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The ICH guidance in practice: stress degradation studies on ornidazole and development of a validated stability-indicating assay☆

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Abstract

The present study describes degradation of ornidazole under different ICH prescribed stress conditions (hydrolysis, oxidation and photolysis), and establishment of a stability-indicating reversed-phase HPLC assay. Degradation was found to occur in alkaline medium, under high acidic conditions, under oxidative stress, and also in the presence of light in acid conditions. Previously the drug is only known to decompose under alkaline conditions. Separation of drug and the degradation products under various conditions was successfully achieved on a C-18 column utilising water-acetonitrile in the ratio of 86:14. The detection wavelength was 310 nm. The method was validated with respect to linearity, precision, accuracy, selectivity, specificity and ruggedness. The response was linear in the drug concentration range of 5–500 μg ml^{-1}. The mean values ($\pm$RSD) of slope, intercept and correlation coefficient were 45251 ($\pm$1.59), 104418 ($\pm$2.49) and 0.9996 ($\pm$0.03), respectively. The RSD values for intra- and inter-day precision studies were <1 and $<2.6\%$, respectively. The recovery of the drug ranged between 100–103% from a mixture of degradation products. The method was specific to drug and also selective to degradation products.

Keywords: Ornidazole; Stress testing; Stability-indicating assay; Reversed-phase HPLC

1. Introduction

The parent drug stability test guideline (Q1A) issued by International Conference on Harmonisation (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability-indicating [1]. Further, it is suggested that stress studies should be carried out to establish the inherent stability characteristics of the molecule such as the degradation pathways, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures.

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The aims of the present study accordingly were to establish inherent stability of ornidazole through stress studies under a variety of ICH recommended test conditions, [1,2] and to develop a validated stability-indicating assay. There is no report yet on these aspects for this drug. Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol (1), (Fig. 1). It is active against protozoa and anaerobic bacteria and is used like metronidazole in a range of infections.

The only information known in literature on decomposition of ornidazole is that it is rapidly hydrolysed in basic solutions to an epoxide (2) and a diol (3), while the drug is stable in acidic medium below pH 6 [3]. Reports also exist on the stability of the drug in 0.9% sodium chloride [4] and PVC bags under different storage conditions [5].

Previously, spectrophotometric [6,7], potentiometric [8] and electrochemical [9,10] methods have been described for determination of the drug in dosage forms. Pulse polarographic [11] and HPLC [12–15] procedures are reported for its analysis in biological fluids.

2. Experimental

2.1. Materials

Ornidazole was supplied by Panacea Biotec Ltd. Lalru, India and was used without further purification. Methanol and acetonitrile (HPLC grade) were purchased from Mallinckrodt Baker Inc. (Paris, KY). Buffer materials and all other chemicals were of analytical reagent grade. Ultra-pure water was obtained from an ELGA (Bucks, UK) water purification unit.

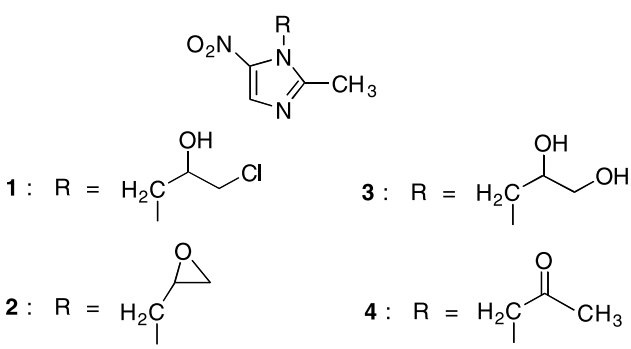

Fig. 1. Structure of ornidazole and its degradation products.

2.2. Instrumentation

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for stress studies. Photostability studies were done in a stability chamber (KBWF 240, WTB Binder, Germany) capable of controlling tolerances in temperature (2 °C) and humidity (5% RH) as specified in the ICH guideline Q1A. The chamber was equipped with illumination bank made of light sources defined under Option 2 in the ICH guideline Q1B [16]. The light bank consisted of a combination of two blacklight lamps OSRAM L73 and eight lamps OSRAM L20. The blacklight lamps (L73) had a spectral distribution between 345 and 410 nm with maximum at around 365 nm. The output of white fluorescent lamps (L20) was similar to that specified in ISO 10977 (1993). Both UV and VIS lamps were put on simultaneously. The samples were placed at a distance of 9 inches from the light bank. The overall illumination at the point of placement was 7000 lux. The samples were exposed for a total period of 30 days.

