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ICH guidance in practice: establishment of inherent stability of secnidazole and development of a validated stability-indicating high-performance liquid chromatographic assay method

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Abstract

The degradation behaviour of secnidazole was investigated under different stress degradation (hydrolytic, oxidative, photolytic and thermal) conditions recommended by International Conference on Harmonisation (ICH) using HPLC and LC–MS. A stability-indicating HPLC method was developed that could separate drug from degradation products formed under various conditions.

Secnidazole was found to degrade significantly in alkaline conditions, oxidative stress, and also in the presence of light. Mild degradation of the drug occurred in acidic and neutral conditions. The drug was stable to dry heat.

Resolution of drug and the degradation products formed under different stress studies were successfully achieved on a C-18 column utilizing water–methanol in the ratio of 85:15 and at the detection wavelength of 310 nm. The method was validated with respect to linearity, precision (including intermediate precision), accuracy, selectivity and specificity.

Keywords: Secnidazole; Stress degradation studies; Stability-indicating assay method; HPLC; LC–MS

1. Introduction

The revised parent drug stability test guideline Q1A(R2) issued by International Conference on Harmonisation (ICH) requires that stress testing on the drug substance should be carried out to establish its inherent stability characteristics and for supporting the suitability of the proposed analytical procedures [1]. It is suggested that stress testing should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH values. It is also recommended that analysis of stability samples should be done through the use of a validated stability-indicating testing methods.

The aim of the current study was to establish inherent stability of secnidazole under different ICH recommended conditions [2] and to develop a validated stability-indicating HPLC assay method [3]. The study was carried out as part of a bigger project, which included similar investigations even of other nitroimidazoles (ornidazole, metronidazole and tinidazole). The results on these drugs were reported previously [4–6].

Secnidazole is chemically 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole (Fig. 1). It is used in the management of protozoal infections and bacterial vaginosis. It is not official in any pharmacopoeia. There is no information in literature on the stability behaviour of this drug under hydrolytic, oxidative and thermal conditions. As of now, information in literature is limited to the fate of the drug under photolytic conditions in organic solvents. The drug followed photochemical rearrangement and degradation pattern similar to metronidazole [7]. In another report, a methanolic solution of the drug was exposed to artificial sunlight for 24 h, and 2-methyl-5-nitroimidazole and 2-hydroxy propanol were found as the major degradation products [8].

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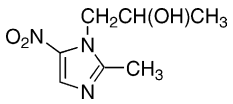

Fig. 1. Structure of secnidazole.

Several analytical procedures have been reported for the determination of secnidazole in pharmaceutical formulations, either alone or in combination with other drugs. These include spectrophotometric [9–14], differential pulse polarographic [15], supercritical fluid chromatographic [16], GLC [17] and HPLC [13,18,19] methods. Adsorptive stripping voltammetry [20] and HPLC [21,22] methods have been reported for its determination in biological fluids. For stability testing, four different analytical methods, viz. derivative spectroscopy, HPLC, TLC densitometry and colorimetry were developed by Moustafa and Bibawy [8]. All these methods were applicable to separation of photolytic degradation products from the drug. But no method accounted for the products formed under conditions of hydrolysis, oxidation and thermal stress, which are required to be considered along with photolysis, according to ICH [1].

2. Experimental

2.1. Materials

Secnidazole was supplied by Aarti Drugs Ltd. (Mumbai, India) and was used without further purification. HPLC grade methanol was purchased from Mallinckrodt Baker Inc. (Paris, USA) and acetonitrile of the same grade was purchased from J.T. Baker (Xalostoc, Mexico). Buffer materials and all other chemicals were of analytical reagent grade. Ultra-pure water was obtained from an ELGA (Bucks, UK) water purification unit.

2.2. Instrumentation

Stress studies under alkaline conditions were performed using precision water bath equipped with MV controller (Julabo, Seelbach, Germany). Degradation experiments in acid and neutral conditions were performed using a Dri-Bath (Thermolyne, Iowa, USA). Dry air oven (NSW Limited, New Delhi, India) was used to study the effect of dry heat. Photolytic experiments were performed in a photostability chamber (KBWF 240, WTB Binder, Tuttlingen, Germany) equipped with light sources defined under Option 2 in the ICH guideline Q1B [23]. The light bank consisted of a combination of two blacklight OSRAM L73 lamps and four OSRAM L20 lamps. 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 visible lamps were put on simultaneously. The study was performed by keeping the samples at a distance of 9 in from the light bank. The overall illumination at the point of placement was 7000 lx, which was tested using a calibrated lux meter (Escorp, New Delhi, India). The near UV energy at the same distance was found to be 0.8 W/m^2 when tested with a calibrated radiometer (PRC Krochmann GmbH, Berlin, Germany). The chamber was set at 40 °C/75% RH during the studies.

