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Simultaneous determination of metronidazole and spiramycin in bulk powder and in tablets using different spectrophotometric techniques

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Metronidazole (MZ) is an anti-infective drug used in the treatment of anaerobic bacterial and protozoa infections in humans. It is also used as a vetinary antiparasitic drug. Spiramycin (SP) is a medium-spectrum antibiotic with high effectiveness against Gram-positive bacteria. Three simple, sensitive, selective and precise spectrophotometric methods were developed and validated for the simultaneous determination of MZ and SP in their pure form and in pharmaceutical formulations. In methods A and B, MZ was determined by the application of direct spectrophotometry and by measuring its zero-order (D^0) absorption spectra at its λ_{max} = 311 nm. In method A, SP was determined by the application of first derivative spectrophotometry (D^1) and by measuring the amplitude at 218.3 nm. In method B, the first derivative of the ratio spectra (DD^1) was applied, and SP was determined by measuring the peak amplitude at 245.6 nm. Method C entailed mean centring of the ratio spectra (MCR), which allows the determination of both MZ and SP. The methods developed were used for the determination of MZ and SP over a concentration range of 5–25 μg ml^{-1}. The proposed methods were used to determine both drugs in their pure, powdered forms with mean percentage recoveries of 100.16 ± 0.73 for MZ in methods A and B, 101.10 ± 0.90 in method C, 100.09 ± 0.70, 100.02 ± 0.88 and 100.49 ± 1.26 for SP in methods A, B and C, respectively. The proposed methods were proved using laboratory-prepared mixtures of the two drugs and were successfully applied to the analysis of MZ and SP in tablet formulation without any interference from each other or from the excipients. The results obtained by applying the proposed methods were compared statistically with a reported HPLC method and no significant difference was observed between these methods regarding both accuracy and precision.

Keywords: mean centring; metronidazole; ratio derivative; spiramycin; spectrophotometry

Introduction

Metronidazole (MZ) (Figure 1) is the reference agent of the nitroimidazole anti-infective family. It is chemically designated as 2-(2- methyl- 5-nitro- 1H- imidazol- 1-yl) ethanol.[1] In humans it is used mainly in the treatment of infections caused by anaerobic bacteria and protozoa and it has a radio-sensitizing effect on hypoxic tumour cells.[2] Metronidazole has also been used as an antiparasitic in veterinary work.[3]

Spiramycin (SP) (Figure 2) is chemically designated as (6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(dimethylamino)-6-methyltetra-hydro-2*H*-pyran-2-yl]oxy}-5,9,16-trimethyl-2-oxo-7-(2-oxoethyl) oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-α-D-glu-copyranoside.[1] It belongs to the class of 16-membered macrolide antibiotics and it is considered to be a medium-spectrum antibiotic with high effectiveness against Gram-positive bacteria. It is isolated from *Streptomyces ambofaciens* and is naturally mixture of three components: SP I together with its 3-acetyl (SP II) and 3-propanoyl (SP III)[4] with minimum of 85% of SP I, and a maximum of 5% for SP II and 10% for SPR III.[5] Spiramycin is absorbed well after oral administration and is distributed in the tissues, especially the lungs, liver and kidney.[6]

Several methods have been reported for the determination of either MZ or SP alone, each in its single dosage form (not in combination). Several methods were reported for the determination of MZ in biological fluids, in pharmaceutical dosage forms, in combination with other drugs and in the presence of its metabolites using spectrophotometry,[7] thin-layer chromatography (TLC),[8] liquid chromatography (LC),[9,10] high-performance liquid chromatography (HPLC),[11] voltammetry,[12] flow injection chemiluminescence analysis,[13] nuclear magnetic resonance spectrometry (NMR)[14] and capillary electrophoresis.[15] Different techniques have also been described for the determination of SP, including titremetry,[16] spectrophotometry,[17] TLC,[18] LC,[19] HPLC,[20,21] capillary electrophoresis,[22] voltammetry[23] and immunological assay.[24]

Combinations of MZ and SP have been developed to take advantage of the complementary antibacterial effects of the compounds *in vitro*. This binary mixture has been determined in plasma, saliva and gingival crevicular fluid by LC-MS/MS[10] and in fish muscle using HPLC with UV detection.[25] Simultaneous determination of MZ and SP by HPLC and HPTLC in tablets has also been reported.[26] A comprehensive literature search revealed the lack of any spectrophotometric techniques for the simultaneous determination of SP and MZ in tablets.

