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Research Article

A STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF METRONIDAZOLE USING ECOFRIENDLY SOLVENT AS MOBILE PHASE COMPONENT

PRATIBHA VERMA[1]*, VARSHA NAMBOODIRY[1], SONI MISHRA[1], ASHOK BHAGWAT[2], SUVARNA BHOIR[2]

[1]School of Science, NMIMS (Deemed to be University), Vile Parle, [2]C.B. Patel Research Centre, Vile Parle, Mumbai 400056, India.
Email: vermapratibha1811@yahoo.in

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ABSTRACT

Objective: This paper describes the simple, economic, selective, precise, and stability-indicating HPLC analytical method to determine Metronidazole and its impurities, as well as gives degradation profile for a formulation by using of Solvent-X (Propylene Carbonate: Methanol 60:40) as a Ecofriendly mobile phase component in place of Acetonitrile (ACN).

Method: The method was efficacious by phosphate buffer (HPLC grade water to which 10µL of 10% H_3PO_4 pH 4.1) and Solvent-X in ratio 90:10 (v/v) on 5µm Phenyl Column (250X4.6mm), with a flow rate of 1mL/min at 310nm of detector wavelength.

Results: Linear regression analysis data for the calibration plot showed there was a good linear relationship between response and concentration in the range 1.0–2.4µg/ mL. The regression coefficient was 0.9997. Metronidazole was found to degrade significantly in alkaline conditions. Mild degradation of the drug occurred in acidic conditions and oxidative stress. The drug was stable to dry heat. The method was validated for accuracy, precision, reproducibility, specificity, robustness, detection and quantification limits, in accordance with ICH guidelines.

Conclusion: Statistical analysis proved the method was precise, reproducible, selective, specific and accurate for analysis of Metronidazole. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply the method is suitable for routine quantification of Metronidazole with high precision and accuracy. The efficiency of method was compared with solvent Acetonitrile in terms of linearity, Accuracy, Precision, Specificity and Robustness.

Keywords: Propylene Carbonate; Acetonitrile; Metronidazole; Validation; Force Degradation

INTRODUCTION

Metronidazole, which is chemically 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethanol, possesses activity against protozoa and anaerobic bacteria. The substance has a wide range of uses, including treatment in amoebiasis and trichomoniasis, vaginosis.

As a well-established drug, Metronidazole has a degradation that has been reported extensively in the literature [1-9]. The drug degrades in alkaline conditions to ammonia, acetic acid, and to a compound that gave pink to violet color with ninhydrin reagent [1]. The photolytic stability of Metronidazole also has been investigated [2-9]. The drug forms rearrangement and degradation products upon UV photolysis [8-9].

Several analytical methods have been reported for the determination of Metronidazole when present alone or in combination with other drugs [10-17]. Techniques include spectrophtometry, supercritical fluid chromatography, electrochemistry and high-performance liquid chromatography (HPLC). Compendial methods for bulk-drug assay involve Non-aqueous titration, and UV spectrophotometry and HPLC assay have been suggested for the determination of drug from various dosage forms [18]. For stability studies, chromatographic [19-22], spectrometric [22], and polarographic methods [22] have been reported. In almost all of these cases, the methods were developed by taking into account the degradation products formed in one or two stress conditions.

The objective of this work was to develop a simple, precise, reliable and rapid Stability-indicating liquid chromatographic analytical method for Metronidazole and to validate the method in compliance with ICH guidelines. The method was successfully validated by using mobile phase as Solvent –X [23] and compare with ACN in terms of precision, accuracy, specificity and ruggedness.

MATERIALS AND METHODS

Chemicals

Metronidazole and impurities working standard (a) and (b), 2-methyl-4-nitoimidazole and 4-nitroimidazole were received as a gift sample from a reputed company along with certificate of analysis. The formulation Metrogly 200mg manufactured by JB Chemicals was procured from local pharmacy for the study of assay of tablets. HPLC grade ACN and Methanol (MeOH) were procured from E-Merck Limited, Mumbai (India). HPLC grade PC of Sigma Aldrich was imported from Germany. Double distilled water was used for solution preparations throughout the project. The Mobile phase was always filtered through 0.45µ membrane filter paper and degassed before use.

