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DETERMINATION OF DILOXANIDE FUROATE AND METRONIDAZOLE IN PRESENCE OF EACH OTHER

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ABSTRACT

Determination of metronidazole and diloxanide furoate in presence of each other was performed using, thin layer chromatography, charge transfer complexation, colorimetry and derivative spectrophotometry.

INTRODUCTION

Metronidazole has strong antiprotozoal and bactericidal actions[1]. Several methods have been reported for metronidazole assay including pharmacological[2], colorimetric[3,4], high pressure liquid chromatographic[5] and spectrophotometric techniques[6].

Diloxanide furoate is the drug of choice in the treatment of asymptomatic intestinal amoebiasis[1]. The most recent methods for determination of diloxanide furoate included biocehmical[7], chromatographic[8,9], titrimetric[10] and spectrophotometric ones[11].

EXPERIMENTAL

Apparatus :

Aluminium sheets coated with silica gel, using developing systems : ethylacetate, chloroform, dioxan, and methanol (8 : 7 : 2 : 3); ultraviolet detector and a Shimadzu recording spectrophotometer U.V. 260 was used.

Materials and Reagents :

Metronidazole Rhone Poûlenc Co., Paris, FRANCE; diloxanide furoate, Pharco Co., EGYPT; Furamebe Forte tablets, Sedico Co., EGYPT, labeled to contain 250 mg diloxanide furoate and 200 mg metronidazole per tablet.

Liebermann's reagent: 5 g sodium nitrite were added to 50 ml of sulphuric acid while cooling and swirling to absorb the brown fumes.

Standard solutions:

Ethanolic stock solutions of either metronidazole or diloxanide furoate (50 mg/100 ml) were prepared for thin layer chromatography and derivative spectrophotometry.

5×10^{-5} M solutions of the two drugs were prepared in methylene chloride for charge transfer complexation.

δ acceptor: Iodine solution, 2.5×10^{-3} M was prepared in methylene chloride.

Methanolic stock solutions (40 mg/100 ml) and 18 N sulphuric acid stock solutions (40 mg/100 ml) of either metronidazole or diloxanide furoate were prepared for colorimetric techniques.

Procedures :

I. Thin layer chromatography :

Ethanolic stock solutions of metronidazole, dilozanide furoate and a laboratory prepared mixture of them were prepared. Sample solutions were applied using the capillary pipette. The chromatogram was developed applying ascending technique. The plate was allowed to dry at room temperature and the spots were detected by U.V. absorption detector.

II. Charge transfer complexation :

Accurately measured aliquots of the standard solution of metronidazole in methylene chloride (1-5 ml) were mixed with equal volumes of iodine solution into 25 ml volumetric flasks. The content of each flask was diluted to the mark with methylene chloride. After 25 minutes, the absorbane was measured at 295 & 360 mm against a reagent blank prepared under the same conditions.

Accurately measured equal aliquots of standard solutions of metronidazole and diloxanide

furoate in methylene chloride (1-5 ml) were transferred into 25 ml volumetric flasks. Aliquots of iodine solution equal to the sum (2-10 ml) were added. The procedure was completed as above.

III. Colorimetry :

a- Methanolic potassium hydroxide[13]:

Calibration curve :

Accurately measured equal aliquots of the methanolic standard solution of metronidazole (1-5 ml) were pipetted into five separate test tubes. 2 ml of 20% methanolic potassium hydroxide were added to each test tube and heated, if necessary to boiling point, to develop the color. Cool, transfer the contents of each test tube into 25 ml volumetric flasks and complete to the mark with methanol. The absorbance was measured at 552 nm against a reagent blank prepared under the same conditions and a calibration curve was constructed.

Accurately measured methanolic standard solutions of metronidazole and diloxanide furoate (1-5 ml) were transfered into separate test tubes. 4 ml of 20% methanolic potassium hydroxide were added to each test tube and the assay was completed as above.

b- Liebermann's test[13] :

Calibration curve :

Accurately measured equal aliquots of the 18 N sulphuric acid stock solution of diloxanide furoate (1-5 ml) were pipetted into five separate test tubes. 3 drops of the Liebermann's reagent were added to each test tube and heated in a waterbath at 100°C for 5 minutes. Cool, transfer the contents of each test tube into 25 ml volumetric flasks and complete to the mark with 18 N sulphuric acid. The absorbance was measured at 392 nm against a reagent blank prepared under the same conditions and a calibration curve was constructed.

