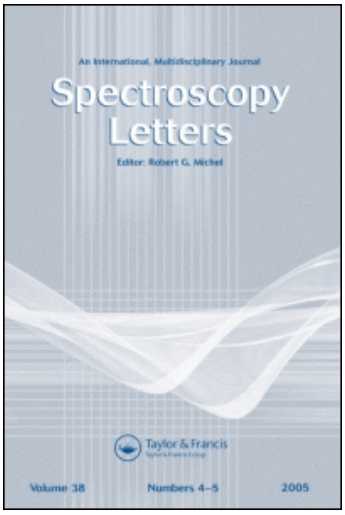

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Synchronous Spectrofluorimetric Determination of Famotidine, Fluconazole and Ketoconazole in Bulk Powder and in Pharmaceutical Dosage Forms

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SYNCHRONOUS SPECTROFLUORIMETRIC DETERMINATION OF FAMOTIDINE , FLUCONAZOLE AND KETOCONAZOLE IN BULK POWDER AND IN PHARMACEUTICAL DOSAGE FORMS

KEY WORDS : Famotidine , fluconazole , ketoconazole , synchronous spectrofluorimetry , bulk powder , pharmaceutical dosage forms.

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ABSTRACT

The aim of this work was to investigate a simple and reliable method for the determination of trace amounts of famotidine , fluconazole and ketoconazole in bulk powder and in their pharmaceutical dosage forms. Also to study the different factors affecting the sensitivity of the synchronous technique in order to improve the measurement.The sample was prepared in methanol in case of famotidine and ketoconazole, and in water , after dissolution in methanol, in case of fluconazole . The

fluorescence intensity was recorded at 384 (ex. at 284 nm) , 285 (ex. at 255 nm) and 364 nm (ex. at 239nm) for famotidine , fluconazole and ketoconazole respectively . The calibration curves were linear over the concentration ranges 15 - 50 , 0. 2 0 - 10 and 0. 02 - 0. 4 μg/ml for famotidine , fluconazole and ketoconazole respectively .

INTRODUCTION

Synchronous fluorescence spectroscopy is a technique introduced by Lloyd in 1971 (1) . Synchronous spectra are narrower and more characteristic than conventional emission spectra (2) . When the scan rate is constant for both monochromators , and therefore a constant wavelength interval ($\Delta\lambda$) is kept between λ_{em} and λ_{ex} the technique is known as synchronous exitation fluorescence spectroscopy .

Several methods for the determination of famotidine , fluconazole and ketoconazole were reported . Concerning the methods for famotidine , the literatures indicate , chromatographic (3 - 10), spectrophotometric(11-12) , flow injection analysis with spectrophotometric detection(13), potentiometric(14) and polarographic determination (15) . Concerning fluconazole,the literatures indicate chromatographic (16 -20), spectrophotometric (21) and microbiological determination (22) . In case of ketoconazole , several methods were reported ; chromatographic (23-24), spectrophotometric(24 - 28), potentiometric (29 - 30) , fluorimetric (31)and polarographic determination(32) .

However , non of the reported methods indicates synchronous spectrofluorimetric determination of any of the cited compounds .

The objective of this research was to apply the synchronous technique for the determination of the cited compounds to obtain selective , rapid , simple and accurate methods for analysis, statistically not differ from those published and compandial methods .

EXPERIMENTAL

Materials and Chemicals

Methanol (HPLC grade) BDH .

Water (Bidistilled) .

Famotidine (99.8%) , fluconazole (99.6%) and ketoconazole (99.9%) supplied by Impex Quimica S.A. Spain .

Gastrodomina Tablets nominally containing 40 mg / tablet of famotidine , manufactured by Medical Union Pharmaceuticals (Egypt) under licence of Prodes , SA Laboratories (Spain) .

Flucoral capsules nominally containing 150 mg / cap of fluconazole , and Ketoderm 2% Gel Shampoo nominally containing 2%w/v of ketoconazole; manufactured by South Egypt Drug Industries Co. (Egypt) .

