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Original Article

A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SATRANIDAZOLE, IDENTIFICATION AND CHARACTERIZATION OF A PHOTOLYTIC DEGRADATION PRODUCT OF SATRANIDAZOLE USING LC-APCI-ION TRAP-MS

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ABSTRACT

Objective: To report the stability of antiprotozoal drug, satranidazole based on the information obtained from forced degradation studies.

Methods: Satranidazole was subjected to forced hydrolytic, oxidative, photolytic and thermal stress in accordance with the ICH guideline Q1A (R2). The drug showed labiality under only photo-neutral condition by forming a single degradation product. Resolution of the drug and degradation product was achieved on a Hypersil Gold C-18 column (4.6 × 250 mm, 5µm) utilizing methanol and potassium dihydrogen ortho phosphate buffer (pH 3.0; 0.01M) in the ratio of 25:75 (v/v) at a flow rate of 1 ml min^{-1} and at the detection wavelength 320 nm. The method was extended to LC-MS/MS for characterization of the degradation product and the pathway of decomposition was proposed. Validation of the LC-DAD method was carried out in accordance with ICH guidelines.

Results: The product was identified as 1-(methylsulfonyl)-7-nitro-2,3-dihydro-1*H*-diimidazo[1,2-*a*:1',2'-*c*]imidazol-9a(9*H*)-ol. The method met all required criteria and could be applied for analysis of commercially available tablets.

Conclusion: The method proved to be simple, accurate, precise, specific and robust and can be applied for determination of instability of satranidazole in bulk and commercial product.

Keywords: Satranidazole stress degradation; Characterization; Degradation products, LC-MS.

INTRODUCTION

The parent ICH stability testing guideline requires the drugs to be subjected to stress decomposition studies followed by identification and characterization of the degradation products (DPs) [1]. In parallel, the ICH guidelines on impurities [2, 3] necessitate characterization of all DPs formed in drug products at ≥0.1%. Therefore, the emphasis today is on techniques that allow characterization of very low quantities of DPs, against the conventional process of isolation and spectral analysis, which is tedious and time consuming. The hyphenated techniques are in focus for the purpose, among which LC-MS tools have been explored more strongly due to their potential to directly characterize small quantities of DPs [4]. Satranidazole is a novel nitroimidazole derivative possessing a C-N linkage at C-2 of the imidazole ring. Chemically, it is 1-methylsulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone [Fig. 1]. It is more active towards anaerobes than many other nitroimidazoles. It shows activity against, common protozoa like E. hystolytica, T.vaginalis and giardia and also acts as antibacterial agent in the treatment of amoebiasis [5].

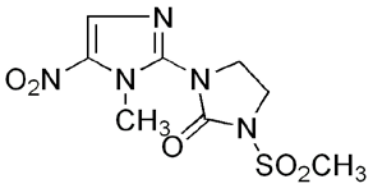


Fig. 1: Chemical structure of Satranidazole

There are two reports on use of RP-HPLC method for study of degradation behaviour of satranidazole along with ofloxacin [6, 7]. Also stability indicating HPTLC methods have been reported for determination of satranidazole in bulk and formulation [8, 9]. Recently, isolation and characterization of alkaline degraded products has been reported [10]. There are no reports available on characterization of light sensitive DPs to the best of our knowledge.

The present manuscript describes the (i) degradation behavior of satranidazole under hydrolysis (acid, base and neutral), oxidation, photolysis and thermal stress conditions, (ii) optimization of LC conditions to separate the drug and its photo-neutral DP on a reversed-phase C18 column, (iii) method validation, (iv) establishment of fragmentation pathway of the drug using LC-MS and MS/MS (v) characterization of photo-neutral DP from findings of LC-MS and LC-MS/MS (vi) Postulated mechanism of formation of photo-neutral DP.

Experimental

Chemicals and reagents

Satranidazole was supplied by Alkem Labs Pvt. Ltd. (Mumbai, India). Acetonitrile and methanol (HPLC grade) were procured from Merck (Mumbai, India) and used without purification. Analytical reagent grade (AR) hydrochloric acid, sodium hydroxide pellets, hydrogen peroxide solution were purchased from S. D. Fine Chemicals (Mumbai, India). Ultrapure water was obtained from a water purification unit (Elga Ltd., Bucks, England). Buffer materials and all other chemicals were of AR grade.

