Original Article

DETERMINATION AND VALIDATION OF SECNIDAZOLE IN TABLETS BY UV SPECTROPHOTOMETRIC

DETERMINAÇÃO E VALIDAÇÃO DO SECNIDAZOL EM COMPRIMIDOS POR ESPECTROFOTOMETRIA NA REGIÃO DO ULTRAVIOLETA

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ABSTRACT: Secnidazole, a 5-nitroimidazole, is a drug used in the treatment against protozoa, and several bacterial infections. This study purpose was to develop and validate a UV spectrophotometric method to determine secnidazole in pharmaceutical tablet dosage forms once there is no method reported in the pharmacopoeia yet. The quantification was performed using methanol as solvent at 325 nm (maximum wavelength) and three kinds of products marketed in Brazil (reference, generic and similar tablets) containing 1g of secnidazole. The method obeyed Beer's law in the concentration range of 4 - 20 μgmL^{-1} respectively. The method was validated according to the International Conference on Harmonization (ICH) and Brazil National Health Surveillance Agency (ANVISA) guidelines, showing accuracy, precision, selectivity, robustness and linearity. Tests such as weight range, friability, disintegration, hardness and dissolution were carried out to check tablets' quality and all the trials showed to be in accordance with the general test guidelines of the Brazilian Pharmacopoeia. The dissolution test was carried out and the developed method was applied. The method developed is suitable for the estimation of secnidazole in tablets without any interference from the excipients and can be used for routine in quality control. Still, it's a simple, fast and low cost method.

KEYWORDS: Quality control. Quantification. Dissolution test. Secnidazole.

INTRODUCTION

Secnidazole (Figure 1), chemically described as 1-(2-hydroxypropyl)-2- methyl – 5 – nitroimidazole (GILLIS; WISEMAN, 1996) is used in the management of protozoal infections and anerobic bacterial infections (BAKSHI; SINGH, 2004; BRUNTON; CHABNER; KNOLLMANN 2012).

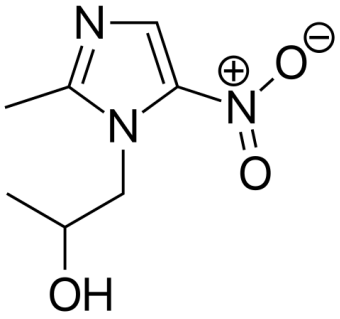

Figure 1. Chemical structure of Secnidazole.

The literature describes different methods for quantitative determination of secnidazole. These include high performance liquid chromatography (HPLC) (KHAN et al, 2015; RAVI et al, 1997; EL WALILY et al, 2000), UV spectrophotometric (SONPETKAR et al, 2012), colorimetric method (EL WALILY et al, 2000), and supercritical fluid chromatography (PATEL et al, 1998). Other methods have been performed for the determination of secnidazole in biological fluids such as polarography (LICHTIG; ANDRADE; VAZ, 1996) and HPLC (RAVI et al, 1997; MONTOVANI et al, 2009). Studies about stability were conducted using HPLC (KHAN et al, 2015; BAKSHI; SINGH, 2004; MIRABAL et al, 2003) and spectrophotometric method (RIVERA et al, 2000). Methods involving determination of secnidazole in tablets usually do not describe the excipients used (SONPETKAR et

Received: 26/12/16
Accepted: 05/05/17

al, 2012; EL WALILY et al, 2000; LICHTIG; ANDRADE; VAZ, 1996) which may compromise the results of analysis.

In Brazil, the manufactured drugs are classified as reference drugs, generic and similar medicines (BERTOLDI; BARROS; HALLAL, 2005) and may have different excipients, since since they have to presente pharmaceutical bioequivalence. Due to this, it is important to have a method for determining secnidazole tablet wherein the excipients do not interfere with the analysis.

As the secnidazole has no monograph for tablets in the official compendiums, the aim of this study was developed to validate and apply a method of UV spectrophotometry cheap, simple and useful for the quantitative determination of secnidazole in preparation of commercial pharmaceutical tablets.