The HPLC system consisted of a 600 E pump, a 996 photo-diode array (PDA) detector, a 717 autoinjector, and a degasser module; data were acquired and processed by use of Millennium software ver. 2.1 (all equipment from Waters, Milford). The chromatographic separations were carried out on a 10 μm Supelcosil LC-18-DB column (250 × 4.6 mm i.d.).

LC-MS studies were carried out on Finnigan Mat LCQ ion-trap equipment. The LC part consisted of a P4000 pump, an AS3000 autosampler, a UV6000LP PDA detector, a SCM1000 degasser (all equipment was from Spectrasystem, USA) and a 5 μm Hypersil-ODS column (150 × 4.6 mm i.d.). The mass determinations were made in positive ESI mode in the mass range of 150–500.

2.3. Degradation studies

For acid decomposition studies, 1 mg ml^{-1} of drug was dissolved in 0.1 M HCl and the solution was heated at 80 °C for 72 h. Same concentration of the drug was subsequently exposed to 1 M HCl at 80 °C for 12 h. Studies were also performed in 5 M HCl at the same temperature for a period of 12 h. The studies in alkaline conditions were done initially at a drug concentration of 1 mg ml^{-1} in 0.1 M NaOH and the solution was heated at 80 °C for 8 h. These were repeated at a lower temperature of 40 °C keeping all other conditions constant. Drug at a concentration of 1 mg ml^{-1} was also dissolved in phosphate buffer (pH 8.0) and the solution was heated at 40 °C for a period of 24 h. For study in neutral conditions, 1 mg ml^{-1} drug was dissolved in water and the solution was heated at 80 °C for 120 h. For oxidative conditions, initial studies were done at a drug strength of 1 mg ml^{-1} in 3% H_2O_2. The drug was kept under the conditions of room temperature for a time period of 24 h. Subsequently, the drug was exposed to 30% H_2O_2 at room temperature for a period of 48 h. Photolytic studies were done at a drug concentration of 1 mg ml^{-1} in phosphate buffer (pH 8.0), 0.1 M HCl and distilled water. One set was kept in a photostability chamber and the second was kept in dark. Samples were withdrawn at different time periods up to 30 days.

2.4. Separation studies

Studies were carried out first on all reaction solutions individually, and then on a mixture of those solutions in which decomposition was observed.

Separations were achieved using isocratic elution. Initial studies on individual reaction solutions were carried out using water-methanol as the mobile phase. However, as good separations in a mixture of reaction samples were not achieved, even on varying methanol concentration, the organic modifier was changed to acetonitrile. Several studies were carried out by decreasing the ratio of acetonitrile from 20% downwards, till satisfactory resolution was obtained. The mobile phase was filtered through 0.45 μm nylon membrane and degassed before use. The injection volume was 20 μl and the mobile phase flow rate was kept constant at 1 ml min^{-1}. The analytical wavelength in most cases was 310 nm, but in select cases where the drug fall was seen without appearance of any peak due to degradation product in the chromatogram, PDA analysis was done to study the behaviour at other wavelengths.

2.5. Validation of the method

2.5.1. Linearity and range

A stock solution of the drug (1 mg ml^{-1}) was prepared. This stock solution was diluted to prepare solutions containing $5-500$ μg ml^{-1} of the drug. The solutions were injected in triplicate into the HPLC column, using water-acetonitrile (86:14) as the mobile phase and keeping the injection volume constant (20 μl).

2.5.2. Precision

Six injections, of three different concentrations (30, 100 and 500 μg ml^{-1}), were given on the same day and the values of relative standard deviation were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

2.5.3. Accuracy

Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with four known concentrations of the drug. The recovery of added drug was determined.

2.5.4. Specificity and selectivity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Overall selectivity was established through determination of purity for each degradation product peak using PDA detector.

2.5.5. Ruggedness

The ruggedness was established through separation studies on the mixture of reaction solutions by different persons on the same chromatographic

Table 1
Retention times and relative retention times of degradation products in different stressed samples when chromatographed using water:methanol (80:20) as the mobile phase

Condition	Retention time ± SD; RSD% (relative retention time)[a]				
pH 8	2.200 ± 0.02; 0.912 (0.1097)	6.008 ± 0.036; 0.602 (0.2997)		11.725 ± 0.011; 0.094 (0.5850)	
HCl (photo)			8.982 ± 0.062; 0.689 (0.4454)		13.948 ± 0.007; 0.05 (0.6917)
Neutral		5.975 ± 0.006; 0.102 (0.3014)		11.642 ± 0.005; 0.04 (0.5872)	
30% H_2O_2			8.280 ± 0.035; 0.423 (0.4059)	11.465 ± 0.034; 0.305 (0.5620)	

[a] Relative to drug at 20.05 min.

system. A study was also done on a different chromatographic system on a different day by one of the two analysts.

