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Spectrophotometric determination of metronidazole and tinidazole in pharmaceutical preparations

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Abstract

Sensitive and simple spectrophotometric methods for the determination of metronidazole (MNZ) and tinidazole (TNZ) in either pure form or in its pharmaceutical formulations are described. The first method is based on the interaction of 3-methylbenzothiazolin-2-one hydrazone (MBTH) with MNZ/TNZ (reduced drug) in presence of copper sulphate and pyridine in acidic medium. The resulting yellowish orange products have λ_{max} of 500 and 490 nm, respectively, for MNZ and TNZ and are stable for about 4 h. The second method describes the reaction between reduced diazotised drugs with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) in neutral medium to yield pink products which have λ_{max} of 520 and 505 nm, respectively, for MNZ and TNZ, respectively. The products are stable for more than 24 h. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed method. Both the methods are highly reproducible and have been applied to a wide variety of pharmaceutical preparations and the results compare favourably with those of official methods.

Keywords: Metronidazole; Tinidazole; MBTH; NEDA; Diazotisation; Spectrophotometry

1. Introduction

Metronidazole (MNZ) (2-methyl-5-nitroimidazole-1-ethanol) and tinidazole (TNZ) (1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitro-1H-imidazole) are used as antiprotozoal, antiamebic and antibacterial drugs. Excellent reviews have been published on the activity and pharmacokinetics of these drugs [1–3]. A survey of literature reveals that there are various methods available for the determination of MNZ and TNZ which includes volumetric [4], gravimetric [5], polarographic [6], gas-chromatographic [7], TLC [8], HPLC and HPTLC [9–14], voltammetric [15], derivative spectrophotometry [16–19], flow injection analysis [20], official methods [21–23] and spectrophotometry [24–55]. Most of the spectrophotometric methods reported suffer from the disadvantages like narrow range of determination, requires heating or extraction, long time for the reaction to complete, use of non-aqueous systems, stability of the coloured product formed, etc. Moreover, ma-

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jority of the spectrophotometric methods reported, are for combined formulations (MNZ or TNZ with some other drugs) and most of them are UV-spectrophotometric methods.

The scientific novelty of the present method is that the reagents used in both the methods are easily available and the chemistry of these reagents is already well established. The reactions involved with these reagents are simple, rapid and sensitive in their range of determinations compared with other established methods. Further, spectrophotometric methods involve simple instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories can not afford to have. The present methods involve the formation of highly stable coloured species (4–24 h) which makes it easier for their determination. As MNZ and TNZ are important class of imidazole compounds known for their antiamebic and antiprotozoal activity, their determination in pharmaceuticals is of great importance.

The idea of the present work is to provide simple, sensitive and rapid spectrophotometric determination of MNZ and TNZ and the methods are free from intereference when excipients are present. In continuation of our research work on spectrophotometric determination of organic compounds of pharmaceutical importance [56–59], the present communication reports sensitive spectrophotometric methods for the determination of MNZ and TNZ in either pure form or in pharmaceutical preparations. This paper makes an attempt to satisfy the growing demand for the determination of these drugs individually, as most of the methods reported are for combined formulations. The methods are based on (i) the oxidative coupling reaction of the reduced drug with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in presence of copper sulphate and pyridine in acidic medium and (ii) the coupling reaction between the diazotised drug (reduced) and *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) in neutral medium.

2. Experimental

A JASCO model UVIDEC-610 UV–vis spectrophotometer with 1.0 cm matched cells was used.

MNZ (Sigma, USA), TNZ (Gift sample from Smithkline Beecham Pharmaceuticals), MBTH (Sigma), NEDA (BDH, Poole, UK), $NaNO_2$ (AR), $CuSO_4$(AR), pyridine (AR), methanol (AR) and hydrochloric acid (AR) were used. All other chemicals and solvents used were of analytical reagent grade. Deionised water was used to prepare all solutions and in all experiments. Commercial dosage forms were purchased from local sources.

2.1. Solutions

Accurately weighed (100 mg) MNZ or TNZ was transferred to a 100 ml beaker containing 4 ml of absolute methanol. Add 0.5 g of zinc dust along with 4 ml concentration hydrochloric acid (HCl). Stir well and wait for 20 min, filter and the filtrate was diluted with water to 100 ml in a volumetric flask. The working standard solution of the reduced MNZ or TNZ containing 50 or 100 μg ml^{-1} was prepared by further dilution. A 0.5% aqueous solution of MBTH and NEDA were freshly prepared. About 0.001 M $CuSO_4$ solution, 10 M HCl, 1% aqueous solution of sodium nitrite and 2% solution of sulphamic acid were used.