MATERIAL AND METHODS

Reagents and excipients

Secnidazole reference substance was supplied by Pharmanostra (China/Hong Kong) with assigned purity of 98,0%. Secnidazole reference product (Secnidal® tablets, Sanofi-Aventis) and secnidazole generic tablets from two different brands (codified as product A and product B) all contained 1g of the active component were purchased from a local market. For ethical reasons, the brands of these products will not be reported. All studies were performed before the expiration of the validity period of the similar samples.

All reagents used were of analytical grade: distilled water, hydrochloride acid (Synth®), methanol (Synth® and Neon®).

The excipients used were: cellulose (Synth®), crospovidone (Henrifarma®), dihydrated calcium phosphate (Synth®), hypromellose (Henrifarma®), macrogol 6000 (Henrifarma®), magnesium stearate (Henrifarma®), microcrystalline cellulose (Henrifarma®), polyethylene alcohol (Synth®), polyvinyl copolymer (Henrifarma®), povidone (Henrifarma®), propylene glycol (Henrifarma®), silicon dioxide (Henrifarma®), sodium lauryl sulfate (Croda®), sodium starch glycolate (Henrifarma®), starch (Synth®).

Equipments

The equipments used were: analytical balance (Shimatzu/AUY220), Digital pH meter (Del Lab® /DLA-PH), disintegrator (Nova Ética®/301-AC), dissolution test apparatus (Quimis®/Q850), friabilator (Nova Ética®/300-1), hardness tester (Logem Scientific®/LSD-D1), spectrophotometry UV/VIS (PG instruments LTDA®/T80), Affinity-1 Fourier Transform infrared spectrophotometer (Shimadzu®) and ultrasonic bath (Crsitófoli).

Identification

Fourier transform infrared spectroscopy (FTIR) was used to identify secnidazole in three different tablet formulations using an Affinity-1 Fourier Transform infrared spectrophotometer (Shimadzu®). Spectra were recorded at room temperature in the 3500-500 cm^{-1} range. After recording a background spectrum, potassium bromide pellets containing about 1-2% of secnidazole were prepared and immediately analyzed on FTIR spectrophotometer. In each sample, 20 scans were recorded with resolution of 4 cm^{-1}.

Weight variation

This test was performed according to the procedure described on general chapters of the Brazilian Pharmacopoeia. Twenty tablets of each pharmaceutical product (reference, A and B) were weighed individually using an analytical balance. The average weight, standard deviation and variation coefficient were then calculated. For tablets with average weight of 1g, the requirements of the Brazilian Pharmacopoeia are met if the weights of no more than two of the tablets differ from the average weight by more than 5,0 % and no tablet differs in weight by more than double that percentage (BRAZIL, 2010).

Friability

This test was performed according to the method described on general chapters of the Brazilian Pharmacopoeia by using a tablet friability apparatus (friabilator). Ten pre-weighed tablets of each pharmaceutical product (reference, A and B) were placed in the friabilator and then rotated at 25 rpm for 4 min. The tablets were accurately reweighed after removing any loss from the samples. The percentage of weight loss was calculated as follows: % Friability = (Loss in weight / Initial weight) × 100. The Brazilian Pharmacopoeia establishes a mean weight loss of not more than 1.5% (BRAZIL, 2010).

Disintegration test

The disintegration time of tablets was performed using a disintegration apparatus as specified in general chapters of the Brazilian Pharmacopoeia (2010). Six tablets of each pharmaceutical product (reference, A and B) were placed in each compartment of the disintegration apparatus basket and then acrylic disks were added.

The basket was attached to the device and subjected to vertical movements using water as immersion fluid at 37° C ± 2 ° C until complete disintegration of the tablets. The requirement of the Brazilian Pharmacopoeia is met if all tablets tested are completely disintegrated in less than 30 minutes (BRAZIL, 2010).

Hardness

The hardness was determined over 10 tablets as described in general chapters of the Brazilian Pharmacopoeia by using a hardness tester. A minimum hardness of 3 kgf was adopted as the acceptance criteria (BRAZIL, 2010).

Drug content

There isn't a method for determination secnidazole in tablets in main official compendiums. However, drug content was estimated using a spectrophotometric method which was properly developed and validated as recommended in international guidelines (ICH 2005).

