornidazole, Secnidazole, Tinidazole and P- hydroxybenzaldehyde .

Nitro heterocyclic compounds having wide variety of applications varying from food preservatives to antibiotics, 5-nitroimidazoles are well-established group of anti protozoan and antibacterial agents. Due to its antimicrobial activity it inhibits the growth of both anaerobic bacteria and certain anaerobic protozoa such as trichomonas, vaginalis, entamoeba histolytica and giardina iamboa[(1)]. Various methods were used in the determination of ornidazole (I) including high performance liquid chromatography[(2,3)], high performance thin layer chromatography[(4,5)], electrophoresis[(6)] and spectrophotometery[(7)], for secnidazole (II) high performance liquid chromatography[(8,9)], electrochemical method [(10)] and spectrophotometery[(11)] and for tinidazole (III) high performance liquid chromatography[(12)], electrochemical method[(13)], spectrophotometery[(14)] and electrophoresis [(6)].

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This paper describes sensitive and simple spectrophotometric method for the determination of ornidazole (1-Chloro-3-(2-methyl-5-nitro-imidazol-1-yl)-propan-2-ol), secnidazole (1-(2-Methyl-5-nitro-imidazol-1-yl)-propan-2-ol) and tinidazole (1-(2-Ethanesulfonyl-ethyl)-2-methyl-5-nitro-1H-imidazole) in either pure form or in their pharmaceutical formulations, the spectrophotometric method is based on the reduction of the nitro group of the cited drugs with zinc dust and hydrochloric acid followed by condensation with P-hydroxy benzaldehyde to form colored dye. The scientific novelty of the present work is that the reagents used in the method is easily available and the chemistry of these reagents is already well established. The proposed method involved is simple, rapid and sensitive in their range of determination compared with other established methods.

Experimental

Apparatus

A Shimadzu 160 A UV/visible double beam spectrophotometer with matched quartz cells of 1-cm optical bath was used.

Materials

ornidazole (I) was supplied from Sigma Co., USA; secnidazole (II) from Rhone-Poulence rorer Co., France; while tinidazole (III) was supplied from Sigma Co., USA they were used as working standards, p-hydroxy benzaldehyde was supplied from Aldrich Chemical Co., USA . The pharmaceutical dosage forms are tibezole tablet 500 mg ornidazole/tab., (Sigma), secnidazole tablet 500mg secnidazole/tab (Egypt Pharma) and prtozol tablet 500 mg tinidazole /tab (El-mehan El-tebiaa)

Reagents

All the reagents and solvents used were of analytical grade. Stock solution of drugs I, II and III containing 1mg ml^{-1} in bidistilled water from all glass apparatus. The stock drug solutions were stable for at least 3 days if they had been kept in cool and dark place at temperature below 20 ^{0}C, P-hydroxy benzaldehyde was prepared by dissolving 0.2% w/v in methanol.

General procedure

50 ml from the standard solution of each drug ($1x10^{-3}$ M) was transferred into 100 ml Erlenmyer flask, 5 ml of 1M hydrochloric acid and 2 g of zinc dust were added with occasional shacking. The flask was allowed to stand for one hour at room temperature. In 100 ml volumetric flask the reaction mixture was filtered through pre-wetted filter paper (whatman No.41) and washed twice with 20 ml of distilled water and complete to the volume with water. In 10 ml measuring flask aliquots of reduced drug standard solution of 10-250 μg ml^{-1} form drugs I, II and 1-300 μg ml^{-1} form drug III were added then the volume was completed to 1 ml with 0.1 M HCl. To each flask 2 ml of p-hydroxy benzaldelyde solution (0.2% w/v) was added and mixed well. The reaction mixture was heated in water

bath at 50 ° C for 30 minu and cooled. The volume was completed to the mark with distilled water. The absorbance was measured at 402 nm against blank prepared similarly without the drug solutions.

