Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 11–18
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

HPLC and LC–MS studies on stress degradation behaviour of tinidazole and development of a validated specific stability-indicating HPLC assay method

Monika Bakshi, Saranjit Singh*

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, S.A.S. Nagar 160062, Punjab, India

Received 3 April 2003; received in revised form 1 August 2003; accepted 1 August 2003

Abstract

The objective of the current investigation was to study the degradation behaviour of tinidazole under different ICH recommended stress conditions by HPLC and LC–MS, and to establish a validated stability-indicating HPLC method. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal decomposition. Extensive degradation was found to occur in alkaline medium, under oxidative stress and in the photolytic conditions. Mild degradation was observed in acidic and neutral conditions. The drug was stable to thermal stress. Successful separation of drug from degradation products formed under stress conditions was achieved on a C-18 column using water–acetonitrile (88:12) as the mobile phase. The flow rate was 0.8 ml min^{-1} and the detection wavelength was 310 nm. The method was validated with respect to linearity, precision, accuracy, specificity and robustness. The utility of the procedure was verified by its application to marketed formulations that were subjected to accelerated stability studies. The method well separated the drug and degradation products even in actual samples. The products formed in marketed liquid infusions were similar to those formed during stress studies.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Tinidazole; Degradation; Stress studies; Stability-indicating method; HPLC; LC–MS

1. Introduction

Tinidazole is chemically 1-(2-ethylsulfonyl-ethyl)-2-methyl-5-nitroimidazole (**1**) (Fig. 1). It is active against protozoa and anaerobic bacteria and is used like metronidazole in a range of infections [1]. The drug is reported to hydrolyse quantitatively in alkaline conditions to 2-methyl-5-nitroimidazole (**2**, Fig. 1) [2]. There exists a report on the conversion of tinidazole to 4-nitro isomer (**3**, Fig. 1) in water in the presence of catalytic amount of base [3]. It is also postulated to decompose by different mechanisms under alkaline and neutral/acidic conditions [4]. Under photolytic conditions, the drug yields intermediate, rearrangement and degradation products [2].

There exist a few reports on stability-indicating assay methods for tinidazole but mainly in the presence of hydrolytic decomposition products. The techniques

* Corresponding author. Tel.: +91-172-214682; fax: +91-172-214692.
E-mail address: ssingh@niper.ac.in (S. Singh).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.japna.2003.08.003

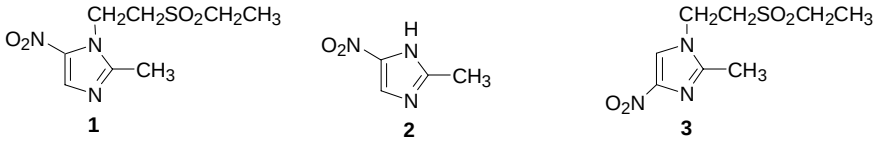

Fig. 1. Structures of tinidazole (**1**) and different hydrolytic degradation products.

employed have been UV [5,6], TLC [7], HPLC [5,8–10] and HPTLC [5,11].

Keeping into view the susceptibility of tinidazole under variety of conditions, it was felt that a HPLC method of analysis that separates the drug from degradation products formed under ICH suggested conditions (hydrolysis, oxidation, photolysis and thermal stress) [12] would be of general interest. Therefore, the aim of the present study was to develop a validated stability-indicating HPLC assay for tinidazole after performing stress studies under a variety of ICH recommended test conditions.

2. Experimental

2.1. Materials

Tinidazole was supplied by Panacea Biotec Ltd., Lalru, India and was used without further purification. 2-Methyl-5-nitroimidazole was procured from Unique Chemicals (Ankleshwar, India). Acetonitrile (HPLC grade) was purchased from Mallinckrodt Baker Inc. (Paris, USA). All other chemicals were of analytical reagent grade. Ultra-pure water was obtained from an ELGA (Bucks, UK) water purification unit.