Twenty tablets of each pharmaceutical product (reference, A and B) were totally powdered and the weight equivalent of the one average weight was transferred into a 100 mL volumetric flask, 40 mL of methanol was added, and the flask was sonicated for 5 min. The volume of the flask was made up with methanol obtaining the concentration of 200 μgmL^{-1}. From standard solutions, aliquots of 100 to 1000 µL were transferred to 10mL flasks and the volume completed with methanol. Samples were determined in spectrophotometer at a wavelength of 325 nm.

The adopted acceptance criterion was not lower than 80.0 percent and neither higher than 120.0 percent of the labeled amount (BRAZIL, 2003).

Dissolution test

In vitro dissolution test was carried out using a discriminative dissolution method described in general methods of the Brazilian Pharmacopeia (2010). The dissolution conditions were: hydrochloride acid 0,1M (900 mL) and a paddle stirring apparatus at rate of 100 rpm.

Ten milliliters of dissolution medium (controlled at 37.0 ± 0.5 °C) were sampled after 60 min, and the samples were analyzed by the spectrophotometric method. Six samples were assayed for each product. According to the Brazilian Pharmacopoeia (2010), the requirements are met in the first acceptance criteria if the quantities of active ingredient dissolved from each one of six dosage units is not less than Q + 5% and the requirements are met in the second acceptance criteria if the average of the quantities of active ingredient dissolved from twelve dosage units is equal to or greater than Q, and no unit is less than Q – 15%.

Validation

The quantification method was validated according to the criteria established by the Resolution n° 899 of May 29th, 2003, National Health Surveillance Agency (BRAZIL, 2003), International Conference on Harmonization (ICH) (ICH 2005), through the parameters of selectivity, linearity, precision, accuracy, robustness, detection limit and quantification limit.

Specificity

Specificity was evaluated by analyzing solutions containing all the components of the different tablets, excepting the secnidazole. The excipients were prepared in methanol and subject to the some analytical conditions the same analytical conditions of the samples. The system response was examined for the presence of interference or overlaps with Secnidazole responders at 325nm.

Linearity

The analytical curves were obtained with eight concentrations of reference solution in the range of 4-20 μgmL^{-1} for the spectrophotometric method. All trials were was prepared in triplicate. The linearity was evaluated by linear regression analysis by the lest square regression method, which was used to calculate the correlation coefficient and slope of the regression line.

Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by analyzing secnidazole work standard solutions at the same concentration and during the same day. Intermediate precision was studied comparing the assay in three different days by two analysts, six replicates at a concentration of 12 μgmL^{-1}. The data were analyzed at 325 nm. The percentages of relative standard (% R.S.D.) of the analytical response were calculated.

Accuracy

The accuracy was determined by the recovery of a known amount of standard secnidazole solution added to samples of product reference, product A and product B. Different levels of secnidazole standard concentrations were used: lower concentration (4 μgmL^{-1}), intermediate

concentration (8 µgmL^{-1}) and higher concentration (12 µgmL^{-1}). The samples were prepared in triplicate, analyzed by the proposed method and the recovery percentages were determined to evaluate the accuracy.

Detection limit (LOD) and quantitation limit (LOQ)

The LOD and LOQ were calculated based on the standard deviation of the response and the slope using three independent analytical curves, as defined by ICH guidelines (ICH, 2005). It is always useful to demonstrate that the analyses are being conducted in a region which is above the LOQ value. It was obtained the LOD and LOQ form equations: *LOD = 3 (SD/a); LOQ = 10 (SD/a),* where *SD* is the standard deviation of the intercept with the axis in three calibration curves and a is the slope of the line.

Robustness

Robustness tests examine the effect operational parameters have on the analysis result. For the determination of the method it was used reagents from different suppliers and operators.

RESULTS AND DISCUSSION

Secnidazole is soluble in water, but with dependent solubility according to pH, as it increases in media pH lower than 7, the solubility of the substance is increased (RIVERA et al, 2000). It was observed that until pH 2,0 the secondary nitrogen ring is protonated, and the molecule is more soluble. From pH 2,1 this nitrogen deprotonates and the solubility decreases (increase log D), and according to ChemSketch software (ACD labs®) the molecule of secnidazole has the same solubility from pH 4,4 to pH 12,8. The result is showed in Figure 2 and agree with the results obtained by Rivera (2000).