Accurately measured 18 N sulphuric acid stock solutions of diloxanide furoate and metronidazole (1-5 ml) were transferred into five test tubes, 6 drops Liebermann's reagent were added to each test tube and the procedure was completed as above.

IV. Derivative spectrophotometry :

Accurately measured equal aliquots of either metronidazole or diloxanide furoate ethanolic stock solutions (1-5 ml) were pipetted into separate 25 ml volumetric flasks. The volume was completed to the mark with ethanol. First derivative curves of metronidazole and diloxanide furoate were scaned against ethanol blank and their absorbance maximum were recorded at λ = 259 & 300 nm and at λ = 272 nm, respectively. Calibration curves were constructed.

1-5 ml aliquots of metronidazole stock solution accompanied by 1 ml aliquots of diloxanide furoate stock solution were pipetted into 25 ml volumetric flasks and completed to volume with ethanol. Absorbance was measured at λ = 259 & 300 mm against solvent blank.

1-5 ml aliquots of diloxanide furoate stock solution accompanied with 1 ml aliquots of metronidazole stock solution were pipetted into 25 ml volumetric flasks and completed to volume with ethanol. Absorbance was measured at λ = 272 mm against solvent blank.

V. Application to pharmaceutical preparation :

Twenty Furamebe Forte tablets were powdered well and triturated. Separate accurate weights equivalent to 8.56 and 40 mg of metronidazole were extracted by shaking with successive portions of methylene chloride for charge transfer complexation reaction and methanol for colorimetry, respectively.

An accurate weight of Furamebe Forte tablets equivalent to 40 mg diloxanide furoate was extracted with 18 N sulphuric acid for colorimetry. Also, accurate weights of the drug, equivalent to 50 mg diloxanide furoate and 40 mg metronidazole, were extracted by ethanol for thin layer chromatography and derivative spectrophotometry.

The above prepared solutions were filtered into 100 ml volumetric flasks and completed to volume with the suggested solvents. The assays were completed as under procedures I, II, III & IV.

RESULTS AND DISCUSSION

I. Thin layer chromatography :

As it appears from Fig. 1, the developing system (ethylacetate, chloroform, dioxan, and methanol 8 : 7 : 2 : 3) gave a good resolution of the laboratory prepared mixture and the Furamebe Forte tablets.

II. Charge transfer complexation :

Mixing the metronidazole with iodine in methylene chloride, resulted in a change of the violet color of iodine to lemon yellow. The absorption spectrum showed maxima at λ = 295 & 360 nm which are characteristic of n-donor-iodine charge transfer complex[12] (Fig. 2), leading to radical ion formation in methylene chloride, according to the suggested following scheme:

This was postulated on the basis of the molar ratio of the drug to iodine (1:2) as it appears in

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$$D + I_2 \text{ ---------> } D - I_2 \text{ -------> } {}^{*}DI_2 \text{--------> } (DI^{+} - I^{-}) \xrightarrow{I_2} [(DI+)I^{-}_3]$$

Outer complex — Inner complex I — Inner complex II

The optimum temperature for the reaction was 20-25°C. Higher temperature decreased the absorbance due to dissociation of the formed complex. The time required for the used drug to react with iodine and give maximum absorbance is 25 minutes. Increasing the iodine concentration lead to a strong increase in the absorbance which gradually became constant. Methylene chloride proved here to be the most suitable medium for the charge transfer complexation reaction leading to radical ion formation. A linear relationship was obtained for the final measured concentration range (0.34-1.71%) and is represented by the regression equation C = B + KA (Table 1).

The validity of the proposed procedure for the determination of the studied drug in their pure state and in pharmaceutical preparation was tested. Statistical anaylsis of the results obtained indicated that the proposed procedure was equally accurate and precise as the official one[14] (Table 2) and no interference from excepients and vehicles was encountered.

According to the chemical structure of diloxanide furoate, it does not undergo charge transfer complexation with iodine. Therefore, the analysis of a laboratory prepared mixture of metronidazole in presence of aquimolar concentration of diloxanide furoate as well as Furamebe Forte tablets gave good results (Table 2).

III. Colorimetry :

The colorimetric methods previously used for the determination of either metronidazole or diloxanide furoate separately are here applied for the mixture of the two.

As it appears from (Fig. 4), the colored product resulting from the reaction of metronidazole with methanolic potassium hydroxide has an absorption maximum at l = 552 nm, while diloxanide furoate has nearly neglected absorbtion under the same conditions. Thus, metronidazole can be determined without interference from diloxanide furoate.