MATERIALS AND METHODS

Instrumentation

High Performance Liquid Chromatography

The analyses were carried out on Jasco HPLC (Jasco International Co., Tokyo, Japan) equipped with binary pump (PU-2080 plus), solvent mixing module (MX-2080-31), multi-wavelength PDA detector (MD-2010 plus), an interface box (LC-NET II/ADC), a rheodyne manual injector (7725i, USA) and chrompass data system

software ver. 1.8.1.6. The separations were carried out on a Hypersil Gold C18 (4.6 × 250 mm, 5 µm) analytical column (Thermo Scientific, Japan).

Mass Spectrometry

The LC-MS analyses were carried out on a 500-MS LC Ion Trap Mass spectrophotometer (Varian Inc., USA) in which the HPLC part comprised of an auto sampler (410, Prostar), solvent delivery module (210, Prostar), column valve module (500, Prostar), PDA Detector (355, Prostar), fraction collector (710, Prostar). The data acquisition was under the control of 500-MS workstation software.

Stress decomposition studies

Forced degradation studies of bulk drug and drug formulation included appropriate solid state and solution state stress conditions in accordance with regulatory guidelines. The stressors, choice of their concentration and preparation of samples were based on guidelines in the publication [11]. Stock solution of drug was prepared by dissolving it in acetonitrile to a final concentration of 2 mg ml^{-1}. Further it was diluted in the ratio of 50:50 (v/v) with the stressor (e.g. HCl, NaOH, H_2O_2 and water etc.). All hydrolytic studies were conducted at 80 °C. The oxidative study was carried out in 30% (v/v) H_2O_2 at room temperature. For thermal stress testing, the drug was sealed in glass vials and placed in a thermostatic block at 50 °C for 21 days. Photolytic studies on the drug in the solid and solution state were carried out by exposure to a combination of UV and fluorescent lamps in a photostability chamber set at accelerated conditions of temperature and humidity (40 °C/75% RH). Parallel blank set was kept in dark for comparison. After subjecting to stress, samples were withdrawn at suitable time interval. The optimized stressed conditions are enlisted in Table 1.

Table 1: Optimized stress conditions for the drug

Stressors	Hydrolytic at 80 °C			Oxidative at RT	Photolytic at 1.2x10⁶ Lux fluorescent and 200 W h/m² UV light at 40 °C/75% RH light				Thermal at 50 °C
	Acid	Neutral	Base		Acid	Neutral	Base	Solid	
Concentration of stressor	2 N HCl	H_2O	2 N NaOH	15 % H_2O_2	0.2 N HCl	H_2O	O.2 N NaOH	-	-
Duration	4 d	4 d	4 d	2 d	13 d	13d	13 d	13 d	21 d

Sample preparation for HPLC and LC-MS analysis

The stressed samples of acid and base hydrolysis were neutralized with NaOH and HCl, respectively to obtain 500 µg ml^{-1} solutions. Neutral hydrolysis, thermal and photolytic samples were diluted with mobile phase to obtain 500 µg ml^{-1}solutions. The oxidative stress sample was diluted with mobile phase to obtain 100 µg ml^{-1} solution. All the prepared samples were passed through 0.45 µm membrane filter before HPLC and LC-MS analyses.

Characterization of Degradation product

The stressed solution, in which sufficient amount of DP was formed, was subjected initially to LC-PDA and further to LC-MS analyses for characterization of DP.