2.2. Procedure

MBTH method, aliquots of the working standard solution of reduced MNZ (25–800 μg ml^{-1}) or reduced TNZ (100–900 μg ml^{-1}) were transferred into 25 ml calibrated flasks. 4 ml of 0.2% MBTH solution was added (2 ml of 0.5% MBTH for TNZ). The solutions were shaken well and add 2 ml of 0.001 M $CuSO_4$ along with 4 ml of pyridine (3 ml of pyridine for TNZ). The solutions were swirled and wait for 5 min (15 min for TNZ). Add 3 ml of 2 M HCl (5 ml of 2 M HCl for TNZ) and made up to the mark with water. After mixing the solutions thoroughly, the absorbance was measured at 500 nm for MNZ and at 490 nm for TNZ against the corresponding reagent blank and calibration graphs were constructed.

NEDA method, aliquots of the working standard solution of reduced MNZ (12.5–450 μg ml^{-1}) or reduced TNZ (12.5–450 μg ml^{-1}) were transferred into 25 ml calibrated flasks. 2.5 ml of 10 M HCl was added (3.0 ml of 5 M HCl for TNZ), cool

in an ice bath and add 2.0 ml of 1% $NaNO_2$ solution, the solutions were cooled and add 5.0 ml of 2% sulphamic acid solution and stir the solution for 5 min and 2.0 ml of 0.5% of NEDA solution was added (2.0 ml of 0.2% NEDA solution for TNZ) and made upto the mark with water. The solutions were mixed thoroughly and the absorbance was measured at 520 nm for MNZ and at 505 nm for TNZ against the corresponding reagent blank and calibration graphs were constructed.

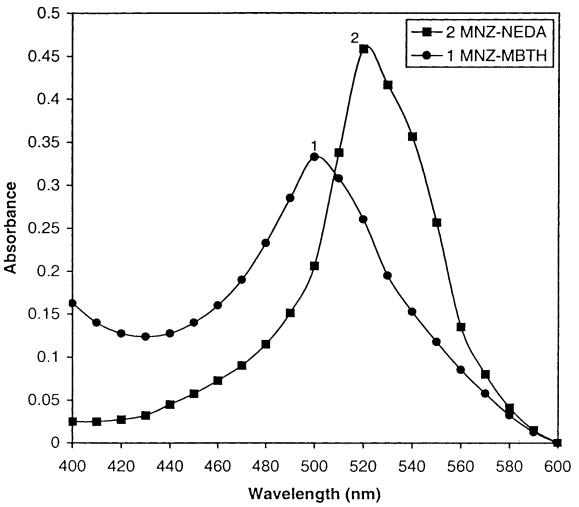


Fig. 1. Absorption spectra of [1](MNZ-MBTH) and [2](MNZ-NEDA) reaction products. Initial concentrations of MNZ were 16 and 9 μg ml^{-1}, respectively.

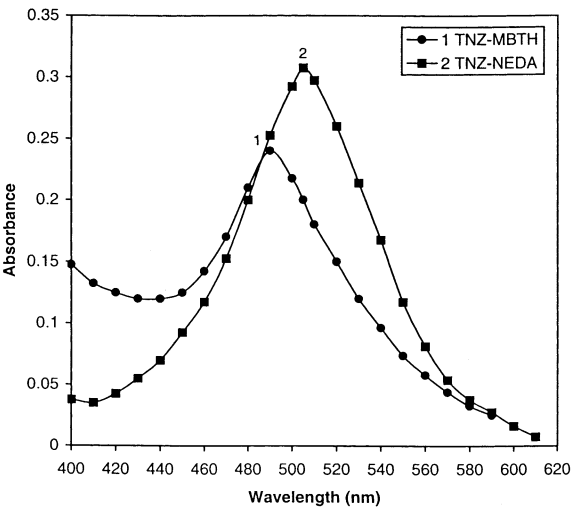


Fig. 2. Absorption spectra of [1](TNZ-MBTH) and [2](TNZ-NEDA) reaction products. Initial concentration of TNZ were 16 and 9 μg ml^{-1}, respectively.

2.3. Procedure for the assay of the drug in commercial samples

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the drug was reduced as mentioned in Section 2.1 and the filtrate was made upto 100 ml and an aliquot of this solution was treated as described above for pure sample in both the methods.