Apparatus

Perkin Elmer Spectrofluorimeter Model LS30 with a sampling system of high efficiency optical system with 7 µl illuminated volume synthetic fused silica flowcell and programmable integral sampling pump .

Measuring Parameters

I-For famotidine :-

1-Exitation range 230 - 360 nm .

2-Emission range 330 - 460 nm .

3- $\Delta\lambda$ = 100 nm .

4- Autozero for methanol over the above mentioned scanning range .

5-Response = 3

6-$\lambda_{max.}$= 284 nm in the synchronous spectrum .

7- Scanning speed for both monochromators = 600nm/minute

II-For fluconazole :-

1-Exitation range 220 - 320 nm .

2-Emission range 250 - 350 nm .

3- $\Delta\lambda$ = 30 nm .

4- Autozero for water over the above mentioned synchronous scanning range .

5-Response = 2

6-$\lambda_{max.}$= 255 nm in the synchronous spectrum .

7-Scanning speed for both monochromators = 600nm/minute

III-For ketoconazole :-

1- Exitation range 210 - 310 nm .

2- Emission range 335 - 435 nm .

3- $\Delta\lambda$ = 125 nm .

4- Autozero for methanol over the above mentioned synchronous scanning range .

5- Response = 2

6- $\lambda_{max.}$= 239 nm in the synchronous spectrum .

7- Scanning speed for both monochromators = 600nm/minute

The optimum instrumental conditions were choosen to give the best signal to noise ratio .

Calibration Curves Construction

1- Famotidine solutions :-

Stock solution of famotidine in methanol (200 µg/ml) was prepared . Aliquots equivalent to different amounts of famotidine

were transferred to 10 ml volumetric flasks and completed to volume with methanol.

2- Fluconazole solution :-

A solution of fluconazole in water (after dissolution of the powder in 1 ml of methanol) was prepared (1000 μg/ml). Several dilutions were made with water to obtain a stock solution of fluconazole having a concentration of 10 μg/ml. Different aliquots were transferred to 25 ml volumetric flasks and completed to volume with water.

3- Ketoconazole solutions :-

Stock solution of ketoconazole in methanol (0. 5 μg/ml) was prepared . Aliquots equivalent to different amounts of ketoconazole were transferred to 10 ml volumetric flasks and completed to volume with methanol.

Procedure

The above mentioned measuring parameters were applied and the fluorescence intensity at 384 nm (ex. 284nm), 285 nm (ex. 255 nm) and 364 nm (ex. 239 nm) were recorded for famotidine , fluconazole and ketoconazole, respectively . Calibration curves were constructed and it was found that there is a linear relationship between the fluorescence intensity and the concentration ranges 15 - 50 , 0. 2 - 10 and 0. 02 - 0. 4 μg/ml for famotidine , fluconazole and ketoconazole , respectively . Using linear regression , the following equations were computed :-

C = F - intercept / slope

Where :-

C = Concentration in μg/ml .

F = Fluorescence intensity at the corresponding exitation and emission maxima .

Constant	Famotidine	Fluconazole	Ketoconazole
Intercept	8.2147	3.005	0.1323
Slope	1.1352	8.9196	262.9
Correlation coefficient (r)	0.9991	0.9999	0.9998

Application Of The Proposed Method For The Determination Of Cited Compounds In Bulk Powder

1- Famotidine solutions :-

Stock solution of famotidine in methanol (100 μg/ml) was prepared . Aliquots equivalent to different amounts of famotidine were transferred to 10 ml volumetric flasks and completed to volume with methanol .

2- Fluconazole solution :-

A solution of fluconazole in water (after dissolution of the powder in 1 ml of methanol) was prepared (1600 μg/ml) . Several dilutions were made with water to obtain a stock solution of fluconazole having a concentration of 16 μg/ml . Different aliquots were transferred to 25 ml volumetric flasks and completed to volume with water

3- Ketoconazole solutions :-

Stock solution of ketoconazole in methanol (3.2 μg / ml) was prepared . Aliquots equivalent to different amounts of ketoconazole were transferred to 10 ml volumetric flasks and completed to volume with methanol .