(Fig. 5) shows that the proposed color obtained from diloxanide furoate-Liebermann's reagent reaction has an absorption maximum at l = 391 nm. while metronidazole gives no absorption under the same conditions. Thus, diloxanide furoate can be determined without interference from metronidazole.

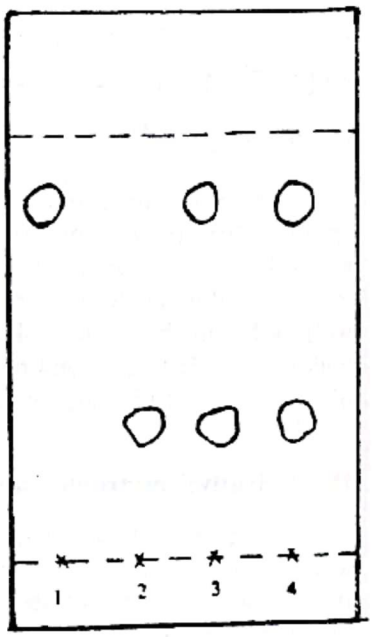
In order to prove the validity and the applicability of the proposed combination of methods, five dilutions of a laboratory prepared mixture of diloxanide furoate and metronidazole as well as Furamebe Forte tablets were analyzed for both diloxanide furoate and metronidazole applying the suggested combination of colorimetric methods (Table 2).

IV. Derivative spectrophotometry :

(Fig. 6a) shows that metronidazole has absorption maxima at l = 230 & 325 nm, while diloxanide furoate has absorption maxima at l = 260 & 315 nm, both are overlapped. Meanwhile, the corresponding first derivative curves (Fig. 6b) show that metronidazole has absorbance at the zero crossing points of diloxanide furoate (l = 250 & 300 nm) and diloxanide furoate has absorbance at zero crossing point of metronidazole (l = 272 nm). Thus, both components can be determined in presence of each other at the above mentioned wavelengths. The optimum parameters for the assay of these drugs are presented in Table 1. For comparison, the official methods[14] were applied. Statistical analysis of the results obtained for either metronidazole or diloxanide furoate (Table 2) showed that the suggested procedures are equally precise and accurate as the official ones.

In order to prove the validity and the applicability of the proposed methods, five dilutions of a laboratory prepared mixture as well as Furamete Forte tabets were analyzed for both metronidazole and diloxanide furoate applying the proposed method. The results obtained show high reliability and reproducibility for both metronidazole and diloxanide furoate in presence of each other.

As a general conclusion, the suggested derivative spectrophotometric method is the most suitable for quantitative determination of the two components in a mixture, being specific for the component at the specified wavelength. The combined colorimetric methods although specific for each component yet they require reagents. Charge transfer complexation procedure depends upon the valence bond between the electrons of the donor and acceptor, thus being non specific and not suitable for binary mixture when both of the compounds react. Thin layer chromatographic procedure gave good qualitative results but requires complicated technique to be quantitative.



1- Diloxanide furoate
2- Metronidazole
3- Laboratory prepared mixture
4- Furamebe Forte tablets

Fig. 1: TLC of Diloxanide furoate and Metronidazole mixture.