RESULTS AND DISCUSSION

Optimization of LC-MS conditions

The main aim of this work was to separate satranidazole and its DP. During the optimization process, preliminary experiments were carried out on HPLC using Hypersil Gold C-18 column (4.6 × 250 mm, 5 µm). For mobile phase selection, several conditions with various mobile phases like methanol/water and acetonitrile/water in different proportions were tried in an isocratic mode. The peaks corresponding to drug and DP did not resolve completely and tailing was noticed. To get acceptable separation between the drug and its DP, potassium dihydrogen ortho phosphate buffer was used. Further studies were carried out using varied proportions of methanol (A) and potassium dihydrogen ortho phosphate buffer (B). The pH of the buffer, flow rate and composition of the mobile phase were systematically varied to optimize the method. Finally, we achieved good resolution of peaks with acceptable shape with the mobile phase consisting of A and B (pH 3.0; 0.01M) (25:75, v/v), in an isocratic mode. The flow rate 1 ml min^{-1}, column temperature 25 °C, wavelength of 320 nm and injection volume 20 µl were found to be suitable to achieve the separation of satranidazole and its DP [Fig. 2]. As the above mentioned C-18 column gave good resolution of drug and its DP, the same column was used for HPLC analyses. The advantage of the method was simple and rapid. Validation of the optimized LC method was done with respect to various parameters outlined in ICH guideline [12] and was extended to LC–MS/MS studies. The chromatographic conditions used for LC-MS analyses were similar to that of LC-PDA analyses, except that the buffer component B was replaced by 0.01 M ammonium formate of the same PH and injection volume was 10 µl. Fig. 4A shows LC-MS chromatogram obtained using mobile phase containing ammonium formate buffer, wherein retention time of drug and DP was not affected.

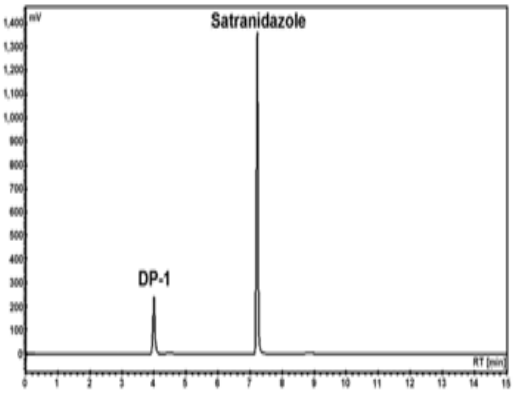


Fig. 2: HPLC chromatogram of photoneutral stressed sample

LC-MS/MS studies were carried out in +APCI ionization mode in the mass range of 50-2000 amu. High purity helium was used as carrier gas and nitrogen was used as nebulizer. Mass parameters were optimized to the following values : Rf loading : 80%; capillary voltage, 80 volts; syringe volume, 250 µL; spray chamber temperature, 50 °C; nebulizer pressure, 35 psi; drying gas temperature, 300 °C; drying gas pressure, 10 psi; vaporizer gas temperature, 350 °C; vaporizer gas pressure, 20 psi; spray shield voltage(±), ± 600.0 volts.

Method validation

Specificity

Specificity is the ability of the analytical method to measure the analyte concentration accurately in presence of all potential DPs. Specificity of the method towards the drug was studied by determination of purity for drug peak in stressed sample using a PDA detector.

The study of resolution factor of the drug peak from the nearest resolving DP was also done. Both drug and DP peaks were found to be pure from peak purity data. Also, the resolution factor for the

drug from degradation peak was greater than 3. Peak purity and resolution factor data is given in Table 5.

Linearity

Linearity test solutions were prepared from stock solution at seven concentration levels of analyte (5, 10, 20, 30, 40, 60, 80, 100, 200 µg ml^{-1}). The peak area versus concentration data is performed by least squares linear regression analysis. The calibration curve was drawn by plotting satranidazole average area for triplicate injections and the concentration expressed as a percentage. Good linearity was observed in the concentration range from 5 to 200 µg $ml^{-1}$of satranidazole. The data was subjected to statistical analysis using a linear regression model; the linear regression equation and correlation coefficient (r2) were y = 1.1178x - 0.2198 and 0.9999 respectively. These results indicate good linearity. The LOD and LOQ for satranidazole were estimated at a signal-to-noise ratio of 3:1 and10:1, respectively. The LOD and LOQ were 0.36 µg ml^{-1} and 1.11 µg ml^{-1}, respectively.