Preparation of standard solutions

A stock solution of Metronidazole and impurities (1.0mg/mL) was prepared by dissolving an appropriate amount of the substance in Solvent-X. Working solutions of different concentrations were prepared from the above stock solution and diluted with the mobile phase.

Equipment

The LC system used for method development and validation consisted of a JASCO HPLC-900 series equipped with PU-980 intelligent pump, AS-950 intelligent auto sampler (1-100 µL) and UV-975 intelligent UV-Vis detector with 8 µL flow cell and Sonicator/Water Bath of DAKSIN 10L250H

Method Development and Optimization

During method development some important parameters were tested for good chromatographic separation such as the pH of the mobile phase, concentration of acid or buffer solutions, percentage and type of organic modifier, etc. The following are the optimized chromatographic condition under which separation was achieved:

Method A: Using Solvent-X as the mobile phase component.

The separation was carried out on a Phenyl (250 x 4.6 mm, 5 µ) column using a mobile phase containing a mixture of Solvent-X:D/W (10µL of 10% H_3PO_4) (10:90, v/v) at a flow rate of 1mL/min .The wavelength was monitored at 310 nm and the column temperature was set at 25°C. The typical RP-HPLC chromatogram of the sample is shown in Fig. 1.

Method B: Using ACN as a mobile phase component.

When Solvent-X was replaced with ACN, all other chromatographic conditions were kept unchanged except that the mobile phase composition used was ACN: D/W (10:90, v/v). The typical RP-HPLC chromatogram of the sample is shown in Fig. 2.

Method Validation

The developed chromatographic methods, A and B, using Solvent-X and ACN, respectively were validated using the following parameters:

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for Metronidazole, impurity (a) and impurity (b) were determined at signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentrations. A precision study was also carried out at the LOQ level by injecting six individual preparations of metronidazole, imp-a and imp-b and calculating the % R.S.D. of the area.

Linearity

Linearity of the method was studied by injecting eight concentrations of the drug prepared in the mobile phase in the range of 1.0 to 2.4 µg/mL for metronidazole and 30-72ng/mL for impurities a and b in triplicate into the HPLC system.

Accuracy

The accuracy of the assay was evaluated in triplicates at three concentration levels, i.e. 50, 100 and 150µg/mL. The percentage recovery was calculated from the slope and Y-intercept of the calibration curve obtained from linearity.

Precision

The precision of the assay method was evaluated by carrying out six independent assays of the Metronidazole test sample against a qualified working standard and calculating the % Relative Standard deviation (% RSD) of the assay.

The precision of the related substance method was checked by injecting six individual preparations of Metronidazole (7.5µg/mL) spiked with 100% of imp-a and imp-b with respect to Metronidazole analyte concentration. % RSD of area of each imp-a, and imp-b was calculated.

The intermediate precision of the method was also evaluated using a different instrument in the same laboratory.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between Metronidazole, imp-a, and imp-b was recorded. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, the flow was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of the column temperature on resolution was studied at 20 and 30° C instead of 25° C. The effect of the percent organic strength on resolution was studied by varying ACN by ±3mL while other mobile phase components were held constant.

Solution stability

The solution stability of Metronidazole in the assay method was determined by leaving both the test solutions of the sample and the reference standard in air tight volumetric flasks at room temperature for 48h. The same sample solutions were assayed after 2, 4, 6, 8, 24 and 48h during the study period. The mobile phase stability was also tested by assaying the freshly prepared sample solutions against freshly prepared reference standard solution for 48h. Mobile phase was kept constant during the study period. The % R.S.D. for the assay of Metronidazole was calculated during the mobile phase and solution stability experiments.