2.2. Instrumentation

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for stress studies. Degradation experiments in acid and neutral conditions were performed using a dry-bath (model DB28120-26, Thermolyne, Iowa, USA). Stability studies were performed in a humidity chamber (KBF 760, WTB Binder, Tuttlingen, Germany). Photostability studies were done in a stability chamber (KBWF 240, WTB Binder, Tuttlingen, Germany) capable of controlling tolerances in temperature (±1 °C) and humidity (±3% RH). The chamber was set at 40 °C and was equipped with illumination bank made of light sources defined under option 2 in the ICH guideline Q1B [13]. Details of the photostability chamber have been provided in a previous publication by our group [14]. The samples were exposed for a total period of 12 days. Thermal stability studies were performed in dry-air oven (NSW Limited, New Delhi, India).

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

LC–MS studies were carried out 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

All degradation studies were done at a drug concentration of 1 mg ml^{-1}. For acid decomposition studies, drug was dissolved in 0.1 M HCl and solution was heated at 80 °C for 12 h. The studies in alkaline conditions were done initially in 0.1 M NaOH at a temperature of 80 °C. Drug was also dissolved in 0.01 M phosphate buffer (pH 10) and the solution was heated

at 80 °C for 6 h. For study in neutral conditions, drug in water was heated at 80 °C for 5 days. For oxidative conditions, initial studies were done in 3% H_2O_2. The solution was kept at room temperature for 6 h. Subsequently, the drug was exposed to 30% H_2O_2 at room temperature for 48 h. Photolytic studies were done in 0.1 M HCl, water and phosphate buffer (pH 10) and the solution was exposed in a photostability chamber for 12 days. Control samples were kept in dark for the same period. Thermal decomposition studies were performed by exposing solid sample of drug to dry heat at 50 °C for 3 months.

The pH of buffered solutions was measured before and after the reaction and no change was observed.

2.4. HPLC analyses

HPLC studies were first performed on stressed samples individually (after appropriate dilution), and later on a mixture of those samples in which sufficient degradation products were formed. Studies on individual reaction solutions were carried out using water–acetonitrile (86:14) as the mobile phase. As good separations were not achieved in a mixture of stressed samples using the above-mentioned mobile phase, therefore, the method was optimised further.

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

2.5. Validation of the developed method

Linearity of the method was established by injecting solutions containing 50–500 μg ml^{-1} of the drug in triplicate. Repeatibility studies were performed by hexaplicate injections of the drug at three concentrations (50, 200 and 500 μg ml^{-1}) on the same day. The studies were also repeated on different days to determine intermediate precision. Accuracy of the method was evaluated by spiking the drug at three concentrations in a mixture of stressed solutions and determining recovery of the added drug. Specificity of the method towards the drug was established through determination of purity for tinidazole peak in a mixture of stressed samples using a PDA detector. The resolution factor of the drug peak from the nearest resolving degradation product peak was also studied. Robustness was verified by studying the resolution of the drug in a mixture of degraded samples on a different chromatographic system on a different day.

2.6. Applicability of developed method to marketed formulations

Two tablet formulations and one infusion of tinidazole were subjected to accelerated conditions of temperature/humidity (40 °C/75% RH) without and with light (according to ICH option 2 [13]) for 3 months. Injectable preparation was also subjected to thermal stress at 60 °C for 1 month.

3. Results and discussion

3.1. Degradation behaviour

HPLC studies on tinidazole under different stress conditions using a mobile phase composition of water–acetoonitrile (86:14) as the solvent system suggested the following degradation behaviour.

3.1.1. Acidic conditions

On heating the drug in 0.1N HCl for 12 h, around 20% degradation was seen but there was no corresponding rise in degradation product peaks (Fig. 2b). No new peaks were seen in mass chromatogram also (Fig. 3b), indicating that the drug was degraded to very low molecular weight non-chromophoric compounds, similar to ornidazole [14].

3.1.2. Alkaline conditions

Tinidazole was found to be very labile in alkali. Complete degradation of the drug was observed in 0.1 M NaOH at 80 °C within 5–10 min. Therefore, subsequent studies were conducted in milder alkaline condition of pH 10 at 80 °C. As shown in HPLC chromatogram in Fig. 2c, degradation of the drug at pH 10 resulted in the rise of two major degradation products at 9.56 and 5.19 min and a minor product at 2.14 min. Parallel LC–MS investigations (Fig. 3c) indicated formation of only major degradation products apart from the drug. Neither the minor peak at 2.14 min nor any additional peak showed up in the