The procedure mentioned under construction of calibration curves was applied and the recovered concentrations were computed using the linear regression equations calculated above , tables 1, 2and 3 .The results

TABLE 1
Determination of famotidine in bulk powder using synchronous spectrofluorimetric technique .

*Amount of famotidine taken µg/ml	% Recovery
22.00	100.24
24.00	99.05
28.00	100.63
32.00	99.61
36.00	100.29
42.00	99.18
46.00	98.21

Mean = 99.60%
S.D. = 0.852
C.L. = 99.60 ± 0.790
* Average of five determinations .

, obtained , indicates that the suggested method is considered to be accurate and precise as the official or published methods , using the Student (t) test and the variance ratio (F) test .

Application Of The Proposed Method For The Determination Of Cited Compounds In Their Pharmaceutical Dosage Forms

Determination of famotidine in Gastrodomina Tablets

Twenty tablets were weighed , powdered and an amount of mixed powder equivalent to 40 mg of famotidine was weighed

TABLE 2
Determination of fluconazole in bulk powder using synchronous spectrofluorimetric technique .

*Amount of fluconazole taken µg/ml	% Recovery
2.80	98.08
3.60	98.80
4.40	99.05
5.20	98.41
5.60	98.45
6.40	99.17
7.20	100.78

Mean = 98.96%
S.D. = 0.8876
C.L. = 98.96 ± 0.820

* Average of five determinations .

and transferred to a 100 ml volumetric flask. Methanol (70 ml) was added and the flask was sonicated for 5 minutes . The volume was completed to 100 ml with methanol. The content were mixed well and filtered through 0.45 µm membrane filter, rejecting the first 5 ml of the filtrate . Aliquots from the filtrate equivalent to 200 and 280 µg of famotidine were transferred to 10 ml volumetric flasks and completed to volume with methanol .

The fluorescence intensity was measured using the procedure mentioned under construction of calibration curves . The procedure was repeated using the standard addition technique , adding exact known amounts of

TABLE 3
Determination of ketoconazole in bulk powder using synchronous spectrofluorimetric technique .

Amount of ketoconazole taken µg/ml	% Recovery
0.064	99.29
0.112	99.92
0.160	101.37
0.208	100.84
0.256	101.74
0.336	99.79
0.384	101.04

Mean = 100.57%
S.D. = 0.910
C.L. = 100.57 ±0.840
* Average of five determinations .

famotidine . Concentrations of labelled and added famotidine were computed using regression equation .

Determination of fluconazole in Flucoral Capsules

The content of ten capsules were emptied , weighed and mixed well . An amount of the powder equivalent to 50 mg of fluconazole was weighed and transferred to a 50 ml volumetric flask . Methanol (1 ml) was added , the flask was shaken for 1 minute and the volume was completed to 50 ml with water. The content of the flask was mixed well , filtered through a filter paper and rejecting the first 5 ml of the filtrate . Aliquot (5 ml) of the filtrate was transferred to a 50 ml volumetric flask and

completed to volume with water. An aliquot (25 ml) of the final solution was transferred to a 250 ml volumetric flask and completed to volume with water (solution A). Different aliquots of solution A (4, 8 and 12 ml) were diluted to 25 ml with water . The fluorescence intensity was measured using the procedure mentioned under construction of calibration curves.

The procedure was repeated using the standard addition technique , adding exact known amounts of fluconazole . Concentrations of labelled and added fluconazole were computed using regression equation .

Determination of ketoconazole in Ketoderm Gel Shampoo

An aliquot (1 ml) of the shampoo equivalent to 20 mg of ketoconazole was transferred quantitatively to a 100 ml volumetric flask with the aid of methanol and completed to volume with the same solvent. From this solution , 1 ml aliquot was diluted to 25 ml with methanol (solution B) . An aliquot (5 ml) of solution B was diluted to 25 ml with methanol . From the final solution , different aliquots (0.7 , 1. 0 and 1. 2 ml) were diluted to 10 ml with methanol. The fluorescence intensity was measured using the procedure mentioned under construction of calibration curves . The procedure was repeated using the standard addition technique , adding exact known amounts of ketoconazole . Concentrations of labelled and added ketoconazole were computed using regression equation .