Precision

Precision of the method was verified by intra-day and inter-day precision studies. Intra-day precision studies were performed by analyses of three different concentrations of the drug in hexaplicate on the same day. Inter-day precision of the method was checked by repeating the studies on three different days. Also, the intermediate precision of the method was determined by changing the brand of the column as well as the analyst handling the whole experiment. The results of intra-day and inter-day precision experiments are shown in Table 2. The developed method was found to be precise as the RSD (%) values for intra-day and inter-day precision studies were less than 0.31 and 0.47, respectively. Even intermediate precision was established for the method as no significant change in the retention time of drug was observed.

Accuracy

Accuracy of the method was assessed employing the standard addition method, at three different levels (50%, 100%, 150%). The mixtures were analyzed in triplicate and the percentage of added drug obtained from difference between peak areas of fortified and unfortified samples of satranidazole. The HPLC area responses for accuracy determination are depicted in Table 3. Good recoveries (98.25-99.61) of the spiked drugs were obtained at each added concentration, indicating that the method was accurate.

Robustness

To determine the robustness of the method, experimental conditions were purposely altered. Three parameters selected were flow rate, composition of mobile phase and solvent from different lots. The mobile phase flow rate was 1ml min^{-1}. This was changed to 1.1 and 0.9 ml min^{-1} and the effect was studied. The effect of mobile phase composition was studied by use of potassium dihydrogen ortho phosphate buffer (pH 3.0; 0.01M) and methanol in ratio of 76:24 and 74:26 (v/v). Also methanol of different lots from same manufacturer was used. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. In all the deliberately varied chromatographic conditions, no significant change in retention time and tailing factor of satranidazole was observed [Table 4].

System suitability test

The system suitability parameters with respect to purity index, capacity factor, resolution factor, theoretical plates, tailing factor were calculated and are given in Table 5. It could be seen from table that the peaks of drug and DP-1 were well resolved.

Table 2: Precision studies

Concentration taken (µg ml^{-1})	Measured concentration (µg ml^{-1}) ± S.D, RSD (%)	
	Repeatability (n = 6)	Intermediate precision (n = 3)
30	29.12 ± 0.092, 0.315	29.15 ± 0.137, 0.471
60	60.49 ± 0.080, 0.132	60.49 ± 0.09, 0.148
90	89.87 ± 0.094, 0.104	89.83 ± 0.09, 0.1

Table 3: Recovery studies

Spiked concentration (µg ml^{-1})	Measured concentration (µg ml^{-1}) ± S.D, RSD (%)	Recovery (%)
30	29.43 ± 0.046, 0.156	98.25
60	59.06 ± 0.187, 0.317	99.48
90	89.65 ± .238, 0.265	99.61

Table 4: Robustness testing of satranidazole

	Chromatographic changes			
	Factor	**Level**	**RT**	**T_f**
A:	Flow rate (ml/min)			
	0.9	-1	7.37	1.26
	1.0	0	7.2	1.19
	1.1	1	6.96	1.13
	Mean ± SD (*n* = 3)		7.17± 0.20	1.19± 0.06
B:	Percentage of Methanol in the mobile phase (v/v)			
	24	-1	7.27	1.20
	25	0	7.2	1.19
	26	1	7.0	1.08
	Mean ± SD (*n* = 3)		7.15± 0.14	1.15± 0.06
C:	Solvents of different lots			
	First lot		7.24	1.22
	Second lot		7.28	1.24
	Mean ± SD (*n* = 3)		7.26± 0.028	1.23± 0.014

Table 5: HPLC system suitability parameters[a]

Code	RT	k	R_s	N	T_f
DP-1	4.0	1.0	10.6	3580	1.03
Satranidazole	7.2	2.6	-	8520	1.19

[a]RT: retention time; k: capacity factor; R_s: USP resolution; N: number of theoretical plates; T_f: USP tailing factor

Degradation behaviour

The drug degraded into DP-1 under photo-neutral condition. The extent of degradation was 28 % under this condition. The chromatogram of photo-neutral stressed sample showing separation of drug and its DP is shown in Fig. 2. The drug was stable under all other stress conditions, including heating in acid, base, water, oxidation, on exposure of acid and base solutions to light, photoexposure of solid drug, and dry heating at 50 °C.