Identify

The FTIR results clearly showed that the absorptions bands of all samples are coincident with secnidazole reference standard. These results confirmed the unequivocal identification of $C_7H_{11}N_3O_3$ on analyzed samples (Figure 3).

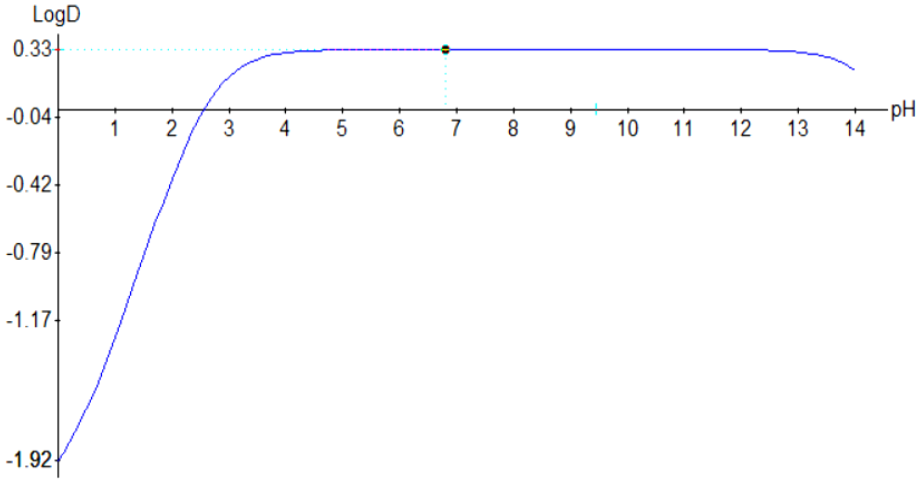

Figure 2. Log D (log ρ) in function of pH calculated for secnidazole molecule using the software ChemSketch.

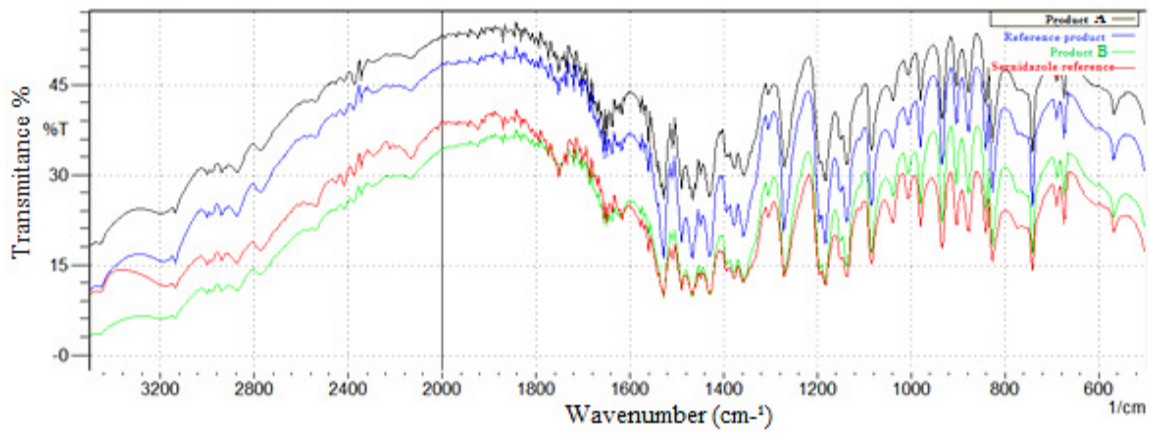

Figure 3. Identification of standard the standard secnidazole and products (reference, A and B).

Weight variation

The Table 1 lists the individual weights of twenty tablets of each pharmaceutical product (reference, A and B) with their average weight, standard deviation and coefficient of variation.

Table 1. Individual weight, average weight, standard deviation and coefficient of variation of tablets claimed to contain 1g of secnidazole.