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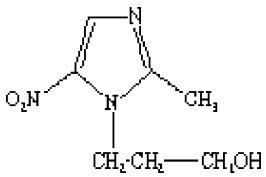

Figure 1. Metronidazole. Mol. formula: $C_6H_9N_3O_3$. Mol. weight: 171.15g mol^{-1}.

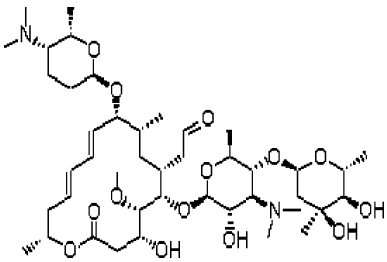

Figure 2. Spiramycin. Mol. formula: $C_{43}H_{74}N_2O_{14}$. Mol. weight: 843.06 g mol^{-1}.

The scientific novelty of the present work is that the methods used are simple, rapid, selective, less expensive and less time consuming than other published LC, TLC and HPLC methods.

The focus of the present study was to develop and validate spectrophotometric methods for the simultaneous determination of SP and MZ in their combined tablet dosage form.

Experimental

Instruments

A double-beam UV–visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to an IBM-compatible computer. The software was UV-PC personal spectroscopy software version 3.7. The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min.

A UV lamp with a short wavelength (254 nm).

Materials

Pure standard

Metronidazole and spiramycin were kindly supplied by El Pharonia Pharmaceuticals, New Borg El-arab City, Alexandria, Egypt. They

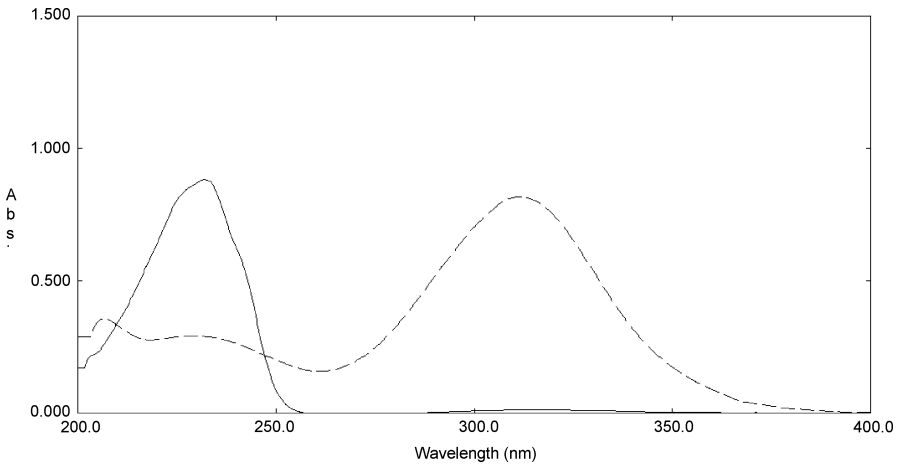

Figure 3. Absorption spectra of 20 µg.mL^{-1} of SP (———) and, and 15 µg.mL^{-1} of MZ (- - - -) in methanol.