The results of recovered added cited compounds are shown in tables 4 ,5 and 6 .

DISCUSSION

Fluorescence is expected in molecules that are aromatic or containing multiple conjugated double bonds with a high degree of

TABLE 4

Recovery of standard famotidine added to Gastrodomina tablets using synchronous spectrofluorimetric technique.

*Amount of famotidine taken as labeled µg/ml	added µg/ml	% Recovery of added famotidine
20.00	17.00	99.50
28.00	10.00	98.22
28.00	12.00	98.37

Mean = 98.70%
S.D. = 0.700
C.L. = 98.70 ±1.740

* Average of five determinations .

TABLE 5

Recovery of standard fluconazole added to Flucoral capsules using synchronous spectrofluorimetric technique.

*Amount of fluconazole taken as labeled µg/ml	added µg/ml	% Recovery of added fluconazole
2.00	6.00	98.27
3.20	4.00	100.00
5.20	3.20	98.40

Mean = 98.89%
S.D. = 0.964
C.L. = 98.89 ±2.390

* Average of five determinations .

TABLE 6

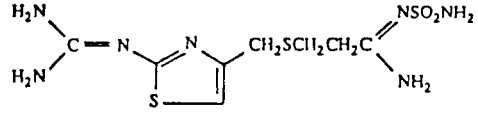
Recovery of standard ketoconazole added to Ketoderm shampoo using synchronous spectrofluorimetric technique.

*Amount of ketoconazole taken as labeled µg/ml	added µg/ml	% Recovery of added ketoconazole
0.112	0.050	99.29
0.160	0.100	97.39
0.192	0.200	98.91
	Mean	= 98.53%
	S.D.	= 1.005
	C.L.	= 98.53 ±2.500

* Average of five determinations .

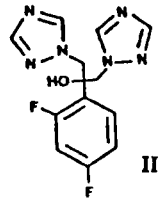
resonance stability . Both classes of substances have delocalized π - electerons that can be placed in low - lying exited singlet states (33) .

Famotidine (I) contains conjugated double bonds system which leads to a fluorescent activity . Althogh this is not a highly conjugated system , but the attachment of two amino groups to the system may enhance the fluorscence due to the delocalization of the π– electrons .



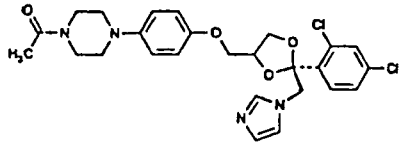
I

Although , compound I showed the abscence of benzene ring , yet , the presence of a benzene ring having di - substitutions of fluorine atoms may be responsible for the fluorescence activity of fluconazole (II) .



II

The presence of benzene ring having a substitutuion of $-OCH_2$ group on one side and $-N^{-CH_2-}_{-CH_2-}$ on the other side may give reasons for the fluorescence activity of ketoconazole (III) .



III

Ketoconazole molecule has a benzene ring , with a di - chloro substitutions , which participate in the fluorescence activity of the molecule , but the chlorine substitution decreases this fluorescence participation of the benzene ring which thought to be due in part to the heavy atom effect . Intersystem crossing , are most common in molecules that contain heavy atoms . Apparently , interaction between the spin and orbital motions become large in the presence of such atoms , and a change in spin is thus more favorable (34).

This fluorescence activity of famotidine , fluconazole and ketoconazole was used to introduce an accurate , rapid , simple and sensitive method for the determination of these compounds .

In the conventional fluorescence , an emission spectrum is recorded as the sample , which contains the analyte , is irradiated at a single exitation wavelength .