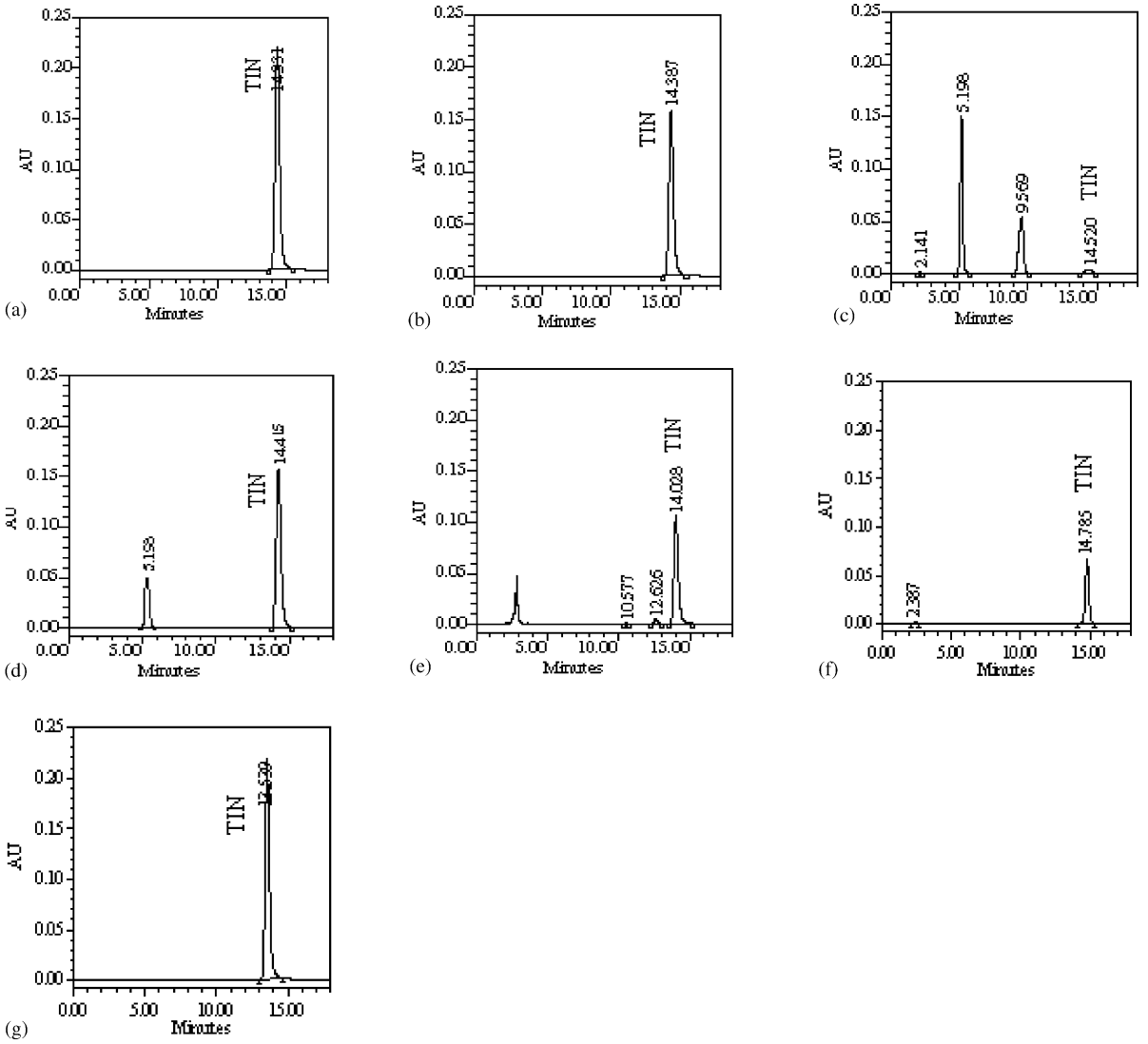

Fig. 2. Representative HPLC chromatograms of tinidazole (TIN). Key: Standard solution (a), sample degraded in 0.1 M HCl (b), sample degraded in buffer (pH 10; c), sample heated in water (d), sample subjected to oxidative degradation (e), sample subjected to photolytic degradation in 0.1 M HCl (f), sample subjected to dry heat (g).