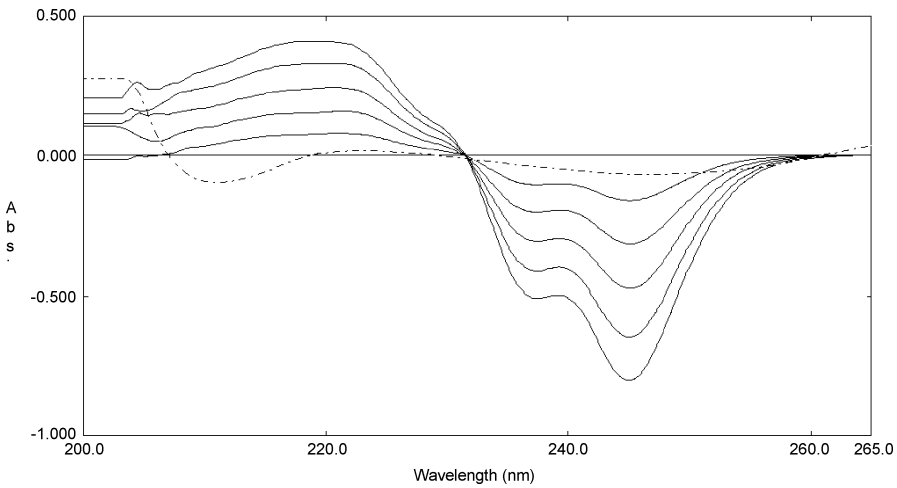

Figure 4. First derivative absorption spectra of SP (5–25 µg mL^{-1}) (———), and 15 µg mL^{-1} of MZ (- - - -) in methanol.

Table 1. Assay validation obtained by applying the proposed methods

	D^0	D1	DD1	MCR	
	MZ	*SP*		*SP*	*MZ*
Range		5–25 µg mL^{-1}			
Slope	0.0555	0.0168	0.0890	0.1264	0.2456
Intercept	−0.0132	−0.0098	0.0014	0.006646	−0.04521
S E of the slope	0.4838×10^{-3}	0.1083×10^{-3}	0.7397×10^{-3}	1.3755×10^{-3}	0.8895×10^{-3}
S E of the intercept	0.0080	0.0018	0.0123	0.0228	0.0148
Correlation coefficient (r)	0.9997	0.9999	0.9999	0.9999	1.0000
LOD	0.3938	0.2950	0.3740	0.4933	0.1639
LOQ	1.1934	0.8930	1.1340	1.4948	0.4967
Accuracy (mean±SD)	100.16 ± 0.73	100.09 ± 0.7	100.02 ± 0.88	100.49 ± 1.26	101.10 ± 0.90
RSD %	0.73	0.69	0.88	1.26	0.89

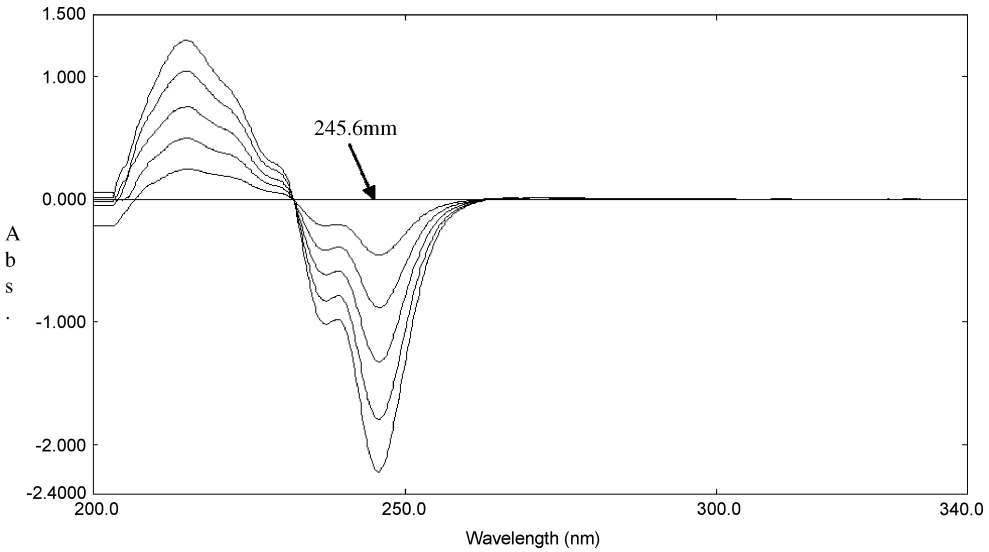

Figure 5. First derivative of the ratio spectra of spiramycin (5–25 µg mL^{-1}) and 20 µg mL^{-1} of metronidazol as a divisor and methanol as a blank.