3. Results and discussion

3.1. Development and optimisation of the stability-indicating method

Table 1 shows the retention times and relative retention times of different degradation products formed under various stress conditions and obtained using water:methanol (80:20) as the mobile phase. Some of the products had close relative retention times although they were formed under entirely different conditions and had different UV spectrum (PDA analysis). To remove the overlapping behaviour, methanol concentration was varied, but no success was achieved. The organic modifier was then changed from methanol to acetonitrile. Trials were done varying the concentration of acetonitrile from 20 to 12%. As the percentage of acetonitrile was decreased, the resolution factor of different degradation products increased. However, it was found that 14% acetonitrile was ideal as it caused sufficient resolution of the drug and the degradation products without causing an undue increase in the run time. The resolution in this solvent system of different degradation products, contained in the mixture of reaction solutions (in which decomposition of drug was seen), is shown in Fig. 2.

3.2. Validation of the developed stability-indicating method

3.2.1. Linearity

With the water:acetonitrile (86:14) solvent system, the response for the drug was strictly linear in the concentration range between 5–500 μg

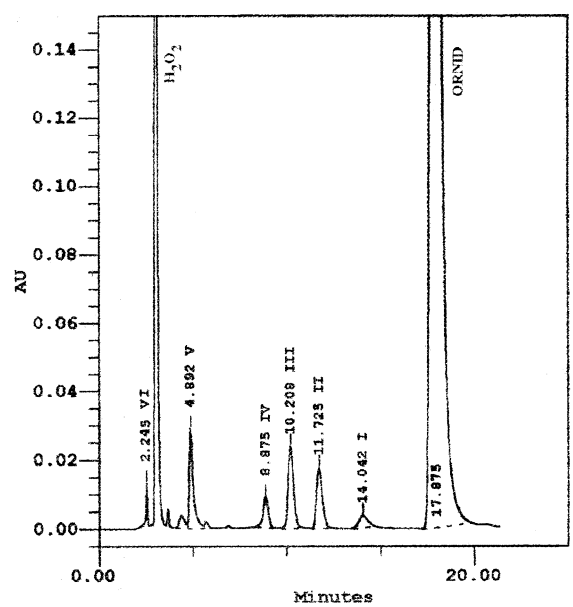

Fig. 2. Chromatogram showing the separation of different degradation products of ornidazole contained in a mixture of reaction solutions. I and IV represent oxidative degradation products; II, V and VI represent the degradation products in phosphate buffer (pH 8) and III represents the photolytic degradation product in 0.1 M HCl. Key: Ornid, Ornidazole; H_2O_2, Hydrogen peroxide.

Table 2
Reproducibility and precision data evaluated through intra-day and inter-day studies

Actual concentration ($\mu g\ ml^{-1}$)	Intra-day measured concentration ($\mu g\ ml^{-1}$) $\pm$ S.D.; RSD% ($n = 6$)	Inter-day measured concentration ($\mu g\ ml^{-1}$) $\pm$ S.D.; RSD% ($n = 3$)
30	34.783 ± 0.301; 0.864	35.531 ± 0.909; 2.560
100	98.706 ± 0.192; 0.195	99.827 ± 2.019; 2.022
500	497.007 ± 0.738; 0.148	507.562 ± 8.053; 1.587

ml^{-1}. The mean ($\pm$ RSD) values of slope, intercept and correlation coefficient were 45251 ($\pm$ 1.59) and 104418 ($\pm$ 2.49) and 0.9996 ($\pm$ 0.03).

3.2.2. Precision

Data obtained from precision experiments are given in Table 2 for intra- and inter-day precision studies. The RSD values for intra-day precision study are $< 1\%$ and for inter-day study are $<$ 2.5%, which confirm that the method is sufficiently precise.

3.2.3. Accuracy

Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 3, excellent recoveries were made at each added concentration, despite the fact that the drug was fortified to a mixture that contained drug as well as the degradation products, formed under various reaction conditions.

3.2.4. Specificity and selectivity

Fig. 2 shows that the method was sufficiently specific to the drug. The resolution factor for the drug peak was > 3 from the nearest resolving peak. The method was also selective to degradation products as all the peaks were pure, which was proved through PDA purity studies.

3.2.5. Ruggedness

Ruggedness was performed to confirm that separation was satisfactory under conditions external to the method. Good separations were always achieved, indicating that the method remained selective for all components under the tested conditions.