Famotidine has an exitation and an emission maxima at 290 and 360 nm respectively (fig. 1 - A) , while in case of fluconazole at 252 and

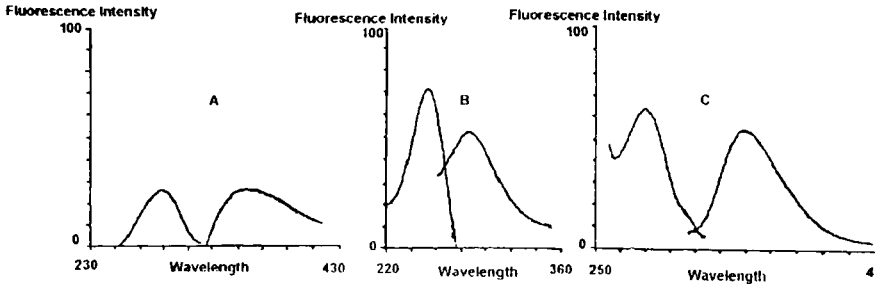


FIG.-1 - Conventional fluorescence spectra (exitation and emission) for famotidine (A), fluconazole (B) an ketoconazole (C) .

286 nm (fig. 1- B) , and in case of ketoconazole at 290 and 365 nm , respectively (fig. 1 - C) .

In synchronous fluorescence spectroscopic technique ,both exitation and emission wavelengths are varried simultaneously while keeping a constant interval ($\Delta\lambda$) between them . The method introduced in this work got advantage from this technique for the determination of famotidine , fluconazole and ketoconazole in bulk powder and in pharmaceutical dosage form , thus a simple , selective , rapid and accurate method for their determination was obtained .

Synchronous spectra has narrower peaks , hence more charateristic , than conventional ones , fig. 2 .The sensitivity and the intersept was improved significantly when using synchronous technique rather than conventional fluorimetry , fig. 3 . Also synchronous fluorescence spectroscopy may be used , not only for quantitative purposes , but also for qualitative purposes in which the conventional fluorescence fails (ketoconazole and famoidine have almost the same exitation and emission spectra while differ significantly in their synchronous spectra, fig. 4 and 5) .

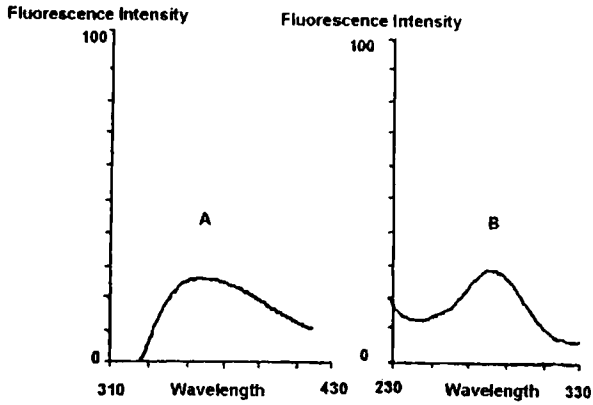


FIG.-2 - Comparison between emission (A) and synchronous (B) spectra of famotidine .

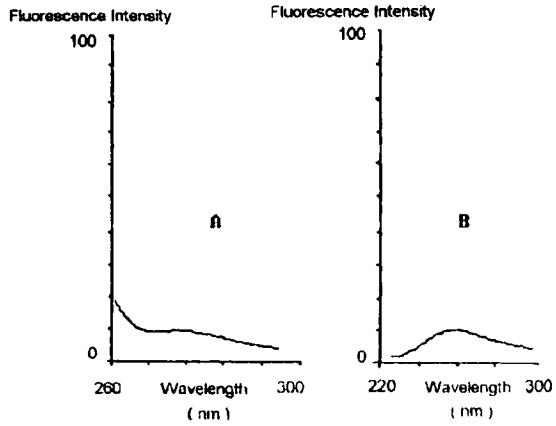


FIG. - 3 - Comparison between emission (A) and synchronous (B) spectra of 1 µg/ml solution of fluconazole .