Tablet	Individual weight Reference Product (mg)	Individual weight Product A(mg)	Individual weight Product B (mg)
1	1250	1255	1215
2	1240	1280	1247
3	1260	1245	1143
4	1241	1251	1150
5	1255	1256	1216
6	1249	1255	1195
7	1250	1257	1141
8	1252	1255	1235
9	1250	1256	1149
10	1255	1257	1198
11	1257	1256	1200
12	1251	1255	1195
13	1251	1254	1199
14	1250	1256	1210
15	1249	1258	1215
16	1257	1256	1198
17	1255	1255	1199
18	1250	1256	1220
19	1256	1256	1215
20	1255	1255	1211
Average weight (mg)	1251,65	1256,65	1197,55
Standard deviation (mg)	4,97	6,23	29,70
Coefficient of variation (%)	0,39	0,49	2,48

The Table 1 showed that all tested units are within the limit established by the Brazilian Pharmacopeia (2010). In addition, none tablet weighed more or less than double the established percentage of 5%. In addition, the coefficient of variation of all tested products was less than 2,5%.

Friability

The results of friability of the analyzed products are shown in Table 2.

Table 2. Friability of tablets claimed to contain 1g of secnidazole.

Product	Total weight before test (g) (n = 10)	Total weight after test (g) (n = 10)	Weight loss (%)
Reference product	12,51	12,50	0,03
Product A	12,57	12,55	0.10
Product B	11,89	11,87	0.15

The results in Table 2 showed that all samples lost less than 0.5% weight when subjected to friability test. Therefore, all samples are within the limit permitted by pharmacopeia establishing a maximum of 1,5% (BRAZIL, 2010).

Disintegration time

The results of disintegration time of the analyzed products showed that the time for complete tablet disintegration in all cases was less than 5 minutes, in accordance with the Brazilian

Pharmacopoeia that requires maximum time of thirty minutes for disintegration test (BRAZIL, 2010).

Hardness

The results of Hardness of the analyzed products are shown in Table 3. It is seen that all that all tested units showed hardness higher, therefore, all samples complied with Brazilian Pharmacopoeia requirements for this test that established a maximum of 3,0 kgf (BRAZIL, 2010).

Drug content

The results of drug content of the analyzed products are shown in Table 4.

Table 3. Hardness of tablets claimed to contain 1g of secnidazole

Tablet	Hardness (Kgf)		
	Reference product	Product A	Product B
1	18,30	18,30	14,80
2	18,70	18,10	18,30
3	18,40	16,00	16,60
4	18,30	17,20	16,50
5	18,60	18,20	18,80
6	18,70	16,30	17,00
7	18,10	18,40	14,90
8	18,40	17,10	14,50
9	18,40	18,10	14,90
10	18,60	18,00	15,00

Table 4. Drug content of tablets claimed to contain 1g of secnidazole.

	Reference Product	Product A	Product B
Percentage of the labeled amount of secnidazole	99,26%	88,00%	93,15%
Coefficient of variation (%)	3,43	4,50	3.13

The label percentage claim found for the procedure is in accordance with Anvisa and the ICH which set the limit between 80 and 120% for drugs that are not described in monographs (BRAZIL, 2003; ICH, 2005).

Rivera et al (2000) determined secnidazole in aqueous solutions in the range of 5,0 to 15µgmL^{-1} and Sonpetkar et al (2012) used the spectrophotometric method for estimation of secnidazole using as solvent a mixture water: methanol (30:70) with concentrations of 1,0 to 4,0µgmL^{-1}. When these procedures were taken, the precision and accuracy were lost, probably due to the excipients used in these formulations and the values of absorbance declined in ten minutes. Because of this, the use of methanol by itself was important to determinate secnidazole in these tablets.

Dissolution test

The results of dissolution test of the analyzed products are shown in Table 5.

The Brazilian Pharmacopeia fixed the maximum time for testing in 60 minutes and limit of 80 % of value for approval. As there is no specific monograph for secnidazole, this criterion was followed for approval.

The observation of Table 5 allows us to conclude that the reference product and product A were approved in the first acceptance criterion established for the dissolution test for the quantities of active ingredient dissolved from each one of the six dosage units were higher than Q + 5% (85%), not being therefore necessary to conduct further tests (BRAZIL, 2010).

The product B was not approved in the first acceptance criterion, since one tested unit presented the quantity of active ingredient dissolved less than Q + 5% (85%). A second experiment was conducted with more six samples and the product was approved in the second acceptance criterion, since the average of the quantities of active ingredient dissolved from twelve tested dosage units was greater than Q (80%), and no unit was less than Q - 15% (65%) (BRAZIL, 2010).