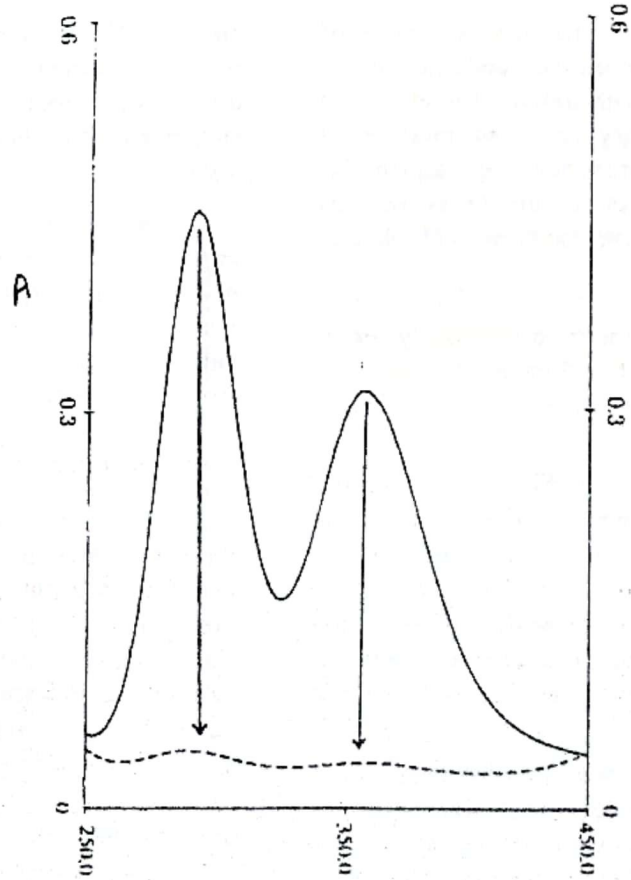


Fig. 2: Absorption spectra of metronidazole (--------) and metronidazole-iodine chargetransfer complex 1.02 mg% (————).

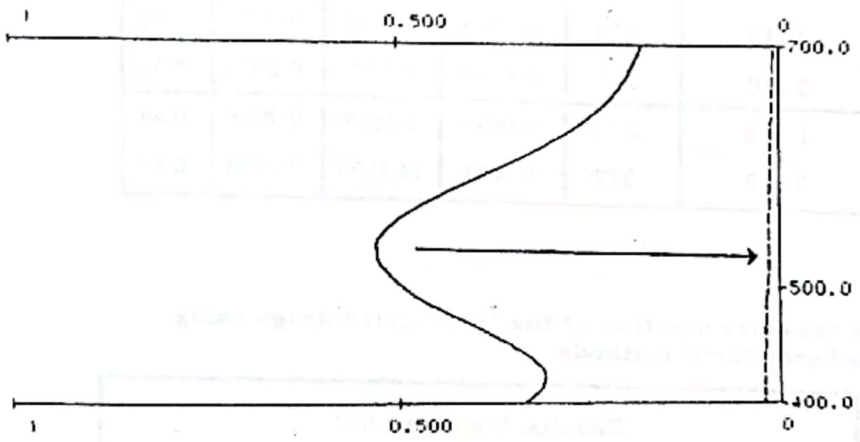


Fig. 4: Absorption spectra of diloxanide furoate with methanolic potassium hydroxide (- - - -) and of metronidazole with methanolic potassium hydroxide (——) and of metronidazole (4.8 mg%), in presence of diloanide furoate with methanolic potassium hydroxide (——).

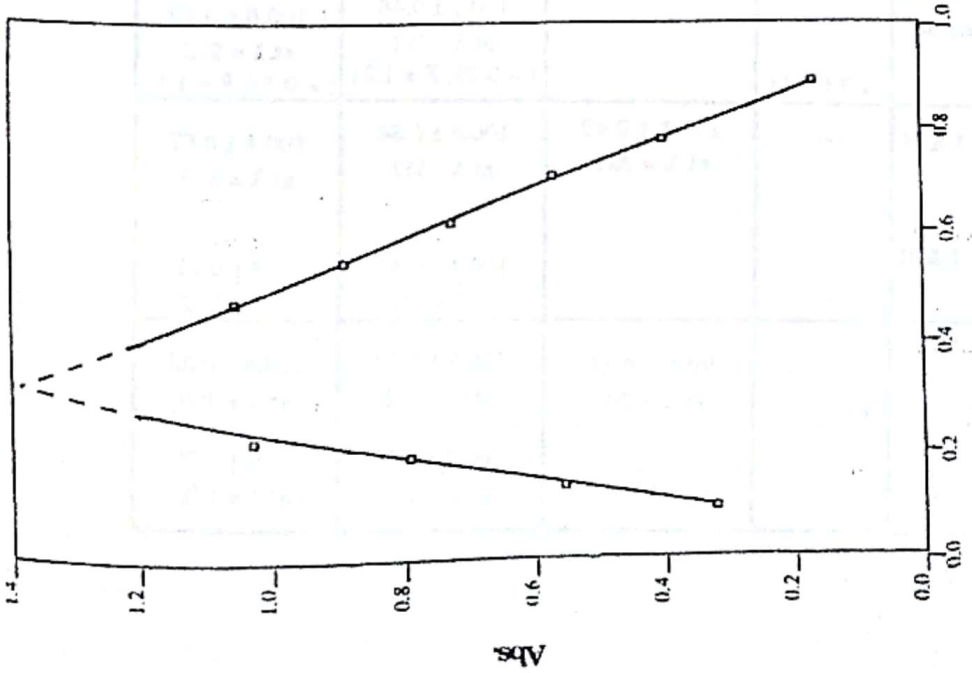


Fig. 3: Continuous variation plot obtained from the metronidazole-iodine (10^{-3}M) reaction carried out in methylene chloride.