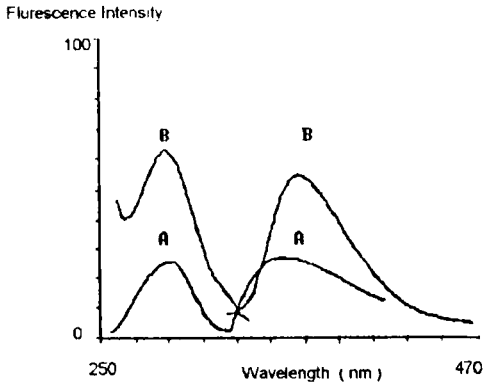


FIG. - 4 - Comparison between conventional fluorescence spectra of famotidine (A) and ketoconazole (B) .

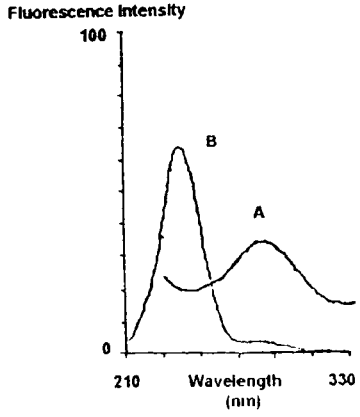


FIG. - 5 - Comparison between synchronous fluorescence spectra of famotidine (A) and Ketoconazole (B) .

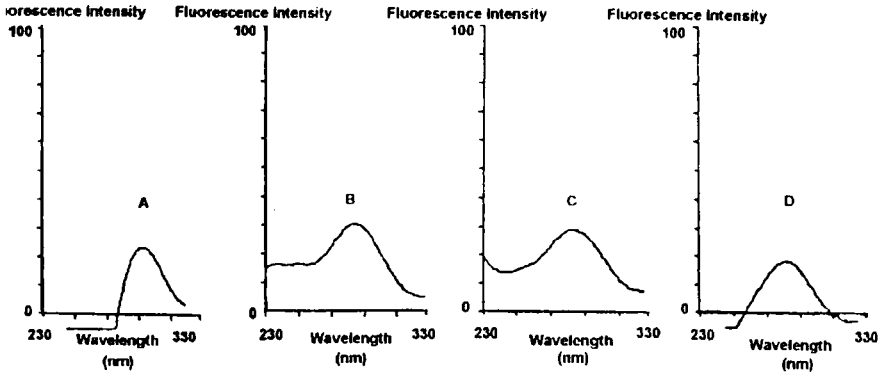


FIG. - 6 - Effect of different $\Delta\lambda$ on the synchronous spectra of famotidine ($\Delta\lambda$= 80 nm (A) , 90 nm (B) , 100 nm (c) and 110 nm (D)) .

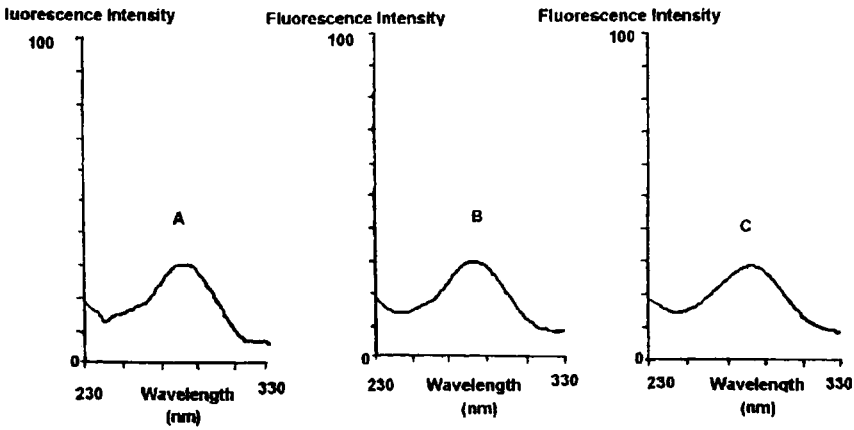


FIG. - 7 - Effect of different responses on the synchronous spectra of famotidine (response = 2 (A) , 3 (B) and 4 (C)) .