The solution stability of Metronidazole and its impurities in the related substance method was tested by leaving spiked sample solutions in tightly capped volumetric flasks at room temperature for 48h. The content of imp-a and imp-b were determined after 2, 4, 6, 8, 24 and 48h during the study period. The mobile phase stability was also determined for 48h by injecting the freshly prepared sample solutions. The content of imp-a, and imp-b in the test solutions were checked.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed LC method for Metronidazole was carried out in the presence of its impurities namely imp-a and imp-b. Stress studies were performed for Metronidazole bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted under stress conditions of heat (60°C), acid (0.5N HCl), base (0.5N NaOH) and oxidation (3.0% H_2O_2) so as to evaluate the ability of the proposed method to separate Metronidazole from its degradation product. The study period was 48 hours for the acid, base hydrolysis, oxidation and for thermal degradation. A Peak purity test was carried out for the Metronidazole peak by using a PDA detector in stress samples. Assay studies were carried out for stress samples against a qualified Metronidazole reference standard.

Induced degradation of Metronidazole:

i) Acid and base-induced degradation: 10ml of sample stock solution was taken in a round bottom flask. 10mL of 1N HCl and 10mL of 1N NaOH were added separately to the stock solution. The reaction was carried out for 70°C for 12hr. After 12 hr, sample was cooled at room temperature and reaction was stopped, diluted with the mobile phase and mixed well. This solution was injected into the HPLC system. This acidic and basic degradation was performed in the dark in order to exclude the possible degradative effect of light.

ii) Hydrogen peroxide-induced degradation: the method described above (i) was followed except that 10mL of 3% H_2O_2 was added in place of HCl [24].

iii) Thermal degradation: The method described above (i) was followed without the addition of H_2O_2 or HCl/NaOH. Thus, all the developed methods were validated and found to meet the acceptance criteria set by the ICH.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective of the chromatographic method is to separate Metronidazole from imp-a and imp-b. Impurities were co-eluted using different stationary phases such as C_{18}, C_8, phenyl and cyano as well as different mobile phases. The chromatographic separation was achieved on an Agilent Phenyl SB (250mm×4.6 mm) 5µm column using a mixture of D/W (10µL of 10% H_3PO_4)–Acetonitrile/Solvent-X (90:10, v/v) as a mobile phase. The flow rate of the mobile phase was 1.0 ml/min, at controlled temperature, the peak shape of the Metronidazole was found to be symmetrical. In optimized chromatographic conditions Metronidazole, imp-a and imp-b were separated with a resolution greater than 2. The typical retention times were about 5.5, 6.5 and 9.9 min for method A and 5.2, 6.2 and 10.8min for method B, respectively (Fig. 1 and 2). The system suitability results are given in Table I. The developed LC method was found to be specific for Metronidazole and its two impurities namely imp-a and imp-b (Fig. 1 and 2).

Limit of detection and limit of quantification

The limit of detection of Metronidazole and all the impurities namely, imp-a and imp-b, was achieved at 15ng/ml for 20 µL injection volume. The limit of quantification for all three impurities namely, imp-a and imp-b, was achieved at 30ng/ml for a 20µL injection volume. The % R.S.D. at the LOQ concentrations for Metronidazole, imp-a and imp-b were 0.2, 0.8 and 1.0 for method A and 0.2, 0.8 and 0.5 for method B, respectively.

Linearity

The linearity calibration plot for the assay method was obtained over the calibration ranges tested and correlation coefficient

obtained was greater than 0.997 for methods A and B. The results show that an excellent correlation existed between the peak area and concentration of the analyte and imp. A linear calibration plot for the related substance method was obtained over the calibration ranges tested, for impurity imp-a and imp-b. The correlation coefficient obtained was greater than 0.999. The above results show that an excellent correlation existed between the peak area and the concentration of imp-a and imp-b.

Accuracy

The percentage recovery of Metronidazole in the bulk drug samples ranged from 98.00 to 102.00% for method A and B.

Precision

The develop method was found to be precise as the % R.S.D. value of Metronidazole for repeatability precision study was within 0.18% for both methods A and B, and of imp-a and imp-b in the related substance was within 1.5% for both methods (A and B). The % R.S.D. value for intermediate precision study was within 0.9 % for both methods A and B; and of imp-a and imp-b were well within 3% for both methods A and B.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), the resolution between critical pair, i.e. imp-a, Metronidazole and imp-b was greater than 2.0, illustrating the robustness of the method A and method B.