Table 1 : Optimum parameters for calibration curves construction

Standard solution of :	Method	Concentration range mg%	λ nm	Regression analysis			C.V%
				B	K	R	
Metronidazole (I)	CTC	0.34 - 1.71	295	0.0009	12.0159	0.9999	0.49
	CTC	0.34 - 1.71	360	- 0.0103	4.3182	0.9999	0.55
	Color.	1.6 - 8	552	- 0.0019	7.8901	0.9998	0.50
	'D	2 - 10	259	0.0002	9.2648	0.9999	0.61
	'D	2 - 10	300	0.0103	10.3770	0.9989	0.51
Diloxanide furoate (II)	Color.	1.6 - 8	392	0.0019	3.9859	0.9999	0.48
	'D	2 - 10	272	0.0021	14.1259	0.9994	0.40

$C = B + KA$

Table 2 : Results for the determination of the investigated drugs using the proposed and official methods

Standard solution of :	Results, % meean ± S.D.			
	Official	CTC	Color	'D
Authentic metronidazole (I)	100.3 ±0.52	99.9 ± 0.49 at λ = 295 t = 0.44, F = 1.13 100.1 ± 0.55 at λ = 360 t = 0.60, F = 1.11	100.0 ± 0.50 at λ = 552 t = 0.93, F = 1.08	100.1 ± 0.61 at λ = 259 t = 0.55, F = 1.4 99.8 ± 0.51 at λ = 300 t = 1.55, F = 1.17
Authentic diloxanide furoate (II)	99.9 ± 0.44	-	100.1 ± 0.48 at λ = 392 t = 0.69, F = 1.21	100.0 ± 0.40 at λ = 272 t = 0.88, F = 1.19
Lab. prep.mix. of I & II for (I)	-	101.1 ± 0.49 at λ = 295	100.5 ± 0.66 at λ = 552	100.4 ± 0.67 at λ = 300
Lab. prep. mix. of I & II for (II)	-	-	100.4 ± 0.57 at λ = 392	99.8 ± 0.71 at λ = 272
Furamebe Forte tab. for (I)	-	99.6 ± 0.58 at λ = 295	100.9 ± 0.67 at λ = 552	100.8 ± 0.33 at λ = 300
Furamebe Forte tab. for (II)		-	100.9 ± 0.68 at λ = 392	99.9 ± 0.75 at λ = 272

* Means of five determinations
* P = 0.05
* t = (2.3), F = (5.5)

