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Application of *p*-Benzoquinone to Spectrophotometric Determination of Secnidazole

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Abstract

A simple, accurate and low cost spectrophotometric method for the determination of secnidazole in raw and pharmaceutical dosage forms has been developed. The proposed method is based on the reduction of the nitro group of the drug to amino group in the presence of Zinc dust and HCl. This reduced product then reacts immediately with *p*-benzoquinone and develops color, which has maximum absorbance at 532 nm. The calibration graph is linear over the concentration range of 12.5-160 μgml^{-1} with molar absorption coefficient of 1.5×10^3 $Lmole^{-1}cm^{-1}$.The common excipients and additives do not interfere in the determination. The proposed method is applied successfully to commercially available tablets and results are statistically compared with those obtained by reference method.

Key words: Secnidazole, p-Benzoquinone, pharmaceutical analysis, spectrophotometry

Introduction

Secnidazole is a 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole and used as antiamoebic, antiprotozoal and antibacterial drugs similar indications as metronidazole but long duration of action [1]. Several methods have been reported for the determination of secnidazole includes spectrophotometric [2-8] spectrofluorometric [6] and HPLC [7-9]. A review of spectrophotometric methods indicates that most of these are time consuming and stringent control of conditions (temperature, pH) is required.

The *p*-benzoquinone was previously reported to be a sensitive reagent for spectrophotometric determination of considerable number of amine containing medicinal compounds [10, 11].

In this work the reduction of secnidazole with Zinc dust and HCl as well as the reaction of its reduced product with p-benzoquinone has been studied to declare the optimum reaction conditions, optical characteristics, precision and accuracy of the proposed method. Officially assay of secnidazole is not reported in pharmacopoeias. That is why we parallel performed an already reported spectrophotometric method [3] for comparing our method statistically. It was found that the results of both methods were not significantly different. In the present work, an attempt was made to provide a simple, accurate and low cost spectrophotometric method for the quantitative determination of secnidazole in raw and pharmaceutical preparations without the interferences of other constituent in the formulations.

Experimental

Apparatus

A Hitachi spectrophotometer model U 1100 with 1 cm silica cells was used throughout this research work.

Materials and Reagents

All reagents were of analytical grade and doubly distilled water was used. Secnidazole was

obtained from Nabiqasim pharmaceuticals (Pvt.) Ltd. Karachi Pakistan. Secnidazole tablets were purchased from local market.

Solutions

Reduced secnidazole standard stock solution was prepared by dissolving 62.5 mg of secnidazole in 30 ml hot ethanol with 5 ml of 5N HCl solution and 1 gm zinc dust. The mixture was heated in water bath at 90 ± 5 °C for 15 minutes, then cool, filter and wash the residue with ethanol .The volume of the filtrate was made up to 100 ml with ethanol in volumetric flask to get the standard solution of 625 $\mu g ml^{-1}$.

An 8 mg ml^{-1} of *p*-Benzoquinone solution was prepared by dissolving an accurate weight in minimum amount of ethanol in 100 ml measuring flask and completing the volume up to the mark with ethanol.

Construction of calibration curve

Into series of 50 ml volumetric flasks, transfer aliquots (1 to 13 ml) of the reduced secnidazole standard stock solution equivalent 12.5-160 $\mu g\ ml^{-1}$ of secnidazole, add one ml of p-benzoquinone solution, complete the volume to 50 ml with ethanol and measure the absorbance at 532 nm against a reagent blank. The calibration curve was constructed by plotting the concentration of the secnidazole in $\mu g\ ml^{-1}$ against the absorbance.

Procedure for the assay of secnidazole in pharmaceutical formulations

Twenty tablets were accurately weighed and powdered. A portion equivalent to 100-600 mg of secnidazole was reduced as mention before under solutions. The resulting filtrate was transferred to a 100 ml volumetric flask and made up the volume with ethanol. One ml of this reduced secnidazole was further treated with one ml of *p*-benzoquinone and volume was completed to 50 ml with ethanol in volumetric flask. An aliquot of this solution was analysed for secnidazole following the procedure described for calibration curve.

Results and Discussion

Determination of absorption maximum

Reduced drugs when treated with *p*-benzoquinone form a purple colour, which absorb maximum at 532 nm. The absorption spectrum of the product against reagent blank is shown in Fig. 1.

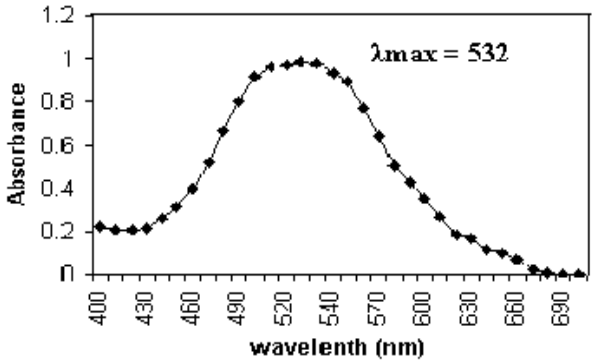
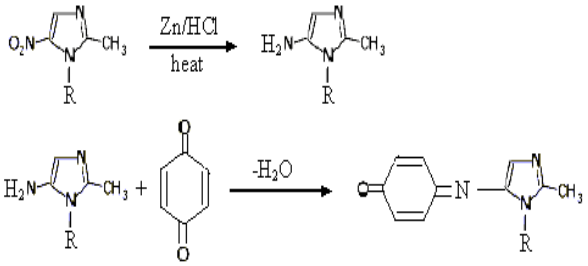


Figure 1. Absorption spectra of secnidazole

Reaction sequence

The reaction between reduced secnidazole and *p*-benzoquinone is shown below.