Solution stability and mobile phase stability

The % R.S.D. of the assay of Metronidazole during solution stability experiments were within 2%. Metronidazole as a bulk product and as a formulation was found to be stable in both mobile phases after 48hrs.

Results of forced degradation studies

Degradation was not observed in Metronidazole bulk drug when subjected to stress conditions under ICH guidelines. Degradation was observed under force degradation condition of Metronidazole formulation under acid and base hydrolysis and oxidation. Peak purity test results confirmed that the Metronidazole peak was homogenous and pure in all the analyzed stress samples. The summary of specificity for the bulk drug is given in Table II

From the above experimental work it is proved that, RP-HPLC methods that use ACN can be replicated using exactly the same amount of Solvent-X instead of ACN to give reasonably good results without any change in other chromatographic conditions for Metronidazole. All methods developed using Solvent-X meet the acceptance criteria set by the ICH. Hence, these methods can be employed in the industry as well as other chemical laboratories for routine analysis. The columns used for method development did not show any deterioration in performance on being exposed to mobile phases containing Solvent-X as a component. However, as compared to the column pressure observed when an ACN based mobile phase was used, a slight increase of upto 60 kg/cm^2 was reported when Solvent-X based mobile phase was used. This may be attributed to the higher viscosity of PC which constitutes 60% of Solvent-X. Solvent-X showed good compatibility with the buffers used during analysis. It was also found to be UV transparent above 210 nm.