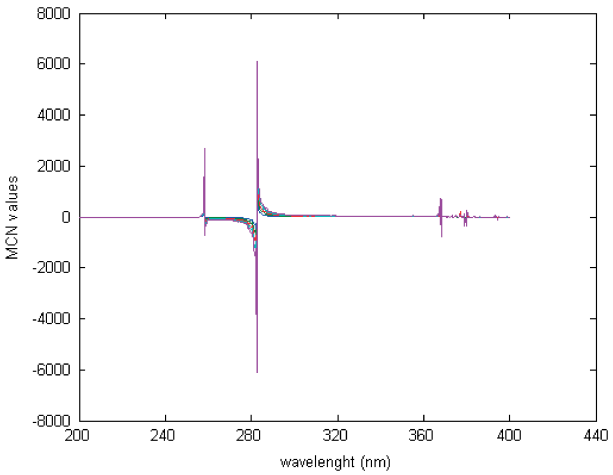

Figure 6. Mean-centred ratio spectra of metronidazole.

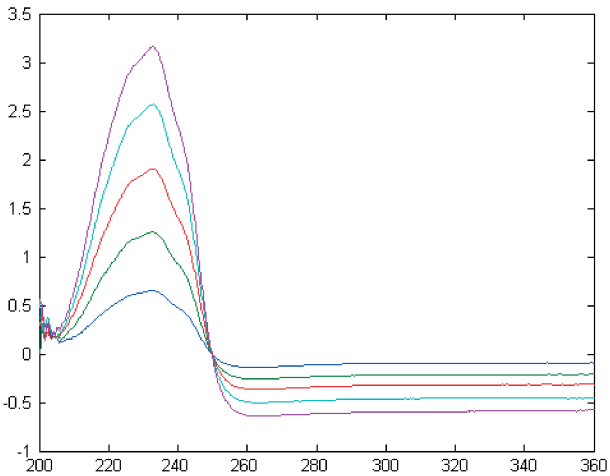

Figure 7. Mean-centred ratio spectra of spiramycin.

Table 2. Determination of spiramycin and metronidazole in laboratory-prepared mixtures by the proposed methods D^0, D1 and DD^1 spectrophotometry

			D^0		D^1		DD^1	
Mixture No	*Amount added of metronidazole µg.mL^{-1}*	Amount found *spiramycin µg.mL^{-1}*	*Found of metronidazole µg.mL^{-1}*	*Recovery %*	*Found of spiramycin µg.mL^{-1}*	*Recovery %*	*Found of spiramycin µg.mL^{-1}*	*Recovery %*
1	20	10	19.77	98.85	9.99	99.90	9.97	99.70
2	10	20	10.06	100.60	19.93	99.65	19.89	99.45
3	15	10	15.23	101.53	10.05	100.50	10.03	100.30
4	10	15	10.18	101.80	14.93	99.53	14.91	99.40
5	20	15	20.06	100.30	14.81	98.73	14.96	99.73
6	15	25	15.17	101.13	25.35	101.40	24.50	98.00
7	20	20	20.06	100.30	20.17	100.85	19.75	98.75
		Mean		100.64		100.07		99.33
		SD		0.99		0.89		0.75
		RSD%		0.98		0.89		0.75

Table 3. Determination of metronidazole and spiramycin in laboratory-prepared mixtures by the MCR method

Mixture No	Amount added of metronidazole **µg.mL^{-1}**	**Amount found of spiramycin µg.mL^{-1}**	**Found of metronidazole µg.mL^{-1}**	**Recovery %**	**Found of spiramycin µg.mL^{-1}**	**Recovery %**
1	20	10	19.92	99.60	9.89	98.90
2	10	20	10.17	101.70	20.05	100.25
3	15	10	15.18	101.20	9.89	98.90
4	10	15	10.17	101.70	14.98	99.87
5	10	30	10.19	101.90	29.48	98.27
6	20	15	20.11	100.55	15.02	100.13
		Mean		101.11		99.39
		SD		0.89		0.81
		RSD%		0.88		0.81

were certified to contain 4412 IU mg^{-1} and 99.90% w/w, respectively, according to the manufacturer's method.

Pharmaceutical dosage forms

Spirazole® tablets, batch no.1288009, were labelled as containing 750 000 IU of SP and 125 mg of MZ, and were manufactured by El Pharonia Pharmaceuticals, New Borg El-Arab City, Alexandria, Egypt.