The HPLC system consisted of a 600E pump, a 996 photodiode array (PDA) detector, a 717 autoinjector, and a degasser module (all from Waters, Milford, USA). Data acquisition and processing was performed by use of Waters Millennium software ver. 2.1. Robustness testing was done on another HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC-10ATVP pump, a SPD-10AVVP UV-VIS dual wavelength detector, a SIL-10ADVP autoinjector, and a DGU-14A degasser module. Chromatograms were recorded using a CLASS-VP software (Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on Spherisorb ODS2 (Waters, Milford, USA) C-18 columns (250 mm × 4.6 mm i.d. with particle size of 5 μm) from different lots.

LC–MS analyses were performed on Finnigan Mat LCQ ion-trap equipment (San Jose, USA). 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 5 μm Waters Spherisorb ODS2 column (250 mm × 4.6 mm i.d.). The mass determinations were made in positive APCI mode in the mass range of 100–500.

2.3. Degradation studies

Drug at a concentration of 1 mg ml^{-1} was used in all degradation studies. The pH of buffered solutions was measured before and after the reaction and no change was observed. Conditions employed for performing stress studies were as follows.

2.3.1. Hydrolytic studies

Acid decomposition studies were performed by heating the solution of drug in 0.1 M HCl at 80 °C for 12 h. The studies in alkaline conditions were done in 0.1 M NaOH and the solution was heated at 80 °C for 8 h. The release of ammonia and acetic acid on alkaline degradation of secnidazole was studied by repeating the hydrolysis steps reported for metronidazole by Baveja and Khosla [24]. For study in neutral conditions, drug was dissolved in water and the solution was heated at 80 °C for 5 days.

2.3.2. Oxidative studies

Initial studies were performed in 3% H_2O_2 at room temperature for 6 h. Subsequently, the drug was exposed to 30% H_2O_2 at room temperature for a period of 48 h.

2.3.3. Photolytic studies

Photolytic studies were done in 0.1 M HCl, water and phosphate buffer (pH 10). One set of solutions was kept in a

photostability chamber and the second was kept in dark for a period of 8 days.

2.3.4. Thermal (dry heat) studies

Susceptibility of the drug to dry heat was studied by exposing the solid drug to 50 °C for 3 months.

2.4. Separation studies on stressed samples

In all HPLC runs, the mobile phase was filtered through 0.45 μm nylon membrane and degassed before use. The injection volume was 10 μl and the mobile phase flow rate was 1 ml min^{-1}. The analytical wavelength was 310 nm.

Preliminary HPLC analyses were performed using a C-18 column and mobile phase composed of water:acetonitrile (86:14). Initial separation studies were carried out on samples of different stress conditions (after appropriate dilution) withdrawn at different time periods individually, and later on resolution of drug and degradation products was studied in a mixture of those stressed samples, where different degradation products were observed. LC–MS studies on stressed samples were also performed using the preliminary HPLC method.

As satisfactory resolution of the drug and the degradation products was not achieved by using the above-mentioned mobile phase, the method was suitably optimised by increasing and decreasing ratio of mobile phase components and also changing acetonitrile with methanol till satisfactory resolution was achieved.

2.5. Validation of the method

Validation of the optimised HPLC method was done with respect to following parameters.

2.5.1. Linearity and range

Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in the range of 50–500 μg ml^{-1} in triplicate into the HPLC system keeping the injection volume constant.

2.5.2. Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentrations of the drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating the studies on three different days. Additionally, the developed HPLC method was checked through separation studies on the mixture of reaction solutions on a different chromatographic system on a different day.

2.5.3. Accuracy

Accuracy of the developed method was tested by fortifying a mixture of decomposed reaction solutions with three concentrations of the drug and determining the recovery of added drug.

2.5.4. Specificity

Specificity of the method towards the drug was studied by determination of purity for drug peak in a mixture of stressed samples using PDA detector. The study of resolution factor of the drug peak from the nearest resolving degradation product peak was also done.

