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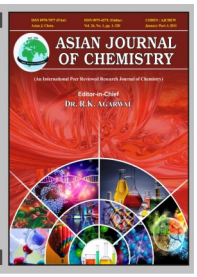

HPLC Method for Simultaneous Analysis of Ranitidine and Metronidazole in Dosage Forms

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A simple, rapid, precise and accurate stability indicating HPLC method for the simultaneous analysis of metronidazole and ranitidine in dosage forms has been developed and validated. Calibration curves for metronidazole and ranitidine exhibited linearity (R^2 = 0.9995 for both compounds) over the concentration ranges investigated. The method was sensitive, selective and accurate for both compounds. Both drugs were found to be stable following acid hydrolysis studies. However, following alkali hydrolysis degradation of both compounds was observed. Furthermore metronidazole appeared to be stable following oxidative studies however ranitidine underwent complete degradation under these conditions. Both drugs were well resolved from the degradation products. The stability indicating chromatographic method has the necessary precision and accuracy for the simultaneous analysis of metronidazole and ranitidine in dosage forms.

Keywords: Metronidazole, Ranitidine, HPLC, Stability-indicating, Validation.

INTRODUCTION

High performance liquid chromatography (HPLC) is a liquid chromatographic technique that has developed over approximately the last 30 years. The principles of HPLC have remained the same as those for liquid chromatography with the exception that automated sample injection, continuous solvent pumping and reusable stationary phases or columns have become the norm[1].

Different chemical and spectroscopic methods have been used for the analysis of metronidazole (MTZ) and ranitidine (RTD) in pharmaceutical dosage forms and/or biological samples[2,3]. However in the pharmaceutical arena, HPLC has been the preferred tool for the analyses of active pharmaceutical ingredients and products as reported in most official compendia[4]. There are several reported HPLC methods for the analysis of ranitidine and metronidazole[5-8], however most of these methods with the exception of the method published by do-Nascimento *et al.*[2] report the analysis of a single compound at any one time from a specific sample matrix[3,5,8-10].

Ranitidine and proton pump inhibitors are both used in the management of ulcerous conditions[11]. Although a proton pump inhibitor is more effective in suppression of intragastric acidity, several adverse effects associated with their long term use have been documented[12-14]. On the other hand ranitidine has been reported to have consistent pharmacokinetic parameters[15] and better efficacy[16,17], with respect to inter-patient variability when compared to a proton pump inhibitor. Metronidazole and ranitidine constitute part of a combination therapeutic regimen for the management of acute and mild cases of ulcers in patients in whom proton pump inhibitor use is contraindicated[11,18]. The duration of therapy usually varies between 7 and 30 days and is dependent on the severity of the condition. Combination therapy usually entails taking a large number of tablets that may result in poor adherence to the drug regimen with a consequent increase in morbidity and mortality. However fixed dose combination therapy has been shown to improve patient adherence[19] and therefore the formulation of a fixed dose combination tablet of metronidazole and ranitidine for the management of ulcers will reduce the pill burden and is consequently, likely to improve patient adherence.

The published methods for simultaneous determination of ranitidine and metronidazole are not stability indicating and there is a dearth of stability indicating HPLC methods that can be used for the analyses of these two compounds in pharmaceutical dosage forms and/or biological samples alone or in combination. This manuscript describes, what is to our knowledge the development and validation of the first stability indicating HPLC method for the simultaneous determination of ranitidine and metronidazole in dosage forms.

EXPERIMENTAL

Ranitidine, metronidazole and ornidazole were purchased from Sigma Aldrich (St Louis, Missouri, USA). Acetonitrile (ACN) (UV cut off 200 nm) and methanol (MeOH) (UV cut off 215 nm) were purchased from Romil Ltd (Waterbeach,

Cambridge, United Kingdom). Sodium hydroxide pellets and potassium dihydrogen orthophosphate were purchased from Merck Chemicals Ltd (Modderfontein, Gauteng, South Africa) and triethlyamine was procured from Saarchem Pty Ltd (Krugersdorp, Gauteng, South Africa). All chemicals were at least of analytical grade and were used without further modification.

HPLC grade water for buffers and sample preparation was purified using a Milli-RO® 15 water purification system (Millipore Co., Bedford, Massachusetts, USA) that consisted of a Super C® carbon cartridge, two Ion-X® ion exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22 µm Millipak® stack filter (Millipore, Bedford, Massachusetts, USA) prior to use.