Table 5. Dissolution test of product reference, A and B.

Tablet	Drug release (%)		
	Reference Product	**Product A**	**Product B**
1	100,98	100,64	99,63
2	95,26	98,96	96,72
3	102,96	99,45	92,61
4	99,20	98,80	92,99
5	98,70	99,70	98,74
6	100,20	99,90	96,45
7	-	-	99,00
8	-	-	96,70
9	-	-	99,80
10	-	-	98,90
11	-	-	101,10
12	-	-	98,90
Average	99,55	99,56	97,62
Standart Variation	2,58	0,67	2,64
Coefficient of variation	0,026	0,007	0,052

Validation

The analytical curve for secnidazole standard in methanol showed proper linearity in the range from 4 to 20 μgmL^{-1}, with r = 0,9989 (correlation coefficient). The equation of the straight line obtained was y = 0,0385x + 0,0098.

The selectivity, verified by the spectrophotometric method, demonstrated no interference by the excipients in the wavelength of 325nm (Figure 4).

The results of precision of the analytical method, are shown in Table 6.

Repeatability (intra-day precision) of the method was found to be reliable based on %R.S.D. (< 2%). Intermediate precision (inter-day) was demonstrated on different days by two analysts. The %R.S.D. values were less than 2%, confirming that the method is sufficiently precise (Table 6) and it is within the parameters established by ANVISA, at most 5.00% (BRAZIL, 2003).

The accuracy of the method was confirmed by determining the average recoveries from the samples by applying the standard addition method. The Table 7 shows that the mean percentage recoveries of products containing secnidazole 1g (Product reference, A and B) were in accordance with fixed limits of 80 up to 120 %, indicating the suitability of the developed method in quantifying the amounts of secnidazole in pharmaceuticals tablets (BRAZIL, 2003).

The limit for detection of drug samples was 0.675 μgmL^{-1}, while it is possible to realize a quantitative analysis of concentrations from 2.25 μgmL^{-1}, showing that all analyzes were performed at concentrations above these limits. To measure the robustness of the studied method, the validation assays were performed using reagents from different suppliers and operators and no change was observed indicating that the method is robust.

The proposed method is simple, sensitive and reproducible demonstrated by the parameters of accuracy, precision, detection limit, and can be applied in the daily the determination of Secnidazole routine in tablet form. It is still an economic and easily executed method.