3. Results and discussion

3.1. Degradation behaviour of secnidazole

HPLC studies of samples obtained on stress testing of secnidazole under different conditions using water:acetonitrile (86:14) as the mobile solvent system suggested the following degradation behaviour.

3.1.1. Hydrolytic studies

3.1.1.1. Acidic condition. It was observed that around 8–10% of the drug degraded on heating it in 0.1 M HCl for 12 h at 80 °C but there was no corresponding formation of degradation products (Fig. 2b) as compared to the standard solution of the drug (Fig. 2a). No new peaks were seen in the mass chromatograms of acid-degraded samples of secnidazole (Fig. 3b), as compared to the standard (Fig. 3a). This indicated that the drug degraded to low molecular weight non-chromophoric compounds.

3.1.1.2. Alkaline condition. The results obtained on degradation of secnidazole in alkaline conditions were found to be very similar to those reported for metronidazole [24] due to the similarities in the structures of two drugs. Secnidazole was found to yield ammonia and acetic acid on alkaline hydrolysis. The major product was seen at around 2 min during HPLC analyses of alkali-degraded samples with some minor intermediary products (Fig. 2c). The degraded samples were subjected to LC–MS studies to detect the non-chromophoric products. The mass chromatogram in Fig. 3c shows that additional product peaks were formed on treatment of drug with alkali, which indicated that decomposition of secnidazole in alkaline conditions to ammonia and acetic acid was mediated through a reaction scheme involving non-chromophoric products resulting from opening of imidazole nucleus.

3.1.1.3. Neutral condition. In neutral condition, only 5–8% degradation of the drug was seen after heating the drug for 5 days at 80 °C (Fig. 2d).

3.1.2. Oxidative studies

The drug was found to be stable in 3% H_2O_2 for 6 h at room temperature. However, almost 70–75% drug degradation was observed on exposure to 30% H_2O_2 for 48 h. Two