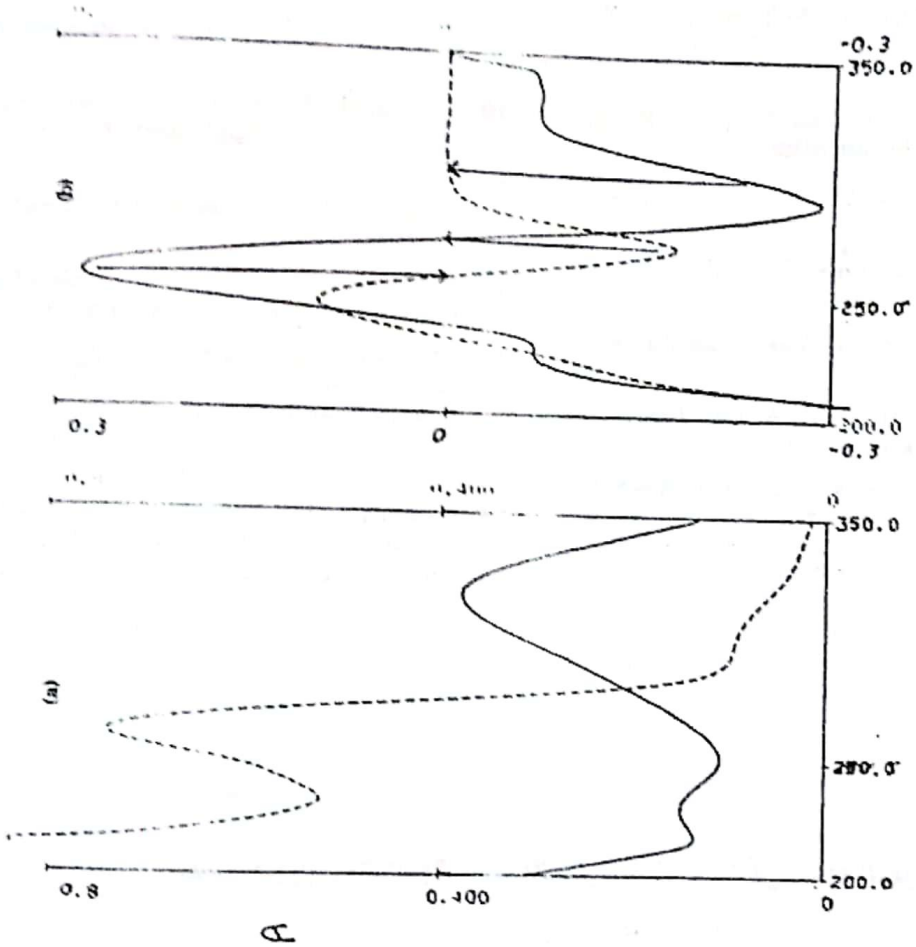


Fig. 6: U. V. absorption (a) and ^{1}D determination of metronidazole, 6 mg%, (——) and diloxanide furoate, 6 mg%, (- - - -) (b).

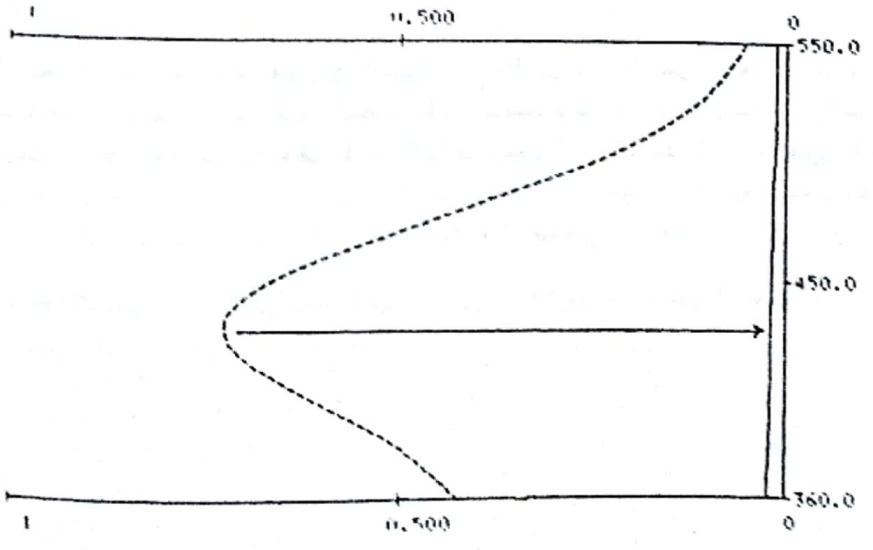


Fig. 5: Absorption spectra of metronidazole with Lieberman's reagent (——) and of diloxanide furoate (4.8 mg%), with Lieberman's reagent (- - - -) and of diloxanide furoate in presence of metronidazole with Lieberman's reagent (- - - -).

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تحليل فيوروات الدايلوكسانيد والميترونيدازول كلا فى وجود الآخر

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فى هذا البحث تم فصل خليط من فيوروات الدايلوكسانيد والميتروتيدازول بطريقة الفصل الكروماتوجرافى على الطبقة الرقيقة والتعرف عليهما كيفياً بإستخدام الأشعة فوق البنفسجية تم استخدام طريقة نقل الشحنات بين اليود مستقبل) والميترونيدازول (معطى) وتكوين متراكب له امتصاصيه عاليه فى تقديره كمياً فى وجود الدايلوكسانيد، كذلك تم تقدير كلا منهما فى وجود الآخر بدمج طريقتين لونيتين تعتمدان على تكوين نواتج ملونة بالتفاعل مع كاشف هيدروكسيد البوتاسيوم الكحولى وكاشف ليبرمان. كما تم تقدير كلاً منهما فى وجود الآخر بإستخدام طريقة المشتقة التفاضلية الأولى لمنحنى الامتصاص.

ولقد أوضحت النتائج المطبقة على مخاليط معمليه وأقراص الفيورامبب - قوى المضاد للأميبا أن الطرق المقترحة بسيطة وتماثل الطرق الدستورية المنشورة فى دقتها وتكرارها.