Assay of pharmaceutical tablets

Twenty tablets of the cited drugs were accurately weighed and average weight of tablet was calculated. The tablets were crushed well to fine powder. A portion of the powdered equivalent to 100 mg of active material was dissolved in 50ml water through vigorous shaking during 5 min; the solutions were filtered through a Whatman No. 41 filter paper into 100 ml volumetric flask and then diluted to volume with distilled water. The procedure was completed as described before for the cited drugs.

Results and Discussion

The utilization of a variety of chromophoric reagents after the reduction of the aryl nitro groups was the basis of the sensitive spectrophotometric determination of many nitro groups containing pharmaceutical compounds. The developed method is based on the reduction of the nitro group of the investigated drug to an amino group with zinc dust and hydrochloric acid flowed by coupling with p-hydroxy benzaldhyde to form colored chromogen which is measured at 402nm. The reaction of the reduced drug solutions with the aldehyde was carried out in the presence of 1M of HCl. The reaction of the primary aromatic amine and p-hydroxy benzaldehyde takes place through the condensation of the protonated primary amino group with the carbonyl group of the reagent to produce the imminium salt. Investigations were carried out to establish the most favorable conditions for the reaction and to achieve maximum color development in the quantitative determination of the drugs I, II and III.

For the reduction procedure, the use of 5 ml 1M HCl with 2 g zinc dust, at room temperature (22±1) °C, was found to be optimal. It was found that one hour is sufficient to yield maximum absorbance. It was found that by increasing the added volume of p-hydroxy benzaldelyde solution (0.2% w/v) the absorbance increased until we reached to the optimum volumes after which any increase in the volumes caused no change in the absorbance due to the complete formation of the ion pair. The optimum volumes of the aldehyde were found to be 1.0, 1.2 and 0.8 ml for I, II and III, respectively, as shown in Fig 1. It was found that, heating the reaction mixture in a water bath for 30 min enhance the color development until we reached 50 ^{o} C after which any increase in temperature caused decrease in absorbance as shown in Fig 2 (a, b). The colored product was proved to be stable for about more than 1 day for drug I. II and III, respectively.

Applying the continuous variation and molar ratio methods for the reaction between the drugs and reagent the stoichiometric ratio of the drugs and reagent were found to be 1: 2, as shown in Fig. 3 (a, b).

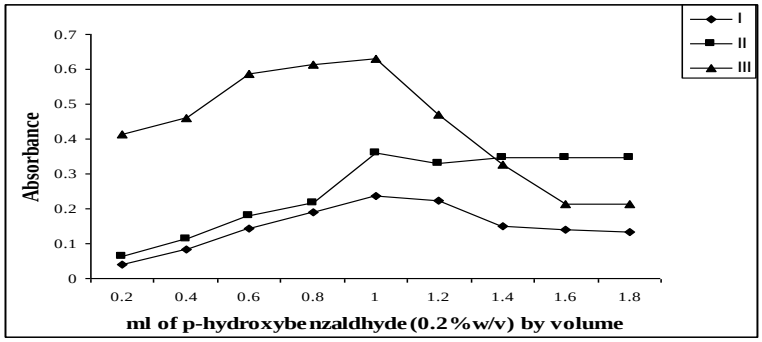

Fig.1. Effect of variable concentrations by volume of p-hydroxybenzaldhyde (2% w/v) on the ion pair formation with the cited Drugs($1x10^{-3}$M) at λ_{max}= 402nm and T=50^{O}C.