mass chromatogram. The study of mass values (Fig. 4) indicated the two major degradation products to have molecular weights of *m*/*z* 128 and 248. The peak with *m*/*z* 128 was characterised to be due to ring nucleus 2-methyl-5-nitroimidazole (**2**) which was also confirmed by spiking and retention matching with the standard in HPLC (RT 5.2 min) and subsequently in LC–MS (RT 4.5 min). The other degradation product with the same mass as of the drug could be attributed to 4-nitro isomer of tinidazole (**3**), which was also confirmed by retention matching (RT 9.5 min in HPLC and 8.2 min in LC–MS) and spiking with a standard product synthesised by the reported procedure [3]. The other nitroimidazoles, metronidazole [15] and ornidazole [14] have been reported to degrade to simple compounds (ammonia and acetic acid) and ornidazole diol, respectively. The difference in degradation behaviour of the three drugs may be due to the changes in the

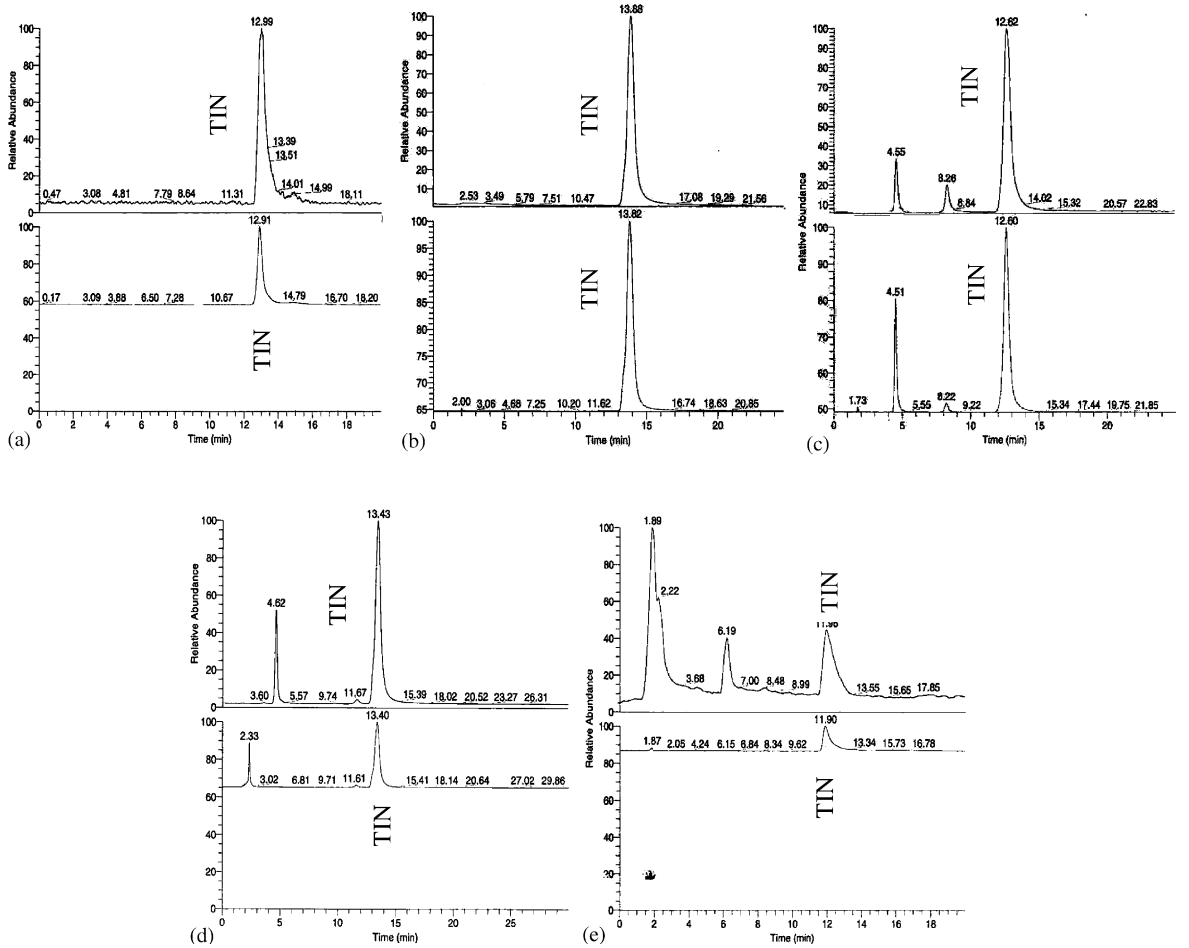

Fig. 3. Representative LC–MS chromatograms of tinidazole (TIN). Key: Standard solution (a), sample degraded in 0.1N HCl (b), sample degraded in buffer (pH 10; c), sample subjected to oxidative degradation (d), sample subjected to photolytic degradation in 0.1N HCl (e). The lower channel in each figure (a–e) represents UV chromatogram and the upper channel represents mass chromatogram taken in positive APCI mode.

side chain even though the basic ring nucleus remains the same.