Study of the stability of commercial tablets

The assay content of satranidazole, commercially available marketed formulation was analyzed by the proposed method after exposure to accelerated storage condition (i.e. 40 °C/75% RH). The peak at retention time 7.2 min for the drug was observed in the chromatogram of the drug samples extracted from tablets and no additional peak was found [Fig. 3]. Experimental results of the amount of satranidazole in tablets, expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are normally present in tablets and packaging material is of good quality [Table 6].

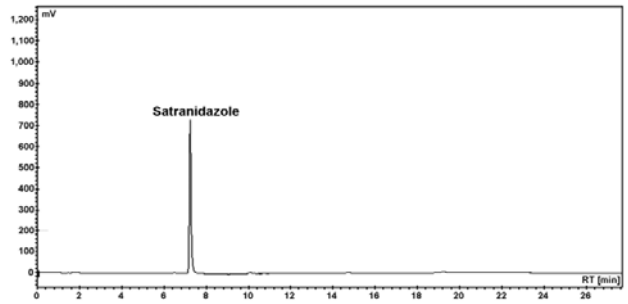
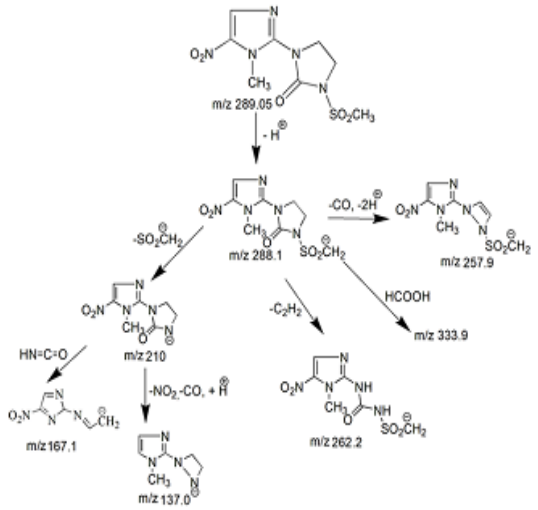


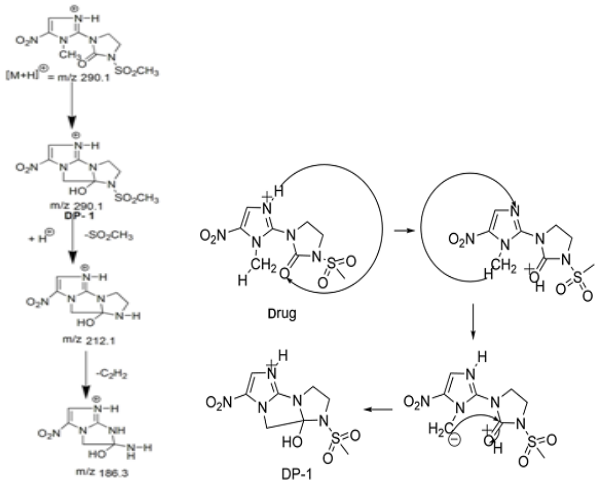
Fig. 3: Chromatogram of marketed formulation

Characterization of Satranidazole and DP-1 by LC-MS/MS

he photo-neutral stressed solution of satranidazole was subjected to LC–MS analyses to characterize the DP. The resulted LC-MS chromatogram is shown in Fig. 4A.



Scheme 1: Mass fragmentation pattern of Satranidazole



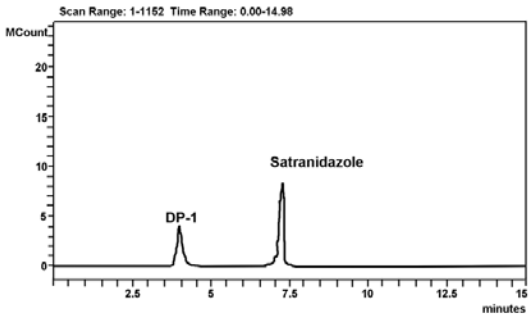
Scheme 2: Proposed fragmentation for DP-1 Scheme 3: Mechanism of formation of DP-1 from the drug