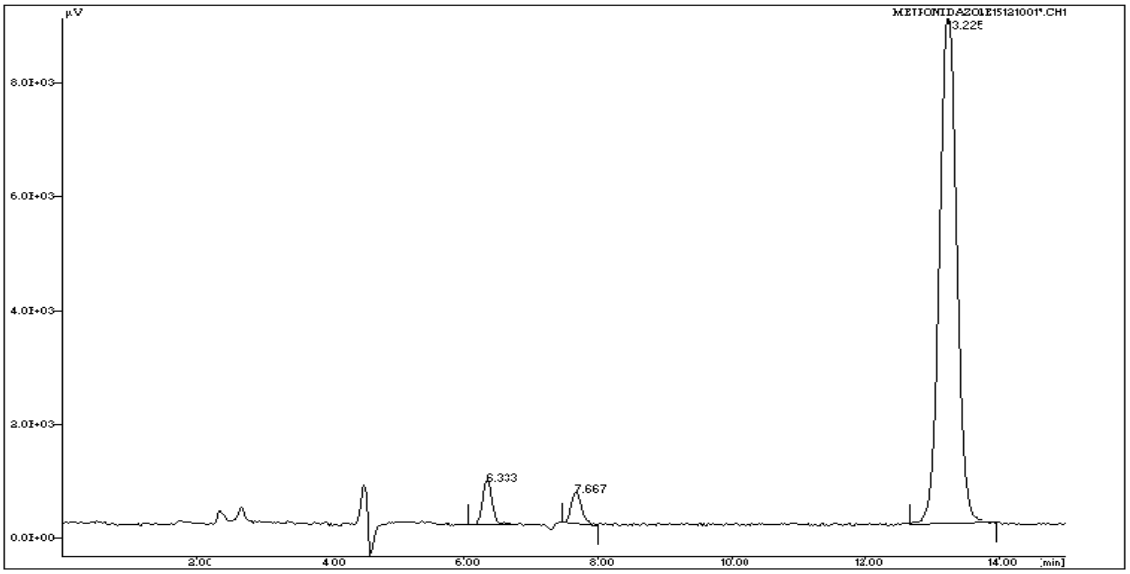

Fig. 1: Chromatogram of Metronidazole and its spiked by Method A

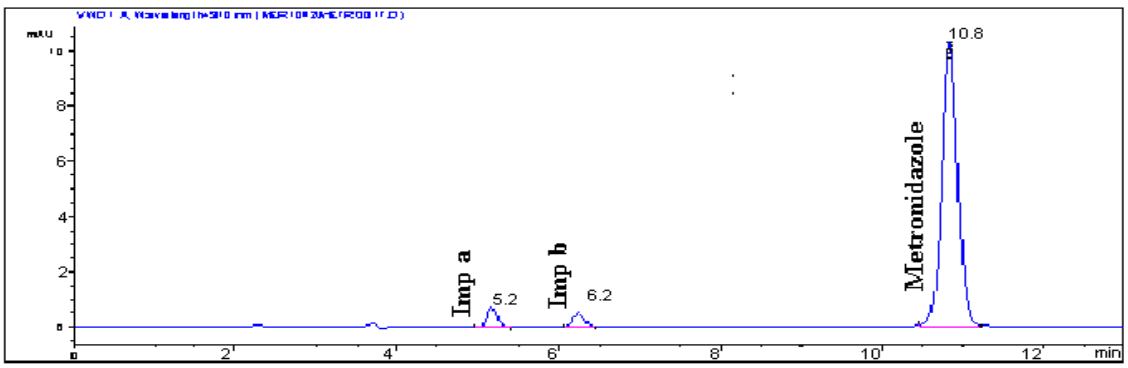

Fig. 2: Chromatogram of Metronidazole and its spiked by Method B

Table 1: System Suitability Report

Parameter	Method (A)*			Method (B)*		
	Impurity a	Impurity b	Metronidazole	Impurity a	Impurity b	Metronidazole
Retention Time (R_t)	5.5	6.5	9.9	5.2	6.2	10.8
Capacity Factor (α)	0.7	0.9	2.0	0.40	0.42	1.90
Selectivity (K')	-	1.23	2.22	-	1.05	4.52
Resolution (Rs)	-	3.04	8.78	-	2.2	7.5
Tailing Factor (T)	1.0	1.20	1.0	0.8	0.9	1.0
Theoretical Plates (N)	16384	15041	28021	23156	27105	38809
HETP (m)	0.0015	0.0017	0.0009	0.0011	0.0009	0.0006

*Method A: MP - Solvent-X: D/W (10µL of 10% H_3PO_4) (10:90, v/v)

*Method B: MP - ACN: D/W (10µL of 10% H_3PO_4) (10:90, v/v)

Table 2: Summary of Specificity

Stress Conditions	Time	Method A		Method B	
		% Assay of active substance	% Mass Balance (active + impurity)	% Assay of active substance	% Mass Balance (active+ impurity)
Acid Hydrolysis (0.5N HCl)	48 Hours	99.7%	99.7%	99.5%	99.5%
Base Hydrolysis (0.5N NaOH)	48 hours	99.8%	99.8%	99.9%	99.9%
Oxidation (3% H_2O_2)	48 hours	99.9%	99.9%	99.6%	99.6%
Thermal 60°C	48 hours	99.8%	99.8%	99.8%	99.8%