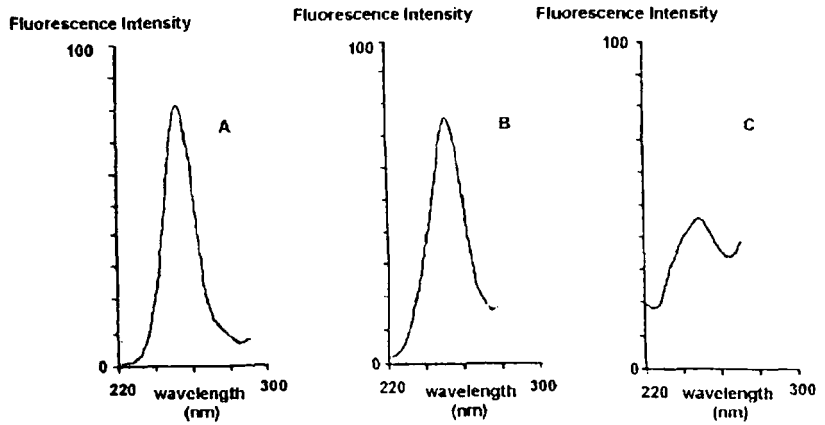


FIG. - 8 - Effect of different $\Delta\lambda$ on the synchronous spectra of fluconazole ($\Delta\lambda$ = 30 nm (A) , 40 nm (B) and 60 nm (C)) .

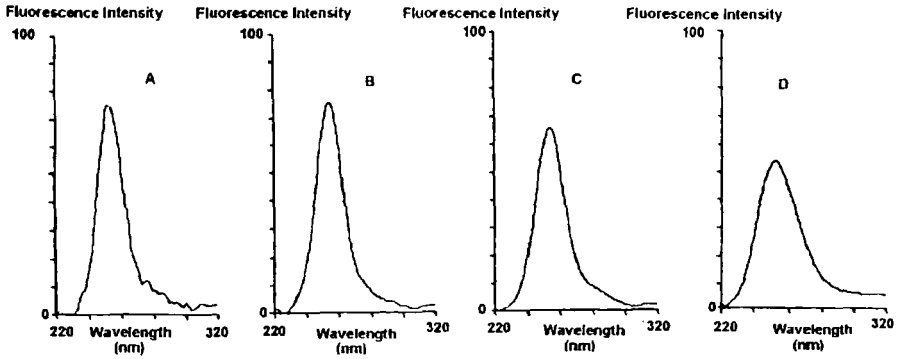


FIG. - 9 - Effect of different responses on the synchronous spectra of fluconazole (response = 1 (A) ,2 (B) , 3 (C) and 4 (D)) .