3.1.3. Neutral condition

In neutral condition, 15–20% degradation of the drug was seen after heating for 5 days at 80 °C with the generation of only one peak at around 5.19 min (Fig. 2d), attributed to 2-methyl-5-nitroimidazole (**2**).

3.1.4. Oxidative conditions

Tinidazole was found to be stable in 3% H_2O_2 at room temperature. However, almost 70–80% drug degradation was seen on exposure to 30% H_2O_2 for 48 h at room temperature. Only two small peaks were seen in the chromatogram (Fig. 2e), similar to ornidazole [14]. Upon LC–MS investigation, an additional peak at 4.62 min was seen in the mass chromatogram (Fig. 3d), confirming the fact that under oxidation conditions, the drug degraded to non-chromophoric compounds, not seen in UV chromatogram.

3.1.5. Photolytic conditions

In acidic solutions, almost 90–95% degradation was seen on exposure to light for 12 days (Fig. 2f). In

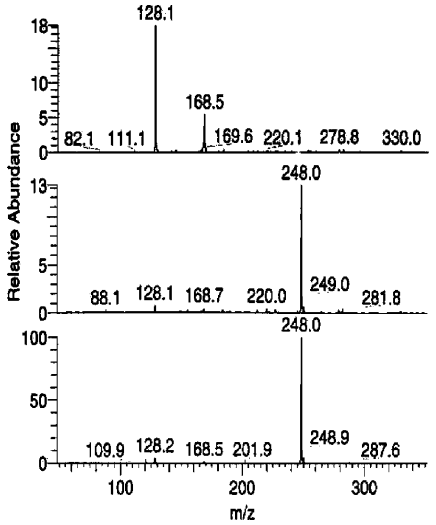

Fig. 4. Mass spectra of tinidazole (TIN) heated in pH 10 buffer at 80 °C for 1 h. The lowest channel represents mass spectrum for tinidazole peak, whereas middle and top channels represent mass spectra for the peaks seen in mass chromatogram in Fig. 3c at 8.2 and 4.5 min, respectively.

neutral and alkaline conditions, the drug got degraded with the formation of only hydrolytic products and no specific photolytic products.

In all the cases, the fall in drug peak was not in correspondence with the rise in degradation product peaks, indicating that even in the presence of light the drug degraded to non-chromophoric products. LC–MS studies of the acidic samples (Fig. 3e) revealed formation of two new products, which did not show up during HPLC analysis with UV detection. Mass values of the two compounds were similar to the products already reported by Salomies [2]. Similar degradation behaviour was seen for samples exposed to light in neutral conditions.

3.1.6. Thermal condition

Negligible degradation was seen on subjecting the drug to dry heat at 50 °C for 3 months.

3.2. Establishment of stability-indicating method in a mixture of solutions of various stress conditions

In order to achieve separation of drug and degradation products in a mixture of stressed samples, acetonitrile concentration was varied, followed by a change in the flow rate. It was found that when acetonitrile concentration was increased in the mobile phase, the peaks started getting merged. When acetonitrile percentage was decreased, there was improvement in the resolution of closely resolving peaks. It was observed that satisfactory resolution was obtained with water–acetoonitrile in the ratio of 88:12, at a flow rate of 0.8 ml min^{-1}, without undue increase in the run time. This method is simpler than the procedure already reported [8] which used a mobile phase composed of phosphate buffer (pH 3)–acetonitrile in the ratio of 82:18. The resolution in our solvent system of different degradation products, contained in the mixture of reaction solutions (in which decomposition of drug was seen), is shown in Fig. 5a.