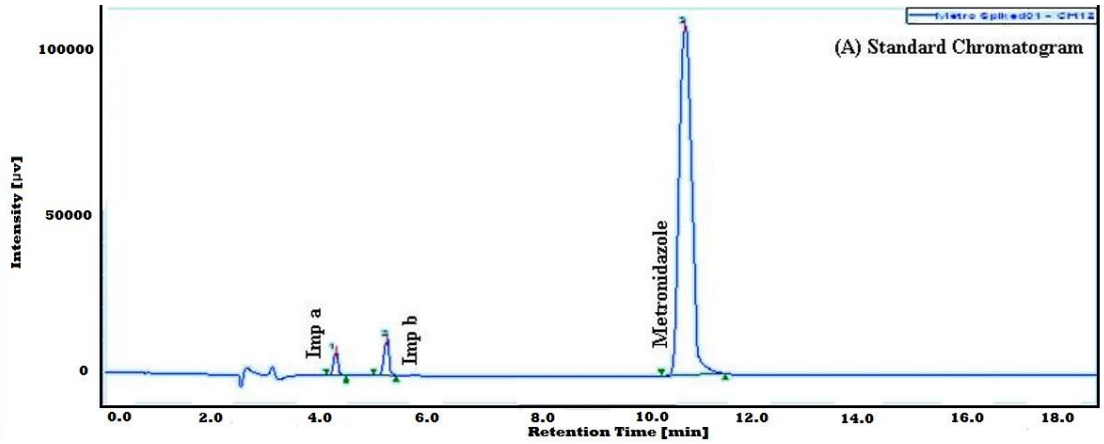

Fig. 3: (a) Standard chromatogram of Metronidazole with spiked impurites

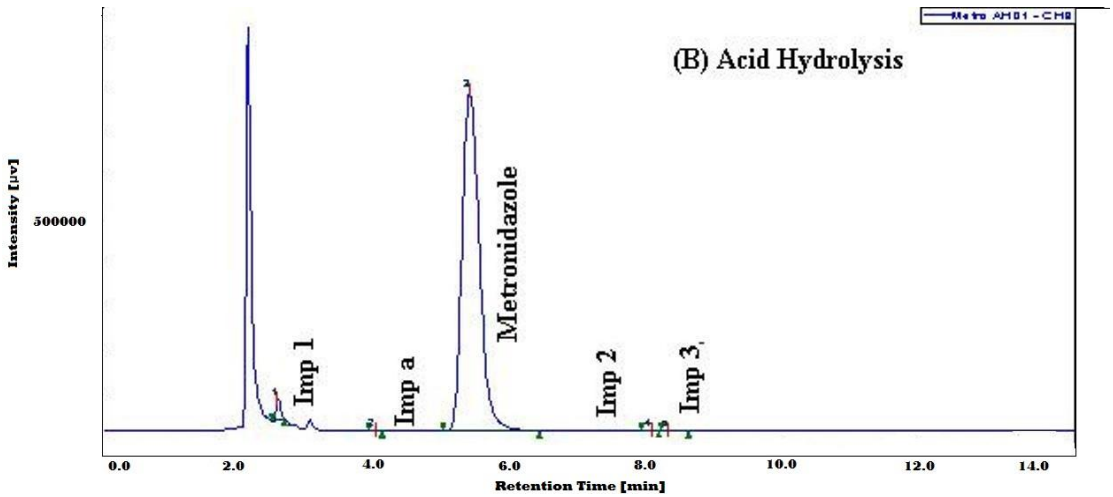

Fig 3: (b) Sample degraded 1.0N HCl

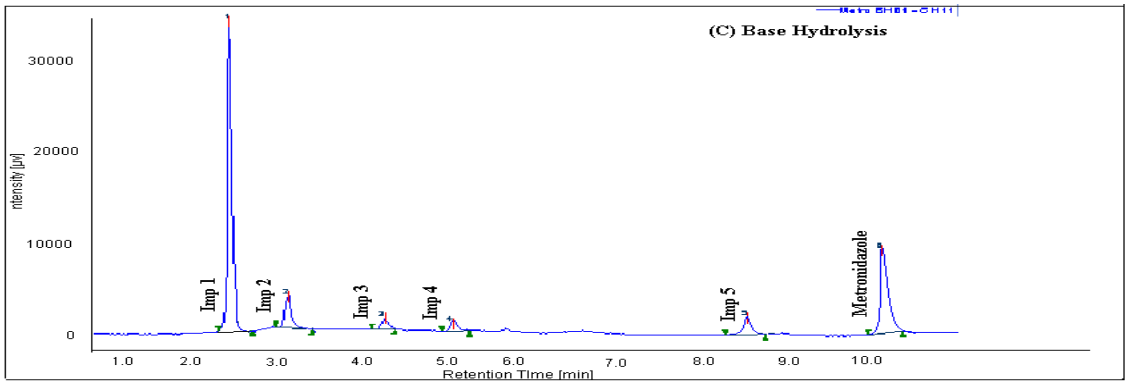

Fig. 3: (c) Sample degraded 1.0N NaOH