Mass fragmentation pathway of the drug

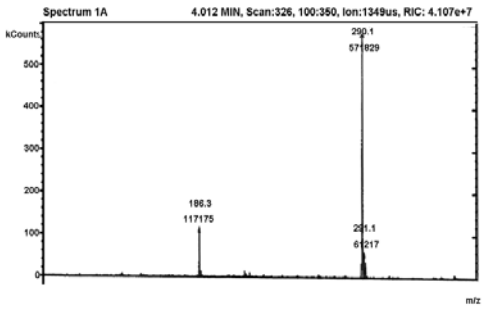
The mass fragmentation pathway of the drug was established from results of LC-APCI-MS in negative mode and APCI/MS/MS analysis using optimized mass parameters. The APCI-MS of $[M-H]^-$ ion at m/z 288 showed abundant fragment ions at m/z 210.1 (loss of SO_2CH_2 from m/z 288.1), m/z 262.2 (loss of C_2H_2 from m/z 288.1), m/z 137 (loss of [NO_2, CO] from m/z 210.1, followed by addition of H^+) and adduct at m/z 333.9 (HCOOH adduct of m/z 288) [Fig. 4C, Scheme 1]. APCI/MS/MS of m/z 288 showed abundant fragment ions at m/z 210, the formation of which is discussed in APCI-MS, m/z 167.1 (loss of HN=C=O from m/z 210.1), m/z 257.9 (loss of [CO, $2H^+$] from m/z 288) [Fig. 5B, Scheme 1].

Table 6: Study of stability of commercial tablets (n = 3)

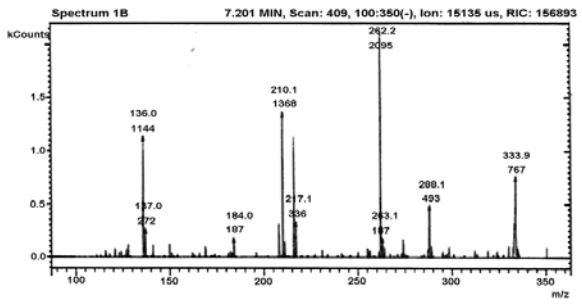
Code	RT	k	R_s	N	T_f
DP-1	4.0	1.0	10.6	3580	1.03
Satranidazole	7.2	2.6	-	8520	1.19



(A)

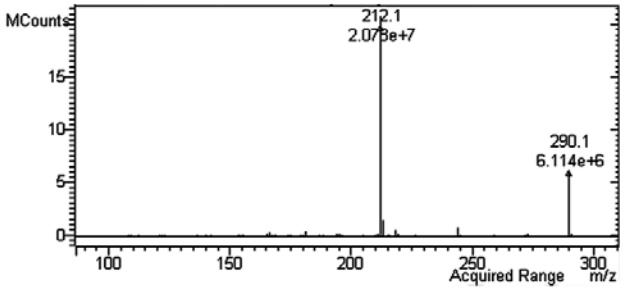


(B)

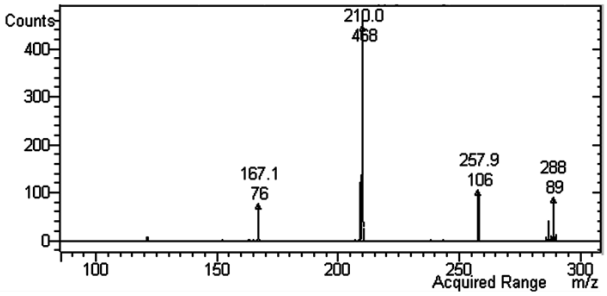


(C)

Fig. 4: LC-MS chromatogram (A) of photo-neutral stressed sample showing separation of DP-1 and Satranidazole and LC-MS spectra (B, C) of DP-1 and Satranidazole, respectively, in +APCI and –APCI mode



(A)



(B)

Fig. 5: MS/MS spectra (A, B) of DP-1 and Satranidazole, respectively, +APCI and –APCI mode

Postulated structure of DP-1

DP1 (m/z 290): The product was obtained due to cyclization in the drug molecule. The LC-APCI-MS of m/z 290.1 in positive mode showed abundant fragment ion at m/z 186.3 (loss of SO_2CH_3 from m/z 290.1 and addition of H^+ and further loss of C_2H_2 from m/z 212) [Fig. 4B, Scheme 2]. The APCI/MS/MS of m/z 290 showed abundant fragment ion at m/z 212.1 (loss of SO_2CH_3 from m/z 290, followed by addition of H^+ to m/z 211.1) [Fig. 5A, Scheme 2]. The probable structure of DP-1 and its postulated mechanism is given in Scheme 2 and Scheme 3, respectively.