3.3. Validation of the method

3.3.1. Linearity

The response for the drug was found to be linear in the investigated concentration range. The mean (±RSD) values of slope and intercept were 29098 (±0.9745) and 83395 (±2.074), respectively. The r^2 value was equal to 0.999.

3.3.2. Precision

Data obtained from precision experiments are given in Table 1, for repeatability and intermediate precision

Table 1
Precision studies

Actual concentration (μg ml^{-1})	Calculated concentration ± S.D. (μg ml^{-1}), RSD (%)	
	Repeatability ($n = 6$)	Intermediate precision ($n = 3$)
50	49.369 ± 0.178, 0.361	46.799 ± 0.528, 1.128
200	200.055 ± 0.539, 0.269	204.216 ± 3.607, 1.766
500	502.696 ± 1.248, 0.248	503.364 ± 0.609, 0.121

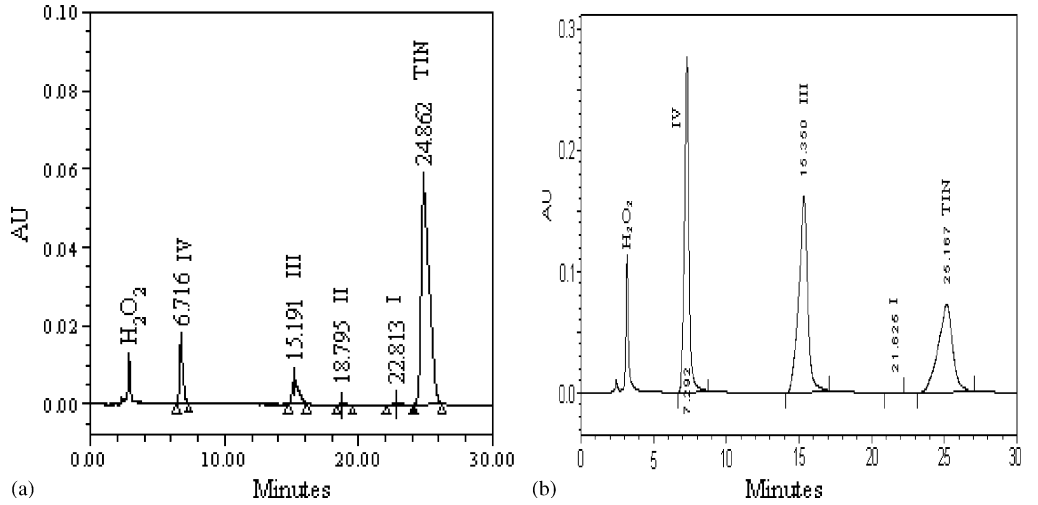

Fig. 5. Chromatogram showing separation of tinidazole (TIN) from degradation products in a mixture of stressed samples. (a) and (b) represent the chromatograms obtained on chromatographic systems 1 and 2, respectively. Key: H_2O_2, hydrogen peroxide; I and II, oxidative degradation products; III, 4-nitro isomer; IV, 2-methyl-5-nitroimidazole.

studies. The RSD values, ranging from 0.248 to 0.361% for repeatability study and from 0.121 to 1.766% for intermediate precision study, respectively, confirm that the method was sufficiently precise.

3.3.3. Accuracy

As shown from the data in Table 2, good recoveries of the drug in the range from 98.87 to 102.99% were made at various added concentrations, 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.

3.3.4. Specificity

Specificity of the method was established by verifying purity of the drug peak in a mixture of stressed samples by PDA analyses. Purity angle (PA) value for the drug peak in a mixture of stressed samples was found to be less than purity threshold (TH) value, indicating the absence of any co-eluting peak in the drug peak. The resolution factor for the drug peak was >2 from the nearest resolving peak.

Table 2
Recovery studies of tinidazole from a mixture of stressed samples ($n = 3$)

Actual concentration ($\mu g\,ml^{-1}$)	Calculated concentration ± S.D. ($\mu g\,ml^{-1}$), RSD (%)	Recovery (%)
50	51.5 ± 0.535, 1.039	102.99
200	205.283 ± 0.846, 0.412	102.64
500	494.333 ± 1.041, 0.211	98.87

3.3.5. Robustness

Resolution of the drug in a mixture of stressed samples was found to be similar when studies were performed on a different chromatographic system on a different day (Fig. 5b), indicating that the method had sufficient ruggedness.