Ornidazole was selected as the preferred internal standard (IS) since it has a similar structure to metronidazole and did not elute close to the peaks for metronidazole and ranitidine and was readily available.

Standard preparation: Stock solutions of ranitidine (100 µg/mL), metronidazole (100 µg/mL) and internal standard (100 µg/mL) were prepared by accurately weighing approximately 10 mg of each compound using a Model AG135 Mettler Toledo top-loading analytical balance (Mettler Instruments, Zurich, Switzerland) and quantitatively transferring each material to a separate 100 mL A-grade volumetric flask. The samples were dissolved in a mixture of MeOH-H_2O (80:20, v/v) followed by sonication for 1 min using a Model B-12 Ultrasonic bath (Branson Cleaning Equipment Co., Shelton, Connecticut, USA). Appropriate aliquots from each stock solution were transferred to 5 mL A-grade volumetric flasks to produce solutions of metronidazole, ranitidine and the internal standard. The resultant solutions were diluted serially using MeOH-H_2O (80:20, v/v) to produce ranitidine:metronidazole solutions of concentration 1:2.5, 2:5, 4:10, 6:15 and 8:20 µg/mL, respectively. The internal standard concentration was 40 µg/mL in all cases.

Preparation of buffers: Phosphate buffer was prepared by accurately weighing 6.8 g potassium dihydrogen orthophosphate into a 1000 mL A-grade volumetric flask and adding 2 mL triethylamine. The solution was made up to volume with HPLC grade water. The buffer pH was adjusted to a pH of 6.7 using a 0.1 M sodium hydroxide solution and the pH was monitored using a Crison GLP 21 pH-meter (Crison Instruments, Barcelona, Spain). The pH of the buffer solution was adjusted prior to the addition of organic modifier to avoid imprecise pH measurement due to drift of electrode response that is attributed to the presence of an organic modifier[20].

Preparation of mobile phase: The mobile phase was acetonitrile-phosphate buffer (pH 6.7; 0.05 M) (9:91, v/v). The mobile phase was degassed using a Model A-25 Eyela aspirator (Tokyo Rikakikai Co., Tokyo, Japan) and filtered through a 0.45 µm Durapore® HVLP membrane filter (Millpore Co., Billerica, Massachusetts, USA) prior to use.

Instrumentation and conditions: The modular HPLC system consisted of a Model P100 dual piston solvent delivery module (Thermo Separation Products, San Jose, California, USA), a model AS100 autosampler (Thermo Separation Products, San Jose, California, USA) fitted with a Rheodyne® Model 7010 injector (Rheodyne, Reno, Nevada, USA), a fixed volume 20 µL loop and a Gastight® 250 µL Model 1725 syringe (Hamilton Co., Reno, Nevada, USA). A linear UV/VIS-500 Model 6200-9060 detector (Linear Instrument Co., California, USA) and Spectra Physics SP 4600 integrator (Thermo Separation Products, San Jose, California, USA) were used for detection and data collection, respectively. Separation was achieved on a Nova-Pak® C18 60 Å 4 µm, 3.9 i.d. × 150 mm cartridge column (Waters Corporation, Milford, Massachusetts, USA) maintained at 22 ± 0.5 °C and a flow rate of 2 mL/min.

Range and linearity: Linearity studies were performed at concentrations of 1, 2, 4, 6 and 8 µg/mL for ranitidine (n = 5) and at 2.5, 5, 10, 15 and 20 µg/mL for metronidazole (n = 5). The linearity and range for the analysis of ranitidine and metronidazole were established by plotting the peak height ratios of metronidazole and ranitidine relative to the internal standard response *versus* the concentration of metronidazole and ranitidine, respectively.

Precision: Repeatability of the method was established following triplicate injection of three homogenous samples covering the calibration range at low, medium and high levels *viz.* 1, 4 and 8 µg/mL for ranitidine and at 2.5, 5 and 20 µg/mL for metronidazole. Intermediate precision was established following analysis of similar concentrations of ranitidine and metronidazole covering the range at low, medium and high levels performed over three consecutive days. A percent RSD of < 5 % was set as tolerance limit for precision.