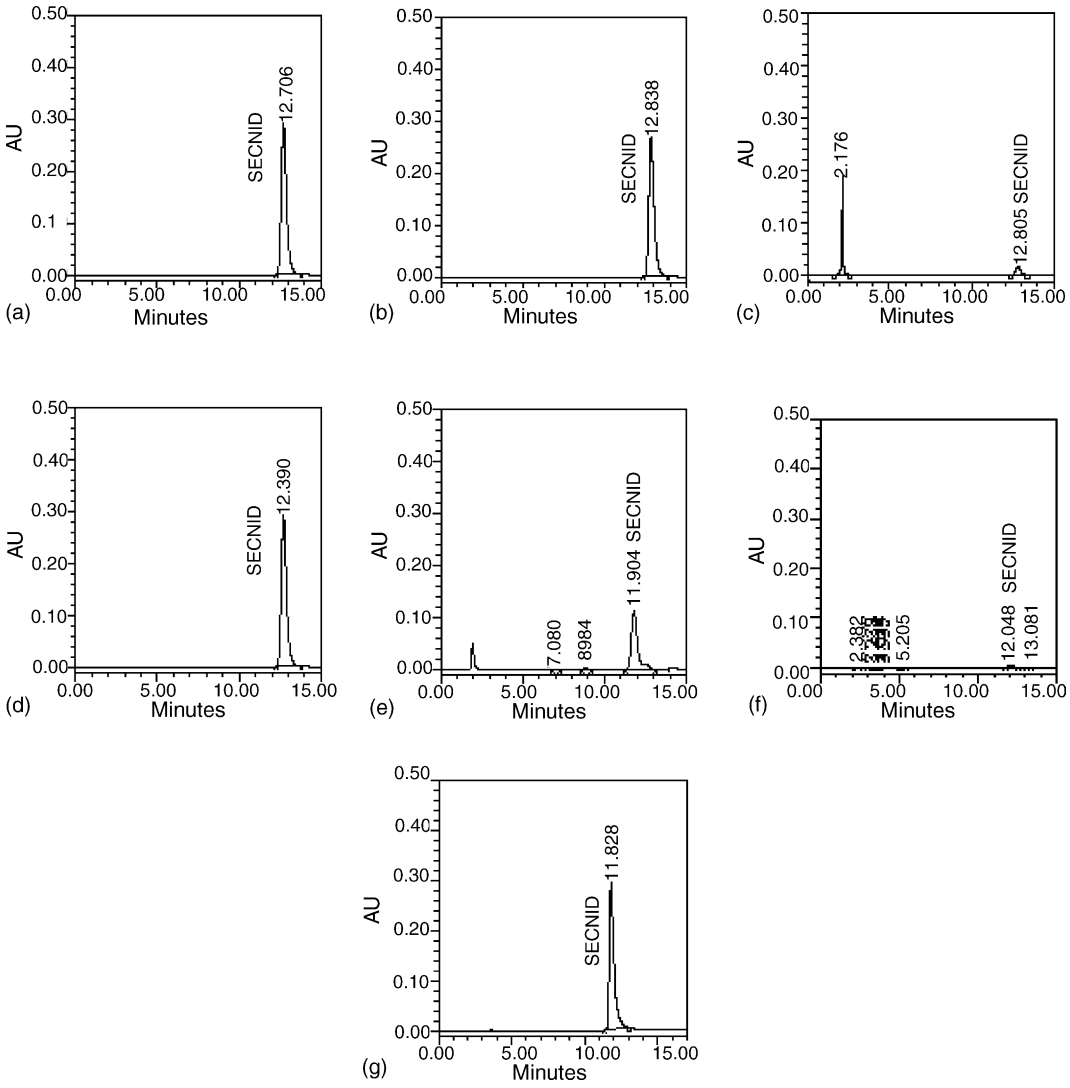

Fig. 2. Representative HPLC chromatograms of secnidazole (SECNID). Standard solution of secnidazole (a), sample degraded in 0.1 M HCl (b), sample degraded in 0.1 M NaOH (c), sample heated in water (d), sample subjected to oxidative degradation (e), sample subjected to photolytic degradation in 0.1 M HCl (f), sample subjected to dry heat (g).

very small degradation product peaks at around 7.05 min and 8.99 min were seen, but there was no significant rise in the height or area of these peaks with time (Fig. 2e). This showed that the drug was degraded in oxidative conditions to non-chromophoric compounds. The LC–MS analyses of degraded samples were done and the results are shown in Fig. 3d. Evidently, along with the small peaks at 6.51 and 8.39 min, which were seen in UV chromatogram, an additional split peak was shown at 3.40–3.67 min in mass chromatogram. This showed that the drug was degraded to very low molecular weight non-chromophoric compounds in oxidative conditions.

3.1.3. Photolytic studies

Secnidazole was found to be highly susceptible to light under acid conditions. Almost 87% of the drug degraded in 4 days with formation of a cluster of minor degradation products between retention time range of 2–6 min (Fig. 2f). In neutral conditions, almost 50% degradation of the drug was seen in 8 days in light-exposed samples as compared to dark controls. There was no degradation product peak seen in any of the samples. Almost 65% drug degradation was seen in alkaline environment in the same time period and a small peak at around 13 min was seen. This peak was not found to increase with time.

In most cases, the fall in drug peak was not in correspondence with the rise in degradation product peaks. LC–MS analyses of photolysed samples of secnidazole were done to detect non-chromophoric products. Additional peaks were seen in mass chromatograms of photolysed solutions of secnidazole (Fig. 3e), which were nonexistent in UV chromatogram. The mass value of these compounds was equal to that of secnidazole, indicating that these are the rearrangement products already reported for secnidazole [7].