3.4. Applicability of developed method to marketed formulations

Analyses of tablet samples using the developed method showed that there was negligible fall of drug and no degradation products were formed on subjecting the formulations to the accelerated conditions. Similar study on injectable preparations revealed 2-methyl-5-nitroimidazole (RT 7.1 min) and the unknown product (RT 2.9 min) to be present even in the control samples. The peaks due to the nucleus and 4-nitro isomer (RT 14.3 min) were seen in samples subjected to thermal stress (Fig. 6). There was insignificant fall of drug in samples exposed to 40 °C/75%

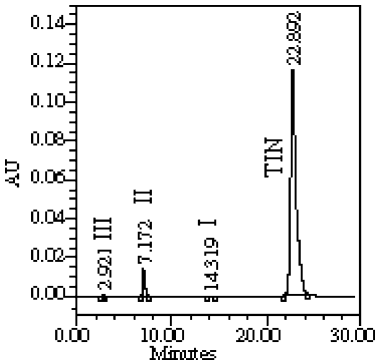

Fig. 6. Chromatogram of tinidazole (TIN) infusion heated at 60 °C for 1 month. I: 4-nitro isomer of the drug; II: 2-methyl-5-nitroimidazole; and III: unknown product.

RH, whereas there was 45 and 10% loss of drug in formulations exposed to light and heat, respectively.

Fig. 6 shows good separation of drug from degradation products when applied to actual stability samples, which confirms the specificity of the developed method.

4. Conclusions

In this study, tinidazole was subjected to stress studies under various ICH recommended conditions. The drug was found to degrade in alkaline and photolytic conditions in the same manner as reported earlier. The additional findings in this study are that the drug undergoes extensive degradation under oxidative stress, degrades to a mild extent in acidic and neutral conditions and is stable to thermal stress.

The drug can be analysed specifically in the presence of different chromophoric degradation products by using isocratic conditions and simple mobile phase containing water–acetoonitrile in the ratio of 88:12. The method was validated for parameters like linearity, precision, accuracy, specificity, ruggedness, etc. and was also applied to real marketed samples. Thus, the method can be employed for analysis of drug during stability studies, but it is not suggested for establishment of mass balance, because most of the degradation products were found to be non-chromophoric in nature and did not appear during HPLC studies.

References

[1] S.C. Sweetman (Ed.), Martindale—The Complete Drug Reference, Pharmaceutical Press, London, Chicago, 2002, p. 603.
[2] H. Salomies, Acta Pharm. Nord. 3 (1991) 211–214.
[3] A.K.S.B. Rao, R.S. Prasad, C.G. Rao, B.B. Singh, J. Chem. Soc. 7 (1989) 1352–1353.
[4] J.P. Salo, H. Salomies, J. Pharm. Biomed. Anal. 14 (1996) 1267–1270.
[5] J.P. Salo, Academic Dissertation, University of Helsinki, Finland, 2003 (http://ethesis.helsinki.fi/julkaisut/mat/farma/vk/salo/, assessed on 21.7.2003).
[6] J.P. Salo, H. Salomies, J. Pharm. Biomed. Anal. 31 (2003) 523–536.
[7] S.N. Sanyal, A.K. Datta, A. Chakrabarti, Drug Dev. Ind. Pharm. 18 (1992) 2095–2100.
[8] H. Salomies, J.P. Salo, Chromatographia 36 (1993) 79–82.
[9] M. Feng, H. Cao, F. Yu, Yaowu Fenxi Zashi 27 (1996) 247–249.
[10] M. Xu, H. Cao, F. Yu, Zhongguo Yiyao Gongye Zazhi 29 (1998) 416–417.
[11] J.P. Salo, H. Salomies, J. Pharm. Biomed. Anal. 14 (1996) 1261–1266.
[12] ICH, Stability testing of new drug substances and products. International Conference on Harmonisation, IFPMA, Geneva, 2000.
[13] ICH, Stability testing: photostability testing of new drug substances and products. International Conference on Harmonisation, IFPMA, Geneva, 1996.
[14] M. Bakshi, B. Singh, A. Singh, S. Singh, J. Pharm. Biomed. Anal. 26 (2001) 891–897.
[15] S.K. Baveja, H.K. Khosla, Indian. J. Technol. 13 (1975) 528.