Accuracy: Accuracy was determined by use of recovery studies. Triplicate injections covering the concentration range at low, medium and high levels were investigated. A tolerance limit of 5 % was set for the % RSD and < 5 % for the bias for these studies.

Selectivity: The selectivity of the method was established by assessing the resolution factor (Rs) for metronidazole and ranitidine following analyses of commercial tablets containing metronidazole and ranitidine individually. Ten tablets of metronidazole and ranitidine were grounded separately using a mortar and pestle. The resultant powder was prepared to produce a final solution of concentration of metronidazole and ranitidine of 10 µg/mL and 4 µg/mL respectively.

LOQ and LOD: The LOQ and LOD were established by use of the signal to noise ratio method. The concentration that produced a signal to noise ratio of 10:1was adopted as the LOQ and a signal to noise ratio of 3:1 was used to establish the LOD of the method.

Forced degradation studies: The analysis of samples obtained from degradation studies was performed by comparing chromatograms generated from samples subjected to forced degradation studies to those generated following analysis of a freshly prepared standard solutions of metronidazole and ranitidine. Approximately 1 mg of metronidazole and ranitidine was accurately weighed separately and quantitatively transferred into 10 mL A-grade volumetric flasks. With the exception of oxidative studies, the drug substance was dissolved and made up to volume with the medium specific for that degradation study, to yield a stock solution with a starting concentration of 100 µg/mL.

Oxidative degradation studies: Metronidazole and ranitidine are poorly soluble in hydrogen peroxide (H_2O_2) and

a solvent may therefore be used to improve the solubility of these compounds in the degradation medium[7]. Consequently each compound was dissolved in 4 mL of MeOH-H_2O (80:20, v/v) and the resultant solutions were made up to volume (10 mL) with a 3 % v/v H_2O_2 solution (Allied Drug Company Ltd, Durban, KwaZulu-Natal, South Africa) and subsequently refluxed at 50 ± 0.5 °C. Sample aliquots (2 mL) were collected prior to refluxing and after every hour during the study after which a 2 mL aliquot of internal standard was added to each sample and the resultant solution was made up to volume (10 mL) with a MeOH:H_2O solution (80:20, v/v) and analyzed immediately using HPLC.

Acid degradation studies: The drug substances were dissolved and made up to volume (10 mL) using 0.1 M hydrochloric acid and the solutions were then refluxed at 50 ± 0.5 °C. Sample aliquots (2 mL) were collected prior to commencing refluxing and after each hour during the experiment. The samples were then transferred to a 10 mL A-grade volumetric flask followed by addition of 2 mL of internal standard that had been prepared as previously described. The resultant solution was made up to volume using a MeOH-H_2O (80:20, v/v) prior to analysis using HPLC.

Alkali degradation studies: The drug substances were dissolved and made up to volume (10 mL) using 0.1 M sodium hydroxide and the solutions were then refluxed at 50 ± 0.5 ºC. Sample aliquots (2 mL) were collected prior to refluxing and hourly after refluxing had commenced and transferred to a 10 mL A-grade volumetric flask followed by addition of 2 mL of internal standard that had been freshly prepared as previously described and the resultant solution made up to volume with MeOH-H_2O (80:20, v/v) prior to analysis using HPLC.

Photolysis: The drug substances were dissolved and made up to volume (10 mL) with MeOH-H_2O (80:20, v/v) and the resultant solutions were then exposed to sunlight. Sample aliquots (2 mL) were collected prior to exposure and hourly following the commencement of exposure and transferred to a 10 mL A-grade volumetric flask followed by dilution with 2 mL of internal standard that had been prepared as previously described prior to analysis by HPLC.

RESULTS AND DISCUSSION

The method revealed a linear response over the concentration ranges investigated *viz.* 1-8 µg/mL for ranitidine and 2.5-20 µg/mL for metronidazole. The equation of the line for ranitidine was y = 0.0626x - 0.0024 (R^2 = 0. 9995) and y = 0.0832x + 0.0171 (R^2 = 0.9995) for metronidazole.

Precision

The repeatability and intermediate precision data obtained for metronidazole and ranitidine are summarized in Table-1. The method generated precise responses as can be seen by the % RSD values for metronidazole and ranitidine. The % RSD values for repeatability studies ranged between 1.04-2.64 % (metronidazole) and 0.56-1.17 % (ranitidine) across the concentration ranges studied. Similar results (< 5 %) were obtained for intermediate precision, the % RSD values ranged between 0.99-3.48 % (metronidazole) and 1.34-3.04 % (ranitidine) across the concentration ranges investigated.