Chemicals and reagents

The methanol used was of spectroscopic grade and was purchased from Prolabo (VWR International, West Chester, Pennsylvania).

Standard solutions

Stock solutions of MZ and SP (1 mg mL^{-1}) were prepared in methanol. *Working solutions of SP and MZ* (0.1 mg mL^{-1}) were prepared by an additional dilution of their stock standard solutions with methanol.

Laboratory prepared mixtures

Solutions containing different ratios of MZ and SP were prepared, containing from 10–25 µg ml^{-1} of each drug.

Table 4. Determination of MZ in Spirazole® tablets by the proposed methods and application of standard addition technique

		D^0			MCR			
Product	*Found[a] %±RSD%*	*Added*	*Found[a]*	*Recovery%*	*Found[a] %±R.S.D%*	*Added*	*Found[a]*	*Recovery%*
MZ in SPirazole® tablets 125 mg MZ and 750,000 IU SP/tablet B.No1288009	100.29 ± 0.98	5	5.07	101.4	100.89 ± 1.01	5	5.06	101.20
		10	9.92	99.2		10	9.95	99.50
		15	14.9	99.33		15	15.2	101.33
Mean ± RSD				99.98 ± 1.23				100.54 ± 1.02

[a] Average of five determinations.

Table 5. Determination of SP in Spirazole® tablets by the proposed methods and application of standard addition technique

	D[1]				DD[1]				MCR			
	Found[a] *%±R.S.D*	*Added*	*Found*[a]	*Recovery%*	*Found*[a] *%±R.S.D*	*Added*	*Found*[a]	*Recovery%*	*Found*[a] *%±R.S.D*	*Added*	*Found*[a]	*Recovery%*
SP in Spirazole® tablets 125 mg MZ and 750,000 IU SP/tablet B.No1288007	99.64 ± 0.63	5	5.03	100.60	100.54 ± 0.45	5	5.02	100.40	99.89 ± 0.50	5	5.01	100.20
		10	9.92	99.20		10	9.90	99.00		10	9.93	99.30
		15	14.94	99.60		15	14.98	99.87		15	14.89	99.27
Mean ± RSD				99.8 ± 0.72				99.76 ± 0.71				99.59 ± 0.53

[a] Average of five determinations.

Procedures

Construction of calibration curve for the D^0 spectrophotometric method (methods A and B)

Aliquots of MZ working solution (0.1 mg mL^{-1} in methanol) equivalent to 50–250 μg mL^{-1} were transferred separately into a series of 10 mL volumetric flasks and the volume was completed to the mark with methanol. The zero–order UV absorption spectrum of each solution was recorded against methanol as a blank. A calibration curve was constructed by plotting absorbance at 311 nm versus the corresponding concentration. The same procedure was used to determine the content of MZ in laboratory prepared mixtures and in Spirazole® tablets.

Construction of calibration curves for the D^1 spectrophotometric method (method A)

Aliquots of SP working solution (0.1 mg mL^{-1} in methanol) equivalent to 50–250 μg mL^{-1} were transferred into a series of 10 mL volumetric flasks; the volume was completed to the mark with methanol. The first derivative absorption spectra (D^1) of the UV-spectrum of each solution against methanol as blank were recorded. D1 curves were recorded at $\Delta\lambda = 4$ and scaling factor = 10. The calibration curve was obtained by plotting the peak amplitude of D^1 spectra at 218.3 nm (corresponding to zero crossing of MZ) of D1 spectra versus the corresponding concentrations. The same procedure was used to determine the content of SP in laboratory-prepared mixtures and in Spirazole® tablets.

Construction of calibration curve for the DD^1 spectrophotometric method (method B)

The absorption spectra of standard solutions of SP (5–25 μg mL^{-1}) and MZ (20 μg mL^{-1}) were recorded against a blank of methanol and stored in the computer. The stored spectra of SP were divided by the stored spectrum of MZ of concentration 20 μg mL^{-1} and the first derivative of the ratio spectra was obtained with $\Delta\lambda = 4$ and scaling factor = 10. A calibration curve was obtained by plotting the peak amplitude at 245.6 nm versus the corresponding concentration of SP and the regression equation was obtained. The same procedure was used to determine the content of SP in laboratory-prepared mixtures and in Spirazole® tablets.