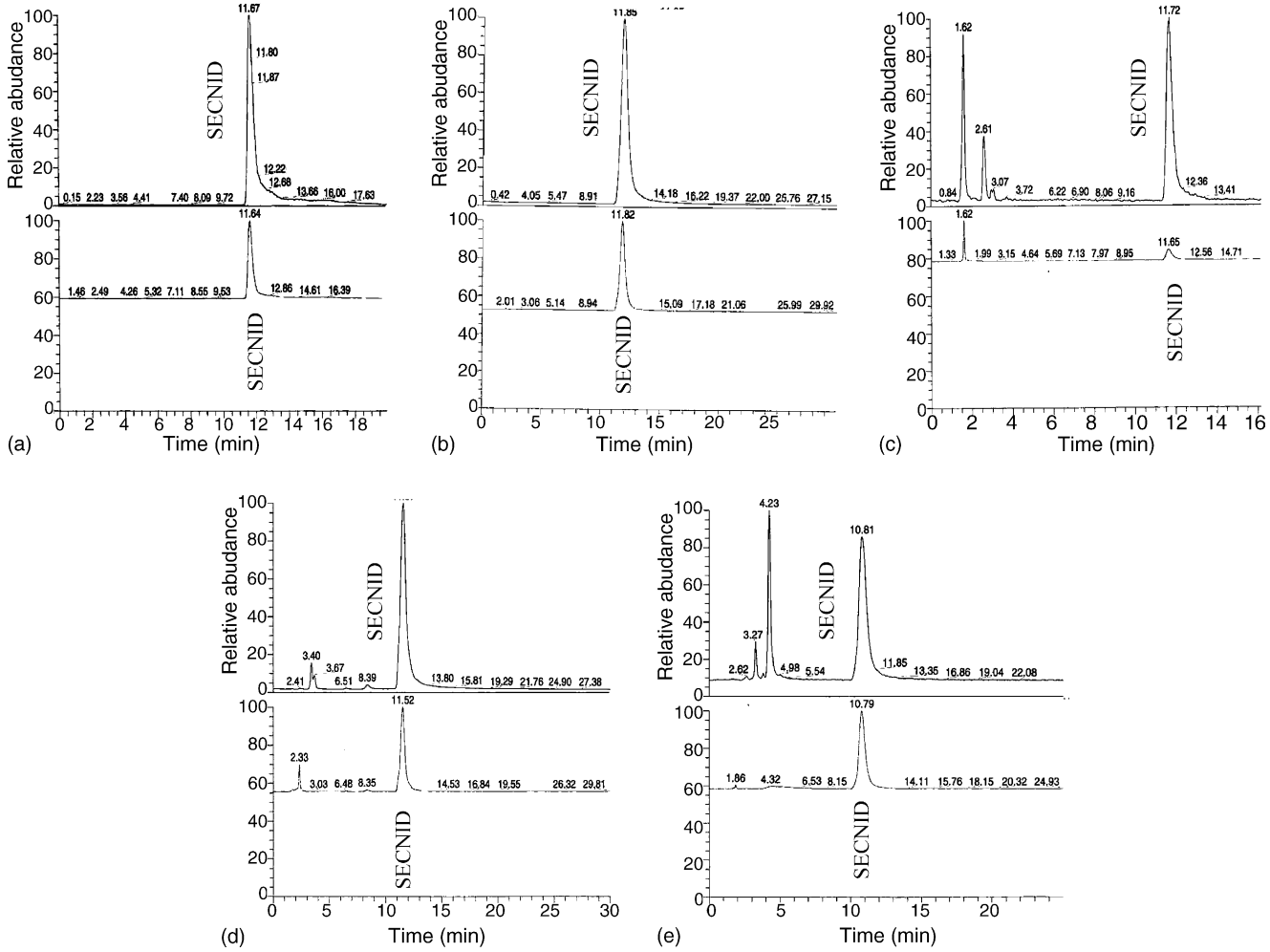

Fig. 3. Representative LC–MS chromatograms showing resolution of constituents in a solution of secnidazole (SECNID). Standard solution of secnidazole (a), sample degraded in 0.1 M HCl (b), sample degraded in 0.1 M NaOH (c), sample subjected to oxidative degradation (d), sample subjected to photolytic degradation in water (e). The lower channel in each figure ((a)–(e)) represents UV chromatogram and the upper channel represents mass chromatogram taken in positive APCI mode.

3.1.4. Thermal stress

Secnidazole was found to be stable to dry heat as negligible (∼2%) degradation was seen after exposing the drug to 50 °C for 3 months (Fig. 2g).

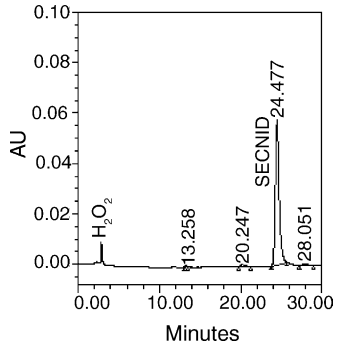

Fig. 4. Chromatogram showing separation of secnidazole (SECNID) and different degradation products in a mixture of reaction samples using water:methanol (85:15) as the mobile phase on a C-18 column.

3.2. Development of stability-indicating method

It was observed that satisfactory resolution of secnidazole and its degradation products formed under various conditions and present in the mixture of stressed samples was not achieved, when the analyses were performed by using water:acetonitrile (86:14) as the mobile phase. Therefore, to achieve the separation, acetonitrile concentration was varied, but no success was achieved. So the organic modifier was changed to methanol and its concentration was varied from 10 to 20%. It was observed that 15% methanol was able to resolve the drug from different degradation products, as shown in Fig. 4.