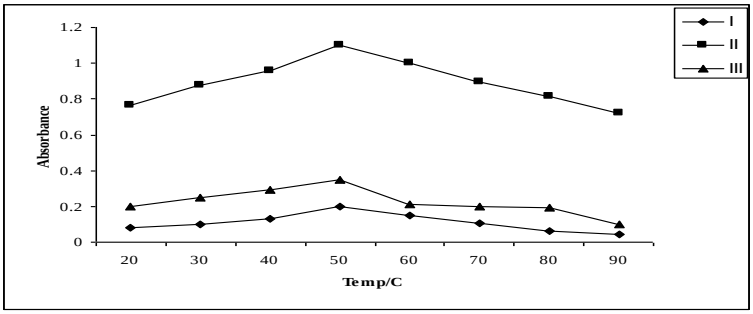

Fig. 2a. Effect of temperature on the reaction between ornidazole (I), secnidazol (II) and tinidazol (III) ($1x10^{-3}$M) and p-hydroxybenzaldhyde (2% w/v) at λ_{max} = 402nm and.

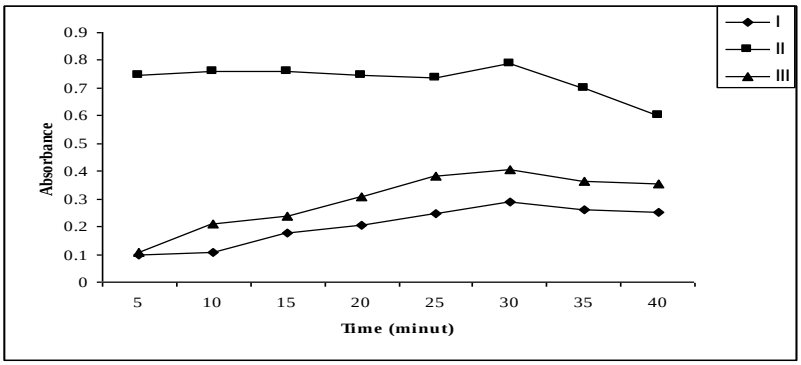

Fig. 2b. Effect of heating time needed for complete formation of the ion pair between hydroxybenzaldhyde (2% w/v) and the cited drugs($1x10^{-3}$M) at λ_{max}= 402nm and T=50^{O}C.

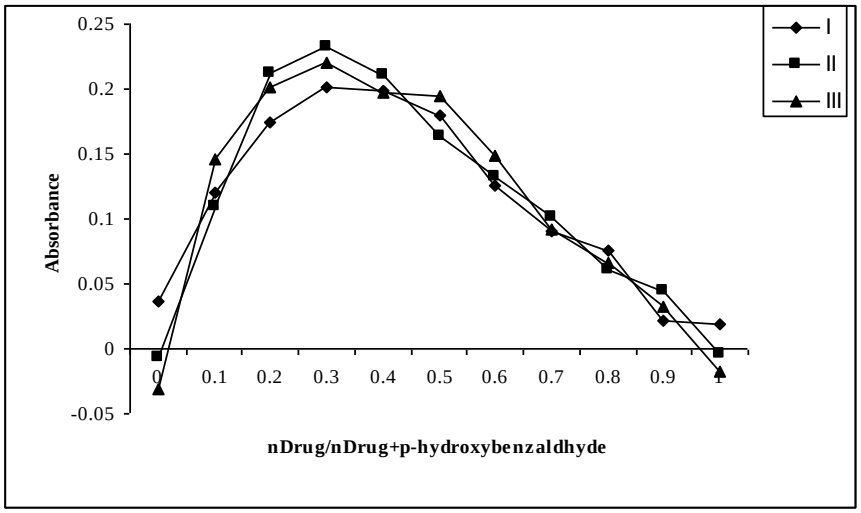

Fig.3 a. Stoichiometric ratio of the reaction of p-hydroxybenzaldhyde with cited drugs using contentious variation method. [Drug]=1x10^{-3}M at λ_{max}= 402nm and T=50^{O}C.