R = CH_2CH_2 (OH) CH_3

Interference studies

To study the potential interference problems from the commonly used excipients and other additives such as glucose, lactose, starch, talc, sodium starch glycolate, microcrystalline cellulose, magnesium stearate and ascorbic acid, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (secnidazole 20 $\mu g\ ml^{-1}$), excipients in different concentrations were added and analyzed. Results of the recovery analysis are

presented in Table 1. Excipients up to the concentrations shown in the Table do not interfere with the assay. In addition recoveries in most cases were 100% and the lower values of the RSD indicate the good precision of the method.

Table 1. Determination of Secnidazole in the presence of excipients

No. of Obs.	Excipients	Amount taken (μgml^{-1})	% recovery ± RSD (n = 5)
1	Talc	50	99.64 ± 0.82
2	Microcrystalline cellulose	300	101.32 ± 0.74
3	Sodium starch glycolate	100	100.84 ± 0.65
4	Glucose	50	99.21 ± 0.56
5	Lactose	300	100.2 ± 0.52
6	Magnesium Stearate	50	98.2 ± 0.58
7	Starch	200	100.02 ± 0.75
8	Ascorbic acid	50	99.82 ± 0.39

Optical characteristics and validation of the method

Optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity, for secnidazole, are given in Table 2. The accuracy and precision of the method was checked by analyzing 5 replicate samples within the Beer's law range containing the same amount of drug. Values of RSD are below 0.9 %. The lower values of RSD indicate the good precision and reproducibility of the method.

Table 2. Optical characteristics and validation data

Parameters	Values
λ_{max}	532
Colour	Purple
Stability of color (minutes)	20
Beer's law limit (μgml^{-1})	12.5-160
Molar absorption coefficient ($L\ mole^{-1}\ cm^{-1}$)	1.5×10^3
Sandell's sensitivity (μgml^{-1} per 0.001 A)	1.25×10^{-1}
Regression equation(Y*)	
Slope (b)	0.0083
Intercept (a)	0.0058
Correlation coefficient (r)	0.9994
RSD** (%)	0.865

Y* = a + bC
Where C is the concentration of analyte (µg/ml) and Y is absorbance unit.
** = Calculated from five determinations

Applicability of the method

The proposed method is successfully applied for the determination of secnidazole in pure and in pharmaceutical dosage forms and results are compared statistically with reported method [3] as shown in Table 3. The RSD values are in range of 0.37 to 0.71 % for the reproducibility and recovery studies which show that the method is precise and accurate. The precision and accuracy of the method was further compared statistically using Student's t-test and variance ratio F-test. At a 95% confidence level, the calculated t-values and F-values do not exceed the tabulated values.

Calibration curve is linear over the concentration range of 12.5-160 µg ml^{-1} as shown in Figure 2.

Table 3. Determination of secnidazole formulations by the proposed and references methods

Formulation	Proposed method		Reported method		t-test	F-test
	Recovery* (%)	RSD (%)	Recovery* (%)	RSD (%)		
Dysen Forte	99.91	0.71	100.00	0.49	0.668	2.125
Secnidal	100.05	0.66	100.14	0.37	0.412	3.021
Senex	99.86	0.68	100.09	0.47	0.872	2.194

*Average of 5 independent analysis

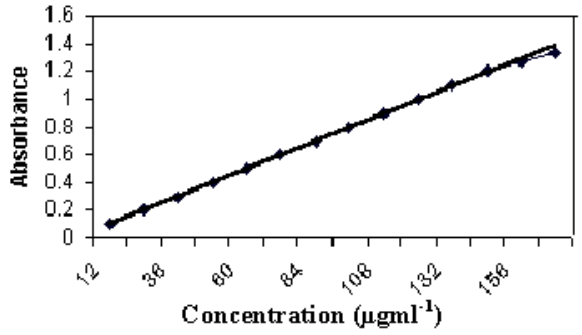


Figure 2. Calibration curve of secnidazole

Conclusion

The proposed spectrophotometric method for the determination of secnidazole is simple accurate, precise and cheap. The statistical analysis is good agreement with those reported method. The colour reaction does not require stringent conditions like temperature and pH. The colour is stable up to 20 minutes which is sufficient time for analyst to perform analysis.A comparison of the present method with the existing spectrophotometric methods is given in Table 4. Which shows that proposed method is cheap, accurate and easy to operate.

Table 4. Comparison of proposed method with existing spectrophotometric methods

S. No.	Reagents	λ_{max}	Limitations	References
1	3-methyl benzothiazolin-2-one hrdrazone (MBTH)	630	MBTH is a costly reagent.	2
2	N-1-naphthyl-ethylenediamine dihydrochloride (NEDA)	536	Involve an additional step of diazotization.	3
3	*p*-dimethyl aminocinnamaldehyde	494	Condensation reaction. Time consuming.	4
4	Bratton and Marshall reagent	470	Three step process involve Zn dust reduction, diazotization and coupling.	6
5	Folin-Ciocalteu reagent	502	Costly reagent and not easily available	7
6	Bromothymol blue	-	Involves extraction with $CHCl_3$ and use of buffer of pH 4.4.	8
7	*p*-Benzoquinone	532	Two step process. Reagents are cheap and easily available.	This work

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