Selectivity: The selectivity of the method is shown in Fig. 1. The chromatogram depicts that the peaks for metronidazole and ranitidine are well resolved from those of internal standard and solvent front. Similarly the peak of metronidazole appeared well resolved from that of ranitidine. The value for peak Rs between metronidazole and ranitidine was found to be 3.3 which indicate that the method has adequate resolution for the simultaneous analysis of metronidazole and ranitidine.

Accuracy: The accuracy data for the method is summarized in Table-2. The method demonstrated adequate accuracy across the concentration ranges investigated. The values for the per cent bias determined at low, medium and high levels ranged between -0.82 to 3.27 % for metronidazole and that for ranitidine ranged between -1.75 to 4.18 %.

LOQ and LOD: The LOQ and LOD values for metronidazole and ranitidine were found to be 0.3 µg/mL and 0.1 µg/mL, respectively.

Oxidative degradation studies: Following exposure to a 3 % v/v H_2O_2 solution at room temperature both compounds did not appear to exhibit appreciable degradation. However significant degradation of ranitidine was observed in comparison to metronidazole samples following 1 h of refluxing the solutions. Metronidazole and ranitidine produced peaks at 3.7 and 4.6 min respectively as shown in Fig. 2a. However following analysis of degradation samples a decrease in the peak height for ranitidine (4.6 min) and the appearance of a new peak at 1.8 min after 1 h of refluxing as shown in Fig. 2b was observed. The total disappearance of the ranitidine peak was observed by the end of the second hour of refluxing (Fig. 2c). This may be due to

TABLE-1
REPEATABILITY AND INTERMEDIATE PRECISION DATA FOR THE SIMULTANEOUS ANALYSIS OF METRONIDAZOLE AND RANITIDINE

Metronidazole			Ranitidine		
Concentration (µg/mL)	Mean concentration determined (µg/mL)	% RSD	Concentration (µg/mL)	Mean concentration determined (µg/mL)	% RSD
Repeatability					
2.50	2.52 ± 0.026	1.04	1.00	1.01 ± 0.005	0.56
10.00	9.69 ± 0.135	1.40	4.00	3.83 ± 0.045	1.17
20.00	20.16 ± 0.533	2.64	8.00	8.00 ± 0.066	0.83
Intermediate precision*					
2.50	2.60 ± 0.090	3.48	1.00	1.01 ± 0.030	3.04
10.00	9.83 ± 0.192	1.96	4.00	3.86 ± 0.040	1.04
20.00	20.15 ± 0.200	0.99	8.00	7.88 ± 0.105	1.34

*Each value is expressed as the mean ± SD of three determinations performed over three consecutive days.

TABLE-2
ACCURACY RESULTS FOR THE SIMULTANEOUS ANALYSIS OF METRONIDAZOLE AND RANITIDINE

Metronidazole				Ranitidine			
Concentration (µg/mL)	Mean concentration determined (µg/mL) (n = 3)	Precision (% RSD)	% Bias	Concentration (µg/mL)	Mean concentration determined (µg/mL) (n = 3)	Precision (% RSD)	% Bias
2.50	2.52 ± 0.026	1.04	-0.90	1.00	1.01 ± 0.005	0.56	-1.75
10.00	9.60 ± 0.135	1.40	3.27	4.00	3.83 ± 0.040	1.17	4.18
20.00	20.16 ± 0.533	2.64	-0.82	8.00	8.00 ± 0.006	0.83	-0.06

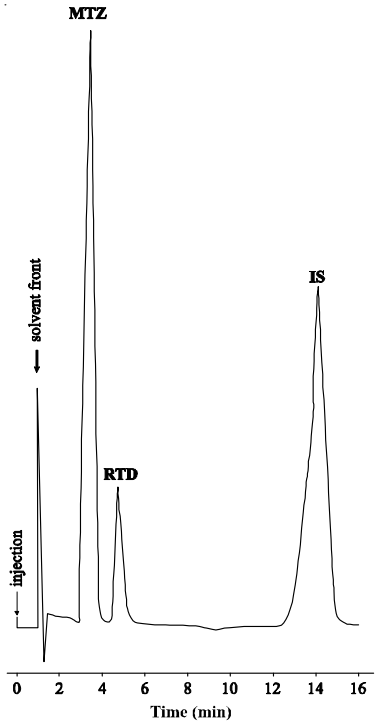

Fig. 1 Typical chromatogram of metronidazole (MTZ), ranitidine (RTD) and internal standard (IS) at concentrations of 10, 4 and 40 µg/mL, respectively