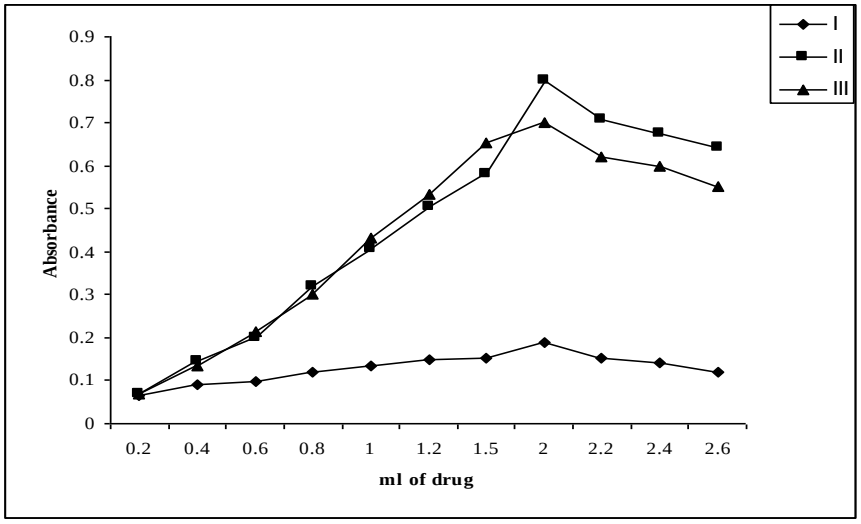

Fig. 3 b. Stoichiometric ratio of the reaction of p-hydroxybenzaldhyde (2% w/v) with cited drugs using molar ratio method. [Drug] = 1x10^{-3} M at λ_{max}= 402nm and T=50^{O}C.

Construction calibration and precision

Typical calibration data for drugs I, II and III with p- amino benzaldelyde reagent obtained form linear regression analysis of the absorbance readings versus concentration of the drugs (1.0 mg ml^{-1}) were made. Beer 's low limits were 10-250 µg ml^{-1} for drug I, III and 10-300 µg ml^{-1} for drug II at λ_{max} 402 nm, respectively. Moreover, the Ringbom concentration ranges can be calculated which gave accurate results and found to be 40-250, 60-220 and 40-150µg ml^{-1} for drugs I, II and III, respectively. The apparent molar apsorbtivities of the resulting colored complex are found to be $6.04x10^{-6}$ and $6.8x10^{-6}$ mol^{-1} cm^{-2} for drugs I, II and III, respectively (Table 1).

TABLE 1. Analytical parameters for the drugs I,II and III determination using the proposed method.

	Reading		
Parameters	**Ornidazole(I)**	**Secnidazole(II)**	**Tinidazole(III)**
λ_{max}(nm)	402	402	402
Molar absorptivity (L mol^{-1} cm^{-1})	$6.0 x10^2$	$1.2 x10^2$	$1.69 x10^2$
Sandell sensitivity (µg cm^{-2})	$6.7 x10^{-6}$	$6.6 x10^{-6}$	$6.8 x10^{-6}$
Beer's low limits (µg ml^{-1})	20-300	20-300	20-250
Ring bom limits (µg ml^{-1})	20-250	60-220	40-150
Retrogression	0.38	0.59	0.684
equation*	0.03	0.097	0.01
Slope(b)	0.991	0.994	0.995
Intercept(a)	0.82	0.52	0.87
Coefficient of determination(r^2)	0.991	0.994	0.995
RSD%	0.82	0.52	0.87

A=a+ b c, where c is the concentration in µg ml^{-1}, A is the absorbance

In order to determine the accuracy and precision of the proposed procedure, solutions containing three different concentrations of each drug were prepared and analyzed in five replicates. The results obtained from the investigation are summarized in Table 2 .The standard deviations (SD), the relative standard deviations (RSD %) considered to be very satisfactory. Table 3 shows the results obtained for the determination of drugs I, II and III in pharmaceutical tablets by the proposed and official methods [15]. There were not any significant differences between the results.

TABLE 2. Assay of the drugs I, II and III in bulk powder by the proposed method.