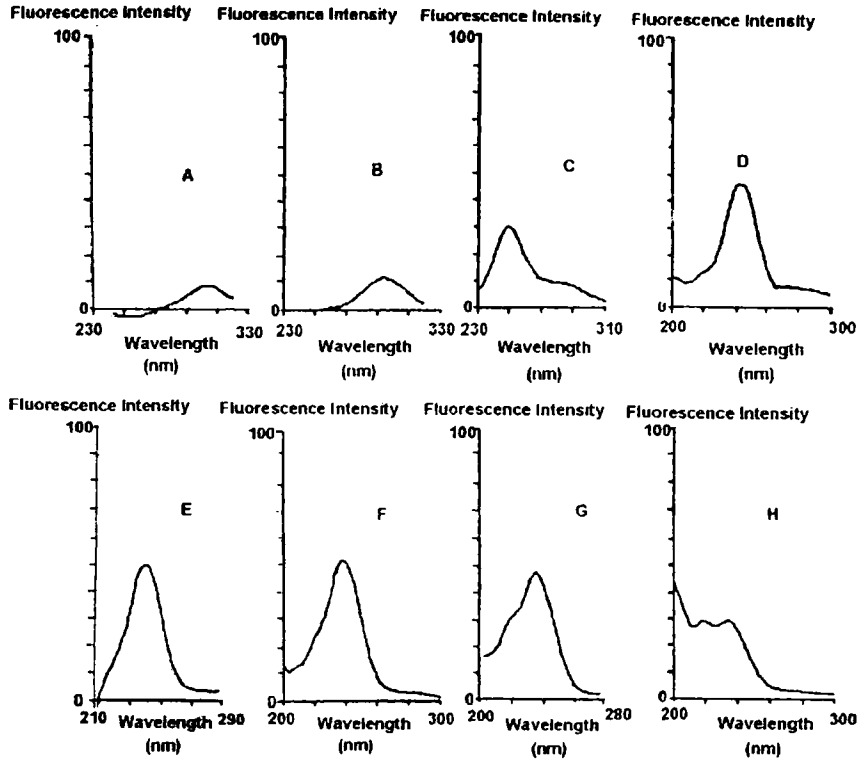


FIG. - 10 - Effect of different $\Delta\lambda$ on the synchronous spectra of ketoconazole ($\Delta\lambda$=50 nm (A), 70 nm (B), 100 nm (C), 115 nm (D), 125 nm (E), 130 nm (F), 140 nm (G) and 160 nm (H)).

Concerning the factors which affect the synchronous fluorescence spectrum , $\Delta\lambda$, monochromators' speed and the response are the most important . In this proposed method , the apparatus used is Perkin Elmer LS 30 . In this model , the monochromators' speed is 600 nm and is not able to be changed . $\Delta\lambda$ and response factors are able to be changed and were choosen , in this work , according to the best signal to noise ratio . The optimum $\Delta\lambda$ and response in case of famotidine , fluconazole and ketoconazole were 100nm & 3 (fig. 6&7), 30nm

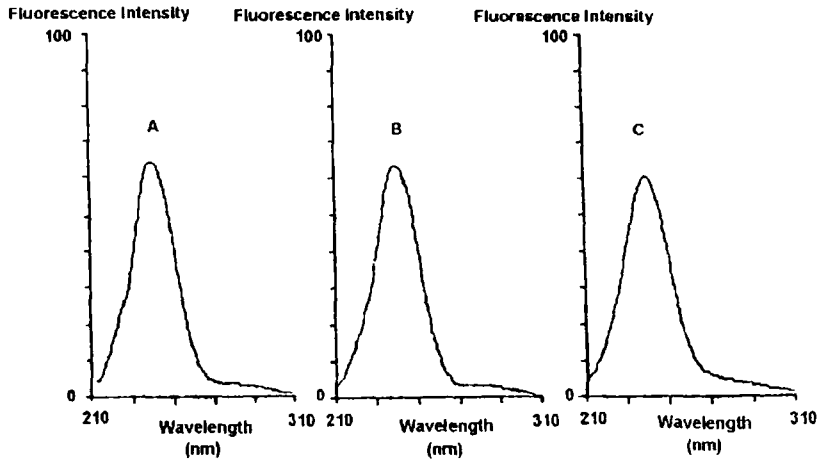


FIG. - 11 - Effect of different responses on the synchronous spectra of ketoconazole (response = 1 (A) , 2 (B) and 3 (C)) .

& 2 (fig. 8&9) and 125nm & 2 (fig.10&11) , respectively . The use of a 7 µl cell during the measurement , may be responsible for the ability of measuring high concentrations of famotidine due to the decrease in the inner - filter effect and also less chance for loosing the energy due to the high concentration of the analyte .

Excellent results have been obtained with amounts as low as 22.4 , 2.8 and 0.064 µg/ml for famotidine , fluconazole and ketoconazole , respectively . The mean percentage recoveries were 99.60 ±0.79 , 98.96 ±0.82 and 100.57 ±0.84 for famotidine , fluconazole and ketoconazole , respectively . The method was successfuly applied for the determination of famotidine in Gastrodomina tablets , fluconazole in Flucoral capsules and ketoconazole in Ketoderm gel shampoo with mean percentage recoveries for added famotidine , fluconazole and ketoconazole 99.70 ±1.74 , 98.89±2.39 and 98.53 ±2.50 , respectively .

The results obtained showed no statistical significant difference concerning accuracy and precision when compared with those obtained by the reference methods .

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