For injections, an appropriate volume of the sample was taken and either of the two methods was followed.

3. Results and discussion

In the MBTH method, the reduced drug was allowed to react with MBTH in the presence of copper sulphate and pyridine in acidic medium. In the NEDA method, the reduced form of the drug was diazotised in acidic medium and coupled with NEDA in neutral aqueous medium.

3.1. Spectral characteristics

The absorption spectra of the yellowish orange coloured product (MNZ-MBTH) with λ_{max} of 500 nm and of the dark pink coloured product (MNZ-NEDA) with λ_{max} of 520 nm are shown in Fig. 1. Similarly, the absorption spectra of the yellowish orange product (TNZ-MBTH) with λ_{max} of 490 nm and of the pink coloured product (TNZ-NEDA) with λ_{max} of 505 nm are shown in Fig. 2. The above mentioned wavelengths were used in the respective studies. The reagent blanks have practically negligible absorption in all the four systems.

Table 1
Optical characteristics and precision data

Parameter/characteristics	MNZ[a]	TNZ[a]	MNZ[b]	TNZ[b]
Colour	Yellowish orange	Yellowish orange	Dark pink	Pink
λ_{max} (nm)	500	490	520	505
Stability (h)	04	04	24	24
Beer's law range (μg ml^{-1})	01–32	04–36	0.5–18	0.5–18
Limit of detection (μg ml^{-1})	0.2898	0.4716	0.1899	0.1804
Limit of quantification (μg ml^{-1})	3.3729	1.6207	0.6328	0.6015
Molar absorptivity (l mol^{-1} cm^{-1})	9.142×10^3	2.774×10^3	7.194×10^3	7.5×10^3
Sandell's sensitivity (μg cm^{-2})	0.0187	0.08917	0.00238	0.00329
Optimum photometric range (μg ml^{-1})	03–29	06–33	1.5–16	1.5–15
Regression equation (y)[c]				
Slope (a)	0.009045	0.01074	0.0413	0.0289
Intercept (b)	0.1276	0.009112	0.01029	0.00346
Correlation coefficient (r)	0.9852	0.9951	0.9978	0.9965
R.S.D. (%)[d]	1.0433	0.6394	0.4502	0.7975
Ranger of error	±1.4481	±0.8875	±0.6249	±1.107

[a] MBTH method.
[b] NEDA method.
[c] $y = ax + b$, where x is the concentration in μg ml^{-1}.
[d] Five replicates.

3.2. Optimum reagents concentration

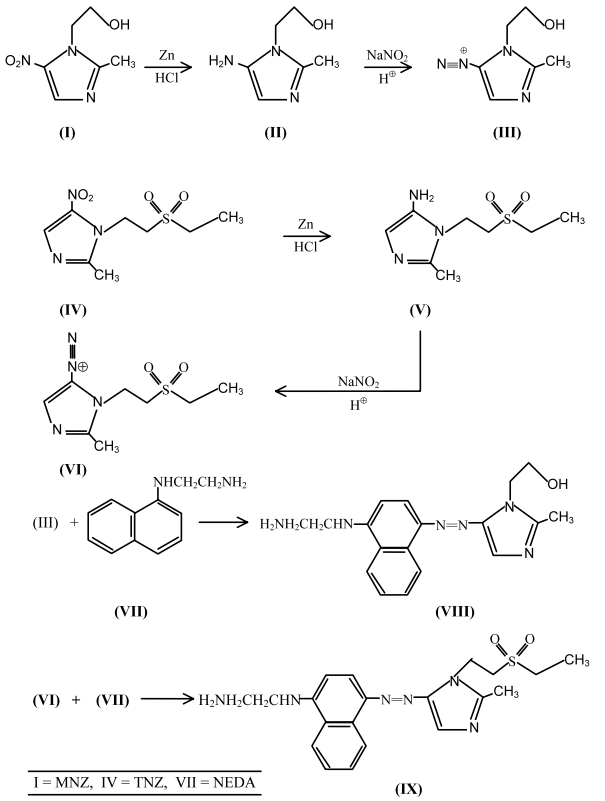
For the MBTH method, it was found that 2–6 ml of 0.2% MBTH solution (1–3 ml of 0.5% MBTH solution for TNZ), 1–3 ml of 0.001 M $CuSO_4$ solution, 3–5 ml of pyridine (2–4 ml of pyridine for TNZ) and 2 M HCl in the range of 2–4 ml (2 M HCl in the range of 3–4 ml for TNZ) were necessary to achieve maximum colour intensity.