Construction of calibration curve for MCR spectrophotometric method (method C)

Aliquots of MZ and SP working solution (0.1 mg mL^{-1} in methanol) equivalent to 50–250 μg mL^{-1} were each transferred separately into a series of 10 mL volumetric flasks. The volume was completed to the mark with methanol. The absorption spectra of the resulting solution were measured in the 200–400 nm range. The scanned spectra of SP were divided by the normalized absorption spectrum of MZ and the obtained ratio spectra were then mean centred. The same was applied to the spectra of MZ as they were divided by the normalized SP spectrum and were then mean centred. The calibration curves for both SP and MZ were constructed by plotting the mean-centred values at 233.2 nm and 282.4 nm for SP and MZ respectively against the corresponding concentration.

Application to tablet formulation

Twenty Spirazole® tablets (labelled as containing 125 mg MZ and 750 000 IU SP, from Pharonia Pharmaceuticals) were weighed and powdered. An accurate weight of the powder equivalent to 50 mg of MZ and 68 mg of SP was transferred into a 50 mL volumetric flask and extracted with 30 mL methanol, in an ultrasonic bath for 30 min, diluted to volume with the same solvent and filtered. Suitable dilutions were made using methanol to prepare tablet solution containing 10 μg mL^{-1} MZ and 13.6 μg mL^{-1} SP.

Results and Discussion

Metronidazole is coformulated with spiramycin in tablets to improve the antibacterial activity of both drugs. Reviewing the literature on the determination of metronidazole and spiramycin in mixture revealed a lack of any spectrophotometric methods for their determination.

The aim of the present work was therefore to develop simple, rapid, accurate selective and reproducible spectrophotometric methods for the simultaneous determination of SP and MZ in their mixture.

For method A

For the determination of MZ and SP in mixture, zero order absorption spectra of both drugs were recorded and it was found that MZ could be successfully determined at about 311 nm without any interference from SP whereas SP could not be determined due to the severe spectral overlap from the MZ absorption spectrum (Figure 3).

Derivative spectrophotometry is a very useful analytical technique for eliminating spectral overlapping by using the first or higher derivatives of absorbance with respect to wavelength.[27] Upon applying the first derivative (D^1) spectrophotometry, SP could be determined by measuring its peak amplitude of D^1 spectrum at 218.3 nm (corresponding to zero-crossing of MZ) (Figure 4). In order to optimize the D^1 method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 4$ and a scaling factor = 10 showed a suitable signal-to-noise ratio and the spectra showed good resolution. A linear correlation was obtained between the absorption and its corresponding concentration for MZ at $\lambda = 311$ nm and between peak amplitude at 218.3 nm and the corresponding concentration for SP in the range of 5–25 μg.mL^{-1} for both drugs. The parameters of the regression equations are shown in Table 1. The linear regression equations were found to be:

$$A = 0.0555\,C - 0.0132 \quad r = 0.9997 \text{ (for MZ)}$$

$$D^1{}_{218.3} = 0.0168\,C - 0.0098 \quad r = 0.9999 \text{ (for SP)}$$

where A and $D^1{}_{218.3}$ are the absorbance and peak amplitude for MZ and SP, respectively, C is the concentration of the drug in μg mL^{-1} and r is the correlation coefficient.

For method B

The zero-order absorption spectra of MZ show no interference from SP, which allowed the determination of MZ by measuring its zero order absorption spectra at 311 nm, as described for method A.

Table 6. Statistical analysis of the results of the proposed methods and reported methods for MZ and SP in pharmaceutical dosage forms

	Mz			SP			
	D^0	MCR	Reported HPLC method[b]	D^1	DD^1	MCR	Reported HPLC method[b]
Mean	100.29	100.89	98.3	99.64	100.54	99.89	99.05
SD	0.96	1	1.48	0.63	0.45	0.5	1.08
N	5	5	5	5	5	5	5
Variance	0.92	1	2.19	0.4	0.2	0.25	1.17
Student's t	1.69 (2.31)[a]	2.21 (2.31)[a]		0.71 (2.31)[a]	1.92 (2.31)[a]	1.06 (2.31)[a]	
F	2.38 (6.3882)[a]	2.19 (6.3882)[a]		2.93 (6.3882)[a]	5.85 (6.3882)[a]	4.68 (6.3882)[a]	