3.3. Degradation behaviour

HPLC studies on ornidazole under different stress conditions using water:acetonitrile (86:14) as the solvent system suggested the following degradation behaviour:

3.3.1. Acidic conditions

The drug gets slowly degraded in strongly acidic conditions over a period of time. On heating at 80 °C in 0.1 M (72 h), 1 M (12 h) and 5 M (12 h) HCl, the height of the drug peak decreased, without corresponding rise in a new peak. This indicated that the drug is hydrolysed under acid conditions, perhaps to non-chromophoric compounds. This is in difference to a report in literature, which demonstrated that the drug is stable below pH 6 [3]. The aim in the literature study was to explore decomposition of ornidazole in the basic medium, so the investigation was carried out between pH 6 and 10. As ornidazole decomposed only above pH 6, so a mention is made that the drug is stable below pH 6. But our studies show that ornidazole has lability in strong acid conditions.

Table 3
Recovery studies

Actual concentration ($\mu g\ ml^{-1}$)	Calculated concentration ($\mu g\ ml^{-1}$) $\pm$ SD; RSD% ($n = 3$)	Recovery (%)
40	40.437 ± 0.337; 0.833	101.09
100	102.960 ± 1.285; 1.248	102.96
200	201.293 ± 1.902; 0.945	100.65
500	501.150 ± 3.167; 0.632	100.23

3.3.2. Degradation in alkali

In alkali, the drug was found to decompose rapidly, similar to the behaviour reported in literature [3]. The reaction in 0.1 M NaOH at 80 °C was so fast that whole of the drug was degraded at 0 min. The degradation rate was still high when the temperature was reduced to 40 °C. Subsequent studies were performed in milder conditions in phosphate buffer, pH 8 at 40 °C. The drug degraded slowly in these conditions and two peaks were generated, at 4.892 and 11.725 min. In later stages, another peak emerged at 2.245 min. As the drug is previously known to decompose in alkaline conditions first to epoxide (1) which is converted further to diol (2), LC-MS studies were, therefore, carried out to identify the resolving peaks. The two peaks at 11.725 and 4.892 min showed mass values of 184 and 202, affirming the formation of epoxide and diol, respectively. The peak at 2.245 min did not show any definite mass value, hence it could not be characterised.

3.3.3. Neutral (water) condition

In neutral condition, mild degradation was seen with appearance of two new peaks, at 4.892 and 11.725 min (Fig. 2), similar to those in alkaline conditions. The peak relative to 11.725 min was found to decrease with time, with an increase in the peak relative to 4.892 min, indicating the conversion of the former to the latter. The additional peak at 2.245 min, which emerged with time in alkaline conditions, was absent in neutral conditions.

3.3.4. Oxidative conditions

The drug was found to degrade in hydrogen peroxide at room temperature. It was decomposed to an extent of 8% in 3% H_2O_2 in 8 h. The degradation increased to 53% in the same time in 30% H_2O_2. In the former, the only specific peak was the one relative to 14.042 min peak in Fig. 2, which was also very small. This peak almost remained constant, though the drug peak was reduced in height. In 30% H_2O_2, there were two small peaks, relative to 14.042 and 8.875 min, which were also found to disappear with time. The indication is that the drug is degraded in oxidative conditions also to nonchromophoric products.

3.3.5. Photolytic conditions

The photolytic studies were done in 0.1 M HCl, water and phosphate buffer (pH 8.0). Under all conditions, a new peak was seen at 10.208 min (Fig. 2). In neutral and alkali solutions, other products due to hydrolysis were also seen, whereas in acid only one peak due to this new product was observed. LC-MS studies indicated the mass of this product as 184, equal to that of the ornidazole epoxide, though it resolved at different retention time. The initial postulation is that the product has a structure 4 (Fig. 1), which appears from dechlorination of the compound, which is a well-established photolytic reaction [17]. Efforts are on in our laboratory to isolate this product and carry out its exact characterisation.

4. Conclusions

This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the rare studies where forced decomposition studies were done under all different suggested conditions and the products were resolved in a single isocratic run. In literature, most of the studies on development of stability-indicating assays involve either forced decomposition studies under only one or two conditions [18–20], or separation of drug from major degradation products whose standards were available [21–23].

The developed method is simple, accurate, precise, specific, selective and rugged. It is proposed for analysis of the drug and degradation products in stability samples in industry. The method, however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation products, as some of the products are shown to decompose further in a complex reaction scheme (like drug → epoxide → diol), while others are indicated to be nonchromophoric.

A new finding of this study is that the drug is unstable in almost all conditions, against the reported behaviour that it is decomposed only in alkaline conditions [3].

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