For NEDA method, 10 M HCl in the range of 1.5–3.5 ml (2–4 ml of 5 M HCl for TNZ), 1–3 ml of 1% $NaNO_2$ solution, 3–4 ml of 2% sulphamic acid solution and 1–3 ml of 0.5% NEDA solution (1–3 ml of 0.2% NEDA solution for TNZ) were necessary for the development of maximum colour intensity.

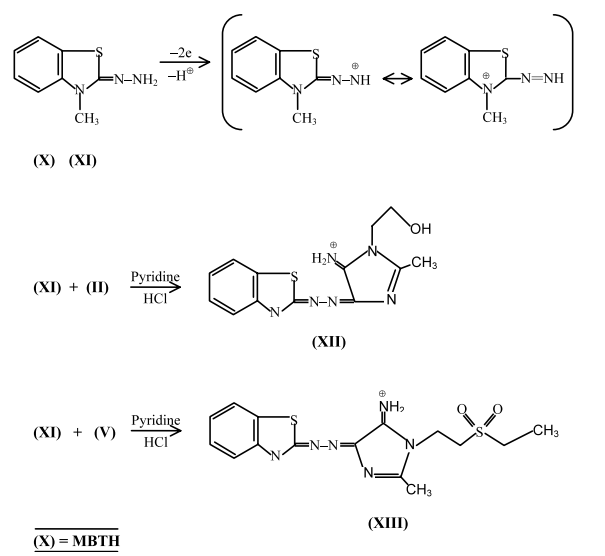
Hence, required volumes of all the reagent solutions were used as mentioned in the recommended procedure. The excess of nitrite could be removed by the addition of 5.0 ml of 2% sulphamic acid solution. An excess of sulphamic acid has no effect on the colour intensity of the product formed. In case of NEDA as a coupling agent, dilution of the coloured solution with different solvents like water, methanol, ethanol, acetic acid and acetonitrile have been tested. However, dilution with water gives maximum intensity and stability of colour.

3.3. Quantification

Beer's law is obeyed over the MNZ concentration range of 1–32 μg ml^{-1} for MBTH and 0.5–18 μg ml^{-1} for NEDA. Similarly, for TNZ, Beer's law is obeyed over the concentration range of 4–36 μg ml^{-1} for MBTH and 0.5–18 μg ml^{-1} for NEDA. The proposed procedures are validated by determining various optical parameters, which are listed in Table 1. The linearity, slope and the intercepts have been calculated using the regression equation $y = ax + b$, where 'y' represents optical density, 'x' the concentration of drug in μg ml^{-1} and 'a' and 'b' represent slope and intercept, respectively. Precision and accuracy of the proposed methods were tested by carrying out determinations of five replicates of pure and com-



Scheme 1.



Scheme 2.

mercial samples of both the drugs, whose concentration lie within Beer's law range. The values of standard deviation (S.D.), relative standard deviation (R.S.D.), range of error at 95% confidence level were calculated.

There was no change in λ_{max}, when the least concentration of the analytes was determined, for the calculation of LOD. LOQ was found to be three times LOD, which is in accordance with Thumb's rule. The experiment for the proposed methods were conducted by the second analyte on different days and the results produced justified the ruggedness of the proposed methods. The two methods have been applied to various pharmaceutical formulations and recovery studies have been made. The optical characteristics and precision data for MNZ and TNZ for both the methods suggested are presented in Table 1.

3.4. Reaction sequence

NEDA method describes the diazotisation of the reduced drug to form the diazonium salt. The salt is then coupled with NEDA to yield a pink azo dye. In the MBTH method, the reduced drug reacts with MBTH in presence of $CuSO_4$ and pyridine in acidic medium to give the coloured product. Actually this reaction is a copper catalysed oxidative coupling of MBTH with the reduced drug and pyridine acts as an activator [60], the sensitivity of the colour reaction being high in presence of pyridine. Under the reaction conditions, MBTH on oxidation loses two electrons and are proton forming the electrophilic intermediate [61], which is the active coupling species. The intermediate undergoes electrophilic substitution with the reduced MNZ/TNZ to form the coloured products. The reaction mechanisms for both the methods are shown in Schemes 1 and 2, respectively.

3.5. Stability

The yellowish orange products were stable upto 4 h when pyridine was used as an activator. In the absence of pyridine, the stability and sensitivity were lower. The pink coloured products were stable upto 24 h. Reproducible results were ob-

Table 2
Determination of MNZ and TNZ in presence of excipients

Excipient	Excipient added (mg)	% Recovery of the drug ± % R.S.D.[a]			
		MBTH method		NEDA method	
		MNZ	TNZ	MNZ	TNZ
Lactose	40	99.7 ± 0.85	99.6 ± 0.65	99.4 ± 0.87	99.8 ± 0.85
Glucose	40	99.8 ± 0.90	99.8 ± 0.60	99.8 ± 0.80	99.7 ± 0.70
Dextrose	40	99.6 ± 0.80	99.7 ± 0.70	99.6 ± 0.85	100.1 ± 0.68
Starch	70	99.8 ± 0.85	99.7 ± 0.80	99.7 ± 1.01	100.2 ± 0.59
Talc	70	99.8 ± 0.90	99.7 ± 0.75	99.8 ± 1.04	99.9 ± 0.78
Gum acacia	60	99.8 ± 0.95	99.8 ± 0.70	99.6 ± 0.91	99.6 ± 1.01
Sodium chloride	50	99.8 ± 0.95	99.7 ± 0.55	99.6 ± 0.88	99.2 ± 1.04
Sodium alginate	50	100.4 ± 1.02	100.2 ± 0.60	100.1 ± 0.91	99.5 ± 0.98
Carboxy methylcellulose	50	100.3 ± 1.02	100.3 ± 0.60	100.1 ± 0.87	99.9 ± 0.61
Vitamin-B_6	30	100.2 ± 1.01	100.2 ± 0.65	100.2 ± 0.51	99.6 ± 1.06
Magnesium stearate	40	100.2 ± 0.90	99.8 ± 0.70	100.1 ± 0.65	99.4 ± 0.99

MBTH method, * 16 μg ml^{-1} of drug taken. NEDA method, * 9 μg ml^{-1} of drug taken.
[a] Average of five determinations.

tained in both the methods in the temperature range of 20–30 °C. An increase in temperature for both the systems upto 40 °C, did not affect the results. However, excellent results were obtained around 25 °C.

3.6. Interference

A detailed study on the interference of various concomitant substances on the determination of these drugs was made. For MBTH method, 16 μg ml^{-1} of MNZ and TNZ were selected to check the interference. In NEDA method, 9 μg ml^{-1} of MNZ and TNZ were used to study the interference. Before adding the reagents, a known amount of the interfering substance was added and the reactions were carried out for both the methods. The extent of interference by various excipients that often accompany the pharmaceutical formulations are tabulated in Table 2. Amines such as aniline, piperidine, morpholine, etc. interfere in the NEDA method, because of diazotisation reaction. The extent of interference by excipients that often accompany the pharmaceutical preparations have been studied for both the methods. It was found that both the methods gave excellent results for the determination of pure MNZ or TNZ in presence of excipients. That means, common excipients do not interfere in both the methods. The results are given in Table 2. An error of 2.0% in the absorbance readings was considered tolerable.

3.7. Application

The reproducibility of the methods were checked by ten replicate determinations at the 16 μg ml^{-1} level of MNZ or TNZ for the MBTH method (9 μg ml^{-1} level for NEDA method) and the R.S.D.(%) was found to vary between 0.45 and 1.1. The applicability of the suggested methods for the assay of a wide variety of pharmaceutical preparations was examined. The results of the assay of tablets and injections are given in Tables 3 and 4. The results of the assay of the pharmaceutical preparations were cross checked by the official methods [21–23]. The results compare favourably with one another and are highly reproducible.

Table 3
Determination of MNZ in pharmaceutical preparations

Commercial formulations analysed	Label claim (mg)	Amount of drug found (mg)[a]				
		MBTH method	NEDA method	Official methods[c]		
				B.P.	I.P.	U.S.P.
Aldezol a	200	198.5 ± 0.6	199 ± 0.7	198.5 ± 0.7	198.5 ± 0.7	199 ± 0.8
	5 ml^{-1} [b]	5.06 ± 0.07	5.02 ± 0.08	–	5.04 ± 0.07	–
Antamebin b	200	198.5 ± 0.6	199.3 ± 0.7	199 ± 0.7	198 ± 0.6	198 ± 0.7
Aristogyl c	200	198 ± 0.7	197 ± 0.6	199 ± 0.6	198 ± 0.6	198 ± 0.7
Compeba d	200	201.5 ± 0.7	202 ± 0.6	202 ± 0.6	203 ± 0.6	202 ± 0.7
Flagyl e	200	198.5 ± 0.6	199 ± 0.7	198 ± 0.6	199 ± 0.5	198 ± 0.7
	500 ml^{-1} [b]	501.5 ± 0.6	501 ± 0.8	–	499 ± 0.8	–
Metrogyl f	200	198 ± 0.6	197 ± 0.6	198 ± 0.6	197 ± 0.6	198 ± 0.7
	100 ml^{-1} [b]	98.5 ± 0.6	98.9 ± 0.7	–	98 ± 0.7	–
Metron g	200	197 ± 0.6	197.5 ± 0.7	197 ± 0.6	917 ± 0.7	198 ± 0.8
Metronidazole I.V. h	5 ml^{-1} [b]	4.95 ± 0.06	4.94 ± 0.07	–	4.95 ± 0.08	–
Unimezol i	400	398 ± 0.6	402 ± 0.7	399 ± 0.6	398 ± 0.7	401 ± 0.7

[a] Average of five determinations ± R.S.D. (%).
[b] Injection marketed by: a, Albert David; b, Raptakos; c, Aristo; d, IDPL; e, Rhone-Poulenc; f, Unique; g, Alkem; h, Core; i, Unichem. U.S.P.-UV spectrophotometry.
[c] B.P.-titrimetry; I.P.-UV spectrophotometry (injections), titrimetry (tablets).

Table 4
Determination of TNZ in pharmaceutical preparations

Commercial formulations analysed	Label claim (mg)	Amount of drug found (mg)[a]		
		MBTH method	NEDA method	I.P. method
Amebamagma a	300	299 ± 0.6	298 ± 0.7	298 ± 0.6
Costini b	500	502 ± 0.5	498 ± 0.6	501 ± 0.7
Enidazol c	500	499 ± 0.7	498 ± 0.7	498 ± 0.8
Fasigyn d	500	498 ± 0.8	498 ± 0.7	497 ± 0.8
Tina 300 e	300	298 ± 0.7	297 ± 0.6	297 ± 0.7
Tini f	300	302 ± 0.6	301 ± 0.7	302 ± 0.7
Tiniba g	300	298.5 ± 0.7	298 ± 0.5	297 ± 0.6
Tinidafyl h	500	498 ± 0.7	497 ± 0.8	497 ± 0.7
Tinipidi I.V. i	2 ml^{-1}	1.98 ± 0.08	1.99 ± 0.07	–
Tizole j	300	298 ± 0.6	297.5 ± 0.7	297 ± 0.8
Trag k	500	497.5 ± 0.7	498.5 ± 0.6	497 ± 0.7
Tridazole l	300	298.5 ± 0.7	298.5 ± 0.6	298 ± 0.6
Zil m	300	297 ± 0.6	298 ± 0.7	297 ± 0.7

Marketed by, a, Wyeth Lederle; b, CFL; c, East India; d, Pfizer; e, Bombay Tablets; f, Kopran; g, Cadila Health care; h, Jagsonpal; i, Parenteral drugs; j, Dolphin; k, Walter Bushnell; l, Franco-Indian; m, Zil. I.P. method-UV-spectrophotometry.
[a] Average of five determinations ± R.S.D. (%).

Table 5
Comparison of UV-visible spectrophotometric methods for the determination of MNZ and TNZ

Sl. No.	Reagents used	Drug analysed	λ_{max} (nm)	B–L-range (μg ml^{-1})	Reference number
1.	Reduced drug with 1% furfural-dehyde	MNZ	395	10–160	[31]
2.	Reduced drug with 1-naphthol	MNZ	480	10–80	[34]
3.	Methanolic solutions of the drug	TNZ	310	20–40	[41]
4.	With 0.5 M NaOH+0.5 M KCl (1:1)	TNZ	368	10–30	[47]
5.	Aqueous DMF solution of the drug	TNZ	317.8	6–30	[49]
6.	Using Bromocresol-purple	MNZ	618	2–24	[50]
7.	Proposed methods				
	NEDA method	TNZ	505	0.5–18	–
		MNZ	520	0.5–18	–
	MBTH method	TNZ	490	4–36	–
		MNZ	500	1–32	–

4. Conclusions

The methods are found to be simple, economical, selective and more sensitive than most of the spectrophotometric methods reported and can compete with other methods in determining drugs in low concentrations (Table 5). The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing MNZ or TNZ showed no interference from the common excipients and additives. Hence, this approach could be considered for the determination of MNZ or TNZ in the Quality Control Laboratories. Thus, the methods can be adopted as an alternative to the existing methods.

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