[a] These values represent the corresponding tabulated values of t and F at $P = 0.05$.
[b] HPLC (using phosphate buffer pH 2.4 and acetonitrile, 70 : 30 v/v).[25]

In order to improve the selectivity of the analysis of SP in presence of MZ, DD1 was also established and validated. The main advantage of this method is that the whole spectrum of the interfering substance is cancelled. In order to optimize the DD^1 method, several divisors were tested as 5, 10, 15, 20, 25 μg mL^{-1} along with the normalized MZ spectrum. The best results were obtained using 20 μg mL^{-1} of MZ as a divisor. The absorption spectra of SP in the range of 5–25 μg mL^{-1} were divided by the absorption spectrum of 20 μg mL^{-1} of MZ. The first derivatives of the obtained ratio spectra were then calculated using $\Delta\lambda = 4$ and scaling factor = 10. DD^1 values showed good linearity and reproducibility at 245.6 nm (Figure 5). The linear regression equation was found to be

$$DD1_{245.6} = 0.089\,C + 0.0014\,r = 0.9999$$

where $DD1_{245.6}$ is the peak amplitude, C is the concentration of the drug in μg mL^{-1} and r is the correlation coefficient.

It was found that first, second and third derivative ratio spectra photometry failed to determine MZ, therefore the MCR method was investigated.

For method C

For further improvement of the selectivity, a new, simple recently developed method was applied. This is based on the mean centring of ratio spectra. It eliminates the derivative step and the signal-to-noise ratio is therefore enhanced.[28]

The MCR method was applied and was able to determine quantitatively both MZ and SP in their laboratory-prepared mixtures and in their pharmaceutical preparation. As shown in Figure 3, the absorption spectra of MZ and SP in methanol severely overlap in the wavelength region of 200–250 nm. So, the absorption spectra of the standard solutions of the SP with different concentrations were recorded in the wavelength range of 200–400 nm and divided by the normalized spectrum of the MZ. The ratio spectra were obtained. Mean centring of the ratio spectra was carried out and the concentration of SP was determined by measuring the amplitude at 233.2 nm (corresponding to a maximum wavelength) (Figure 6). For the prediction of concentration of SP in laboratory prepared mixtures and samples of pharmaceutical preparations the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of SP.

The absorption spectra of the standard solutions of the MZ with different concentrations were recorded in the wavelength range of 200–400 nm and divided by the normalized spectrum of the SP. The ratio spectra were obtained. Mean centring of the ratio spectra was carried out and the concentration of MZ was determined by measuring the amplitude at 282.4 nm (corresponding to a maximum wavelength) (Figure 7). The same procedure was used for the prediction of the concentration of MZ in laboratory-prepared mixtures and dosage form samples, except that the spectra of the mixture were used instead of the spectra of a standard solution of MZ. The effect of divisor concentration on the analytical parameters such as slope, intercept and correlation coefficient of the calibration graphs was also tested. Different of divisors were tested but it was observed that changing the concentration had no significant effect on their linear calibration range and the calculated analytical parameters. A normalized spectrum of each of MZ and SP was therefore used as a divisor spectrum in the proposed method.

The specificity of the proposed methods was proved by the analysis of laboratory-prepared mixtures of MZ and SP in different ratios, as presented in Tables 2 and 3.

All the proposed methods were successfully applied for the determination of MZ and SP in Spirazole® tablets (Tables 4 and 5) and the results obtained were statistically compared with those obtained by the reported HPLC method[25] and there is no significant difference regarding both accuracy and precision as shown in Table 6.

Conclusion

The proposed methods were simple, rapid, sensitive and precise and could be easily applied in quality-control laboratories for the simultaneous determination of MZ and SP. The MCR method has the advantage of eliminating the derivative steps and therefore the signal-to-noise ratio is not degraded. So the proposed methods could be successfully applied for the routine analysis of these drugs both in their pure bulk powders and in dosage form in quality-control laboratories without any preliminary separation step.

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