Compound	Wt. $_{taken}$ μgml^{-1}	Wt. $_{found}$ μgml^{-1}	Recovery%	SD	RSD*
Ornidazole	20	20.13	100.65	0.005	2.13
	50	50.13	50.13	0.002	0.21
	100	99.92	99.92	0.043	0.61
	150	149.95	99.96	0.008	0.56
	200	200.10	100.05	0.010	0.59
Secnidazole	20	19.20	96.00	0.005	0.59
	50	50.31	100.62	0.008	0.63
	100	99.87	99.87	0.010	0.47
	150	150.21	100.14	0.020	0.72
	200	200.10	100.50	0.080	0.21
Tinidazole	20	20.25	101.25	0.010	2.30
	50	50.05	100.10	0.080	0.80
	100	100.96	100.96	0.003	0.18
	150	149.92	99.94	0.090	0.48
	200	200.10	100.05	0.015	0.63

*number of replicates n =5

TABLE 3. Determination of I, II and III in pharmaceutical preparations using proposed and official methods .

Pharmaceutical	Wt. taken $\mu g\ ml^{-1}$	Wt. found $\mu g\ ml^{-1}$ proposed	Wt. found $\mu g\ ml^{-1}$ official	SD_1*	SD_2**	F-test	t-test	% Recovery Proposed
Tibezole tablets (500 mg/tablet)	50	50.14	50.25	0.040	0.070	1.75	2.27	100.25
	70	70.97	70.93	0.035	0.090	2.57	2.18	101.38
	120	119.50	120.32	0.007	0.007	1.07	2.75	99.58
	150	149.12	149.00	0.008	0.009	1.13	2.10	99.41
	200	200.25	199.25	0.020	0.035	1.75	2.37	100.13
Secnidazole tablets (500 mg/tablet)	50	49.99	50.11	0.092	0.13	1.41	2.81	99.98
	70	69.69	70.21	0.069	0.079	1.14	2.7	99.55
	120	120.35	119.45	0.001	0.002	1.90	2.23	100.29
	150	150.25	149.25	0.006	0.007	1.17	2.22	100.16
	200	199.25	200.21	0.079	0.088	1.11	2.10	99.63
Protozol tablets (50 mg/tablet)	50	50.05	49.99	0.082	0.095	1.15	2.09	100.10
	70	69.99	70.21	0.012	0.043	3.50	2.68	99.98
	120	120.87	119.58	0.029	0.085	2.90	2.55	100.73
	150	150.99	150.24	0.092	0.099	1.07	2.08	100.66
	200	199.99	200.23	0.025	0.045	1.80	2.35	99.99

SD_1* for proposed method
SD_2** for official method
Tabulated t-value at 95% confidence limit=2.77, n=5, degree of freedom =4
Tabulated F-value at 95% confidence limit=6.39, n=5

Conclusion

The method found to be simple, economical, selective and more sensitive than most of the spectrophotometric reported methods. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. This approach could be considered for the determination of the cited drugs in the quality control laboratories.

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دراسات تحليلية لتقدير بعض العقاقير المضادة للطفيليات فى مستحضراتها الصيدلية

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يهدف هذا البحث إلى إيجاد طريقة بسيطة، جيده و حساسة لتقدير ثلاثه عقاقير مضادة للطفيليات هم اورنيدازول، سكنيدازول و تنيدازول فى الخامات الدوائية و المستحضرات الصيدلية.

تعتمد الطريقة على اختزال هذه العقاقير بإستخدام تراب الزنك فى وجود حمض الهيدروكلوريك كوسط حمضى ثم تكثيفه مع 3- هيدروكسيد البنزالدهيد عند درجة حرارة 50° مئوية لتكوين صبغة ملونة و إستخدام طريقة التقدير الطيفى لتقدير تركيز هذه العقاقير.

وقد تمت دراسة تأثير العوامل المختلفة مثل درجة الحرارة ، تركيز المتفاعلات و الوقت . و بعد اختيار الظروف المناسبة تم تطبيق هذه الظروف لتقدير العقاقير فى المواد الخام و المستحضرات الصيدلية.