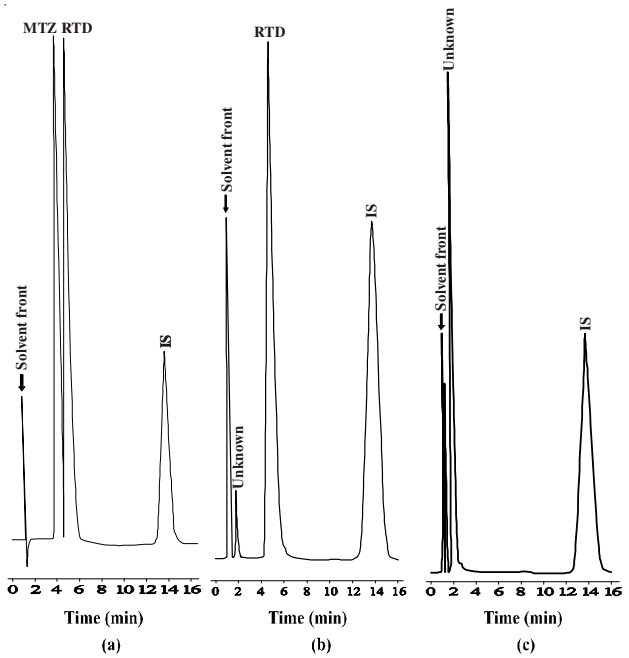

Fig. 2. (a) Chromatograms of standard solutions of metronidazole (MTZ) and ranitidine (RTD) (20 µg/mL) (b) Chromatogram of ranitidine following exposure to oxidative conditions at 1 h of refluxing (c) Chromatogram of ranitidine at 2 h of refluxing in oxidative conditions

degradation of ranitidine to form rantidine S-oxide and these data are similar to those observed[9] following incubation of ranitidine syrup in 3 % H_2O_2.

Unlike ranitidine, metronidazole appears to be relatively stable in a 3 % v/v H_2O_2 solution and the chromatograms revealed neither a change in retention time (Rt) nor the peak characteristics for metronidazole. Similar results were observed following incubation of metronidazole for 6 h in 3 % v/v hydrogen peroxide solution and 30 % degradation was detected following incubation in a 30 % v/v H_2O_2 solution[10].

Acid degradation: Analyses performed following acid hydrolysis of ranitidine revealed no degradation under these conditions. The peak characteristics and retention time for ranitidine were similar to those of the standard solutions used for comparison. These results are in agreement with those observed following incubation of ranitidine in 0.1 N hydrochloric acid[9]. Similarly no degradation was detected following the analysis of metronidazole samples. However, these results are in contrast to those reported by Bakshi and Singh[10] where 20 % degradation of metronidazole was reported when the molecule was heated in 0.1 M hydrochloric acid. The lack of degradation observed for metronidazole may be ascribed to the short refluxing time, *viz*. 8 h and low temperature, *viz*. 50 °C.

Alkali degradation: Metronidazole was observed to degrade when exposed to alkaline conditions. The chromatograms observed following exposure of metronidazole to alkali conditions revealed the presence of a new compound at a retention time 6.9 min that can be seen in Fig. 3a. Sample analyses over the first 3 h revealed the appearance of new peak at a retention time of 1 min and both these peaks are likely to result from the degradation of the parent compound as the analytical response for the parent compound (3.7 min) (Fig. 3b) was reduced and both peaks disappeared completely following 5 h of refluxing (Fig. 3c). This may be due to the formation of non-chromorphic degradants since metronidazole degrades under alkali conditions to produce acetic acid and ammonia[10,21] and 95 % degradation of metronidazole and the presence of non-chromorphic degradants following incubation of the drug in 0.1 M sodium hydroxide at 80 °C has been reported[10].