3.3. Validation of the developed stability-indicating HPLC method

The results of validation studies on the stability-indicating method developed for secnidazole in the current study involv-

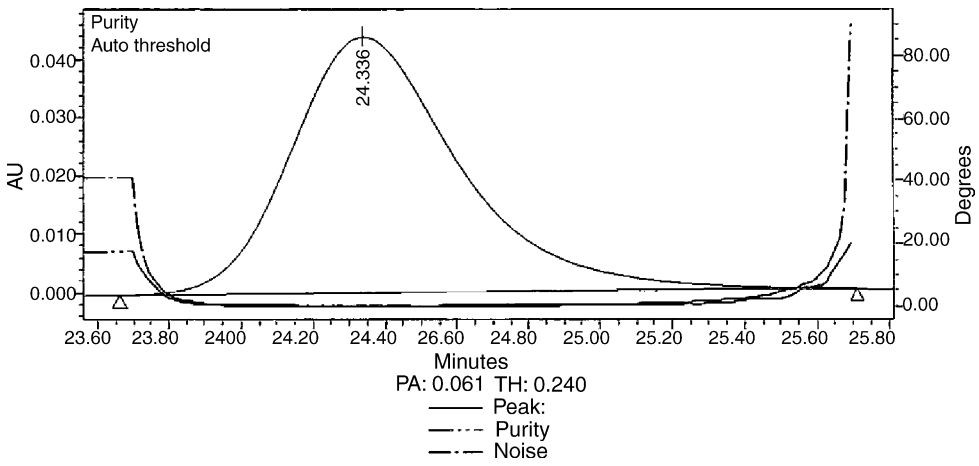

Fig. 5. Purity plot for secnidazole in a mixture of stressed samples.

Table 1
Precision studies

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), RSD (%)	
	Repeatability (n = 6)	Intermediate precision (n = 3)
50	50.274 ± 0.134, 0.267	49.532 ± 0.674, 1.361
200	197.221 ± 0.539, 0.274	196.463 ± 1.036, 0.527
500	503.958 ± 0.697, 0.138	502.309 ± 2.975, 0.592

ing water:methanol (85:15) as the mobile phase are given below.

3.3.1. Linearity

The response for the drug was linear (r^2 = 0.9994) in the concentration range between 50 and 500 $\mu g\,ml^{-1}$. The mean (±RSD) values of slope, intercept and correlation coefficient were 26,100 (±0.856) and 35,517 (±0.808) and 0.999 (±0.127), respectively.

3.3.2. Precision

The results of repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were <0.5 and <1.5%, respectively. Separation of the drug and different degradation products in a mixture of stressed samples was found to be similar when analyses were performed on a different chromatographic system on a different day.

Table 2
Recovery studies (n = 3)

Spiked concentration ($\mu g\,ml^{-1}$)	Measured concentration ± S.D. ($\mu g\,ml^{-1}$), RSD (%)	Recovery (%)
50	49.225 ± 0.248, 0.503	98.45
200	200.430 ± 2.418, 1.207	100.22
500	495.650 ± 2.616, 0.528	99.13

3.3.3. Accuracy

The HPLC area responses for accuracy determination are depicted in Table 2. Good recoveries (98.45–100.22%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

3.3.4. Specificity

Purity plot for secnidazole peak in a mixture of stressed samples (as shown in Fig. 5) proved that the method was specific to the drug as purity angle (PA) value was found to be less than purity threshold (TH) value. The resolution factor for the drug from the nearest resolving peak was >3 (Fig. 4)

4. Conclusions

In this study, intrinsic stability of secnidazole was established through employment of ICH recommended stress conditions. The drug was found to degrade extensively in alkaline and oxidative conditions, and also in the presence of light (especially in acidic environment). Mild degradation was also seen in acidic and neutral conditions but the drug was stable to thermal stress.

An HPLC method was developed, which is simple and can be used to analyse the drug in stability samples. However, the method is not suggested for establishment of mass balance, because some of the degradation products of secnidazole are non-chromophoric in nature and did not show up during HPLC analyses with UV detection. The method is otherwise suitable for LC–MS studies as it involves a buffer-free mobile phase.

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