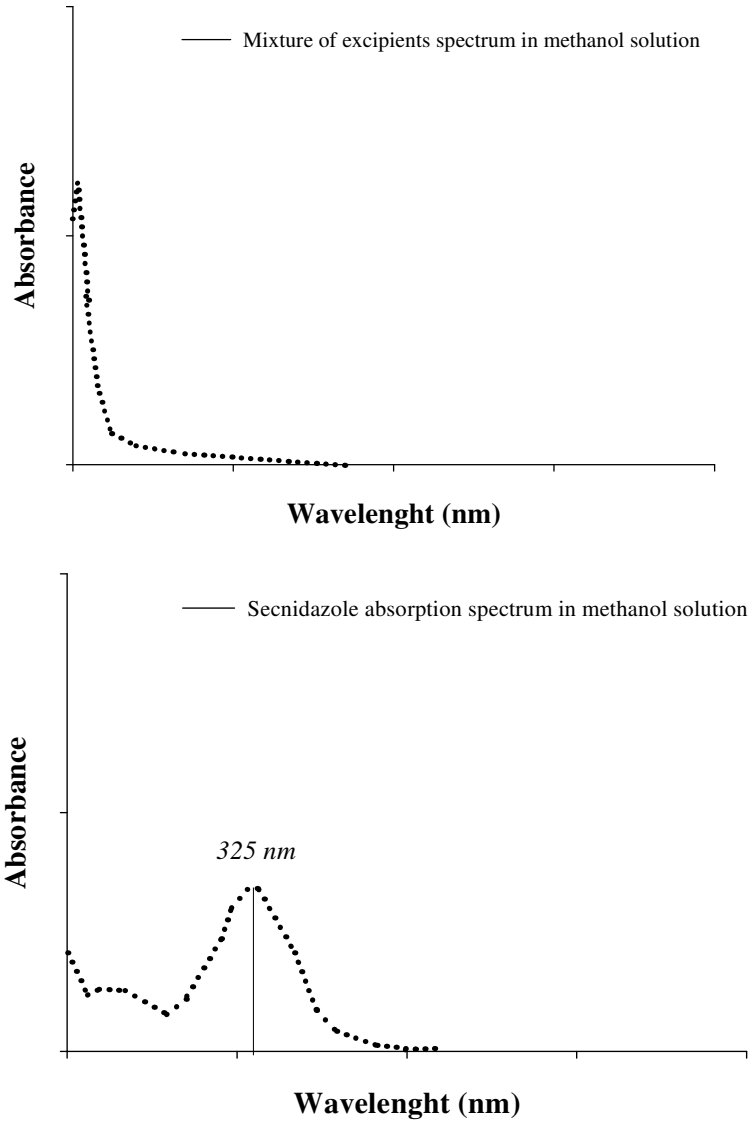

Figure 4. UV spectrum of mixture of excipientes and secnidazole.

Table 6. Precision of the propose method for tablets secnidazole 1g.

Parameter	Product	Absorbance		Average	% R.S.D.
Repeatability (Intra-day) accuracy of the analysis method λ= 325 nm	**Reference standard**	0,428 0,448 0,458	0,454 0,446 0,444	0,446 (n = 6)	1,03
Intermediate precision (Inter-day) accuracy of the analysis method λ= 325 nm	**Reference standard**	0,462 0,496 0,500 0,485 0,481	0,477 0,497 0,485 0,477	0,484 (n = 9)	1,20

Table 7. Method accuracy results for secnidazole 1g tablets.

Samples at 10 μgmL^{-1}	Reference standard concentrations (μgmL^{-1})	Recovery	Standard Deviation (n= 3)	Mean recovery (%)
Reference product	2,00	98,20	1,52	98,73
	6,00	100,80		
	10,00	97,19		
Product A	2,00	97,00	3,45	94,16
	6,00	96,20		
	10,00	89,30		
Product B	2,00	86,60	3,91	92,13
	6,00	94,90		
	10,00	94,90		

CONCLUSIONS

The quality tests for tablets of the products reference, A and B were considered satisfactory in relation to their identification. average weight, friability, disintegration test, hardness.

A UV spectrophotometric method were developed to determine secnidazole in tablets. This method can be applied to the drug content of secnidazole tablets and dissolution test.

The proposed method is simple, quick, accurate, precise, robust and showed adequate detection and quantification limits. Moreover, the method can be easily applied in routine quality control laboratories.

RESUMO: Secnidazol, um nitroimidazólico, é um fármaco utilizado no tratamento para protozoários, e várias infecções bacterianas. Este trabalho propôs o desenvolvimento e validação de um método espectrofotométrico na região do ultravioleta para a determinação de Secnidazol na forma farmacêutica de comprimidos, uma vez que não há nenhum método relatado nas farmacopeias. A quantificação foi realizada utilizando metanol como solvente a 325 nm (máximo de comprimento de onda) e usando três tipos de produtos comercializados no Brasil (de referência, genéricos e comprimidos similares) contendo 1g de Secnidazol. O método obedeceu a lei de Beer no intervalo de concentração de 4 - 20 μgmL^{-1}, respectivamente. O método foi validado de acordo com a Conferência Internacional de Harmonização (ICH) e diretrizes da Agência Nacional de Vigilância Sanitária do Brasil (ANVISA), apresentando exatidão, precisão, seletividade, robustez e linearidade. Testes como variação de peso, friabilidade, desintegração, dureza e dissolução foram realizados para verificar a qualidade de comprimidos e mostrou-se de acordo com os testes gerais da Farmacopeia Brasileira. O teste de dissolução realizado e o método desenvolvido pode ser aplicado. O método desenvolvido é adequado para a estimativa de secnidazole em comprimidos sem qualquer interferência dos excipientes e pode ser usado para a rotina de controlo de qualidade. Ainda, é um método simples, rápido e de baixo custo.

PALAVRAS-CHAVES: Controle de qualidade. Quantificação. Teste de dissolução. Secnidazol.

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