CONCLUSION

Stress degradation studies on satranidazole, carried out according to ICH guidelines, provided information regarding degradation behaviour of the drug. The drug was susceptible to only photo-neutral degradation, whereas it was stable in all other conditions. One DP [DP-1] was formed in photo-neutral condition, and was separated in a single run by an isocratic LC-DAD method. The method proved to be simple, accurate, precise, specific and robust. It was successfully employed for the analysis of marketed formulation stored for three months under accelerated conditions of temperature and humidity. The product DP-1 was characterized with the help of LC–MS/MS data and comparison of the same to that for the drug. The product was a new DP and characterized as 1-(methylsulfonyl)-7-nitro-2,3-dihydro-1H-diimidazo[1,2-a:1',2'-c] imidazol-9a(9H)-ol. The complete fragmentation pattern of drug is shown in Scheme 1 and characterization of DP-1 and its postulated mechanism of formation from drug is shown in Scheme 2 and Scheme 3, respectively.

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REFERENCES

1. International Conference on Harmonization (ICH), Q1A (R2): Stability testing of new drug substances and products, IFPMA, Geneva. Biomedical chromatography : BMC 2003.
2. Substances I. International Conference on Harmonization (ICH), Q3A (R2): Impurities in New Drug Geneva. Biomedical chromatography : BMC 2006.
3. Products I. International Conference on Harmonization (ICH), Q3B (R2): Impurities in New Drug Geneva. Biomedical chromatography : BMC 2006.
4. Wu Y. The use of liquid chromatography-mass spectrometry for the identification of drug degradation products in pharmaceutical formulations. Biomedical chromatography : BMC 2000;14(6):384-96.
5. Veni R, Kumari KJ, Babu BH. A new RP-HPLC method for the estimation of satranidazole in bulk drugs and pharmaceutical formulations. International Journal of Pharmacy Technolgy 2011;3(3 SRC - GoogleScholar):3299-306.
6. Godse KV, Nadkarni NJ, Jani G, Ghate S. Fexofenadine in higher doses in chronic spontaneous urticaria. Indian dermatology online journal 2010;1(1):45-6.
7. Sharma K, Singh RR, Kandaswamy M, Mithra C, Giri S, Rajagopal S, et al. LC-MS/MS-ESI method for simultaneous quantitation of three endocannabinoids and its application to rat pharmacokinetic studies. Bioanalysis 2011;3(2):181-96.
8. Patel M, Patel KM, Patel GS, Suhagia BN, Prajapati AM, J. Development and validation of a stability‐indicating HPTLC‐densitometric method for satranidazole. Chromatogr Relat Technol 2007;30(18 SRC - GoogleScholar):2755-67.
9. Patel A, Cao Q-L, Koenig PR, Hijazi ZM. Intracardiac echocardiography to guide closure of atrial septal defects in children less than 15 kilograms. Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions 2006;68(2):287-91.
10. Kale SS, Deshpande AS, Mulgund SV, Jain KS. Development of stability indicating HPLC method and characterization of major degradation products of satranidazole. Inventi Rapid Pharm Ana Qual Assur 2012;3(1 SRC - GoogleScholar).
11. Singh SP, Lai D, Cartas M, Serio D, Murali R, Kalyanaraman VS, et al. Epitope-tagging approach to determine the stoichiometry of the structural and nonstructural proteins in the virus particles: amount of Vpr in relation to Gag in HIV-1. Virology 2000;268(2):364-71.
12. International conference on Harmonization (ICH), Q2 (R1): Validation of Analytical Procedures-Text and Methodology, Geneva, Switzerland. Biomedical chromatography : BMC 2005.