Similarly analysis of ranitidine solutions following exposure to alkaline conditions revealed the presence of two new peaks, one at 6.9 min prior to the commencement of refluxing (Fig. 4a) and a second peak at 2 min following 1 h of refluxing (Fig. 4b). Subsequent analysis of these samples showed that the appearance of the two peaks corresponded to a decrease of response for the parent peak (Fig. 4c) followed by complete disappearance of the drug and degradant peaks following 5 h of refluxing. These results are in agreement with those previously reported following incubation of ranitidine syrup in 0.1 N sodium hydroxide[9].

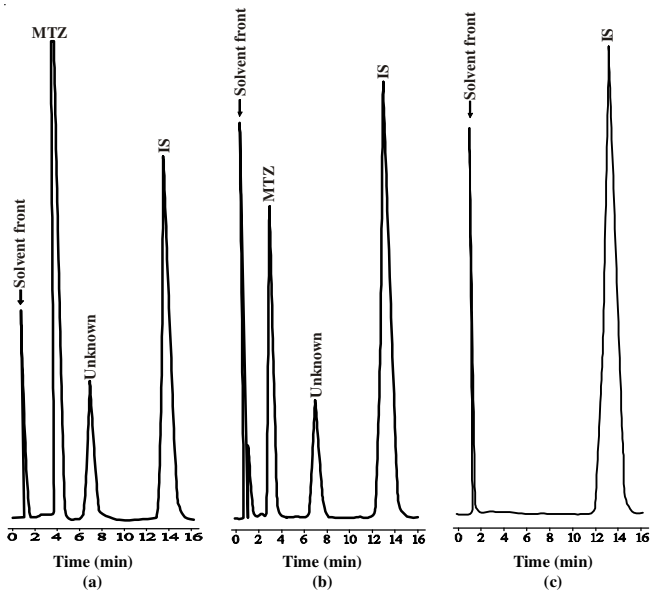

Fig. 3. (a) Chromatograms of metronidazole (MTZ) following 0 h of refluxing under alkaline conditions (b) At 3 h of refluxing (c) At 5 h of refluxing under alkaline conditions

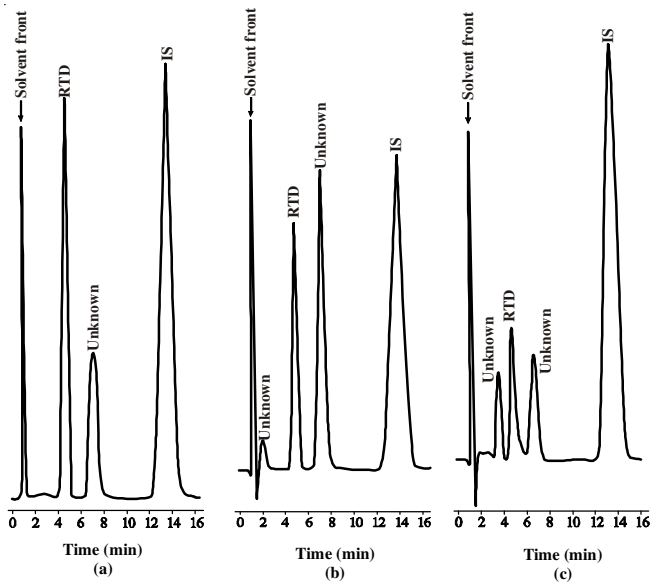

Fig. 4. (a) Chromatograms of ranitidine (RTD) following 0 h of refluxing under alkaline conditions (b) At 1 h of refluxing (c) At 3 h of refluxing under alkaline conditions

Photo degradation: The analyses of samples following exposure to light revealed that both compounds are susceptible to photolysis. The peaks heights of metronidazole and ranitidine decreased as refluxing continued when the solutions were compared to those of a standard solution of the same concentration. The lack of detection of the degradants may be due to the formation of non-chromorphic degradants or degradants with a different λ_{max} in comparison to the parent compound[9,10].

Conclusion

A simple, sensitive HPLC method that has the necessary selectivity, precision and accuracy for simultaneous assessment of ranitidine and metronidazole in pharmaceutical formulations has been developed and described. Furthermore the method has the advantage of versatility in that it can be used for the assessment of the individual compounds in solid dosage forms and/or raw materials without variation of chromatographic conditions. The method is the first stability indicating method for the simultaneous analysis of ranitidine and metronidazole in dosage forms. Additional instrumentation such as use of a mass spectrometer and/or photodiode array detector may be required for detection and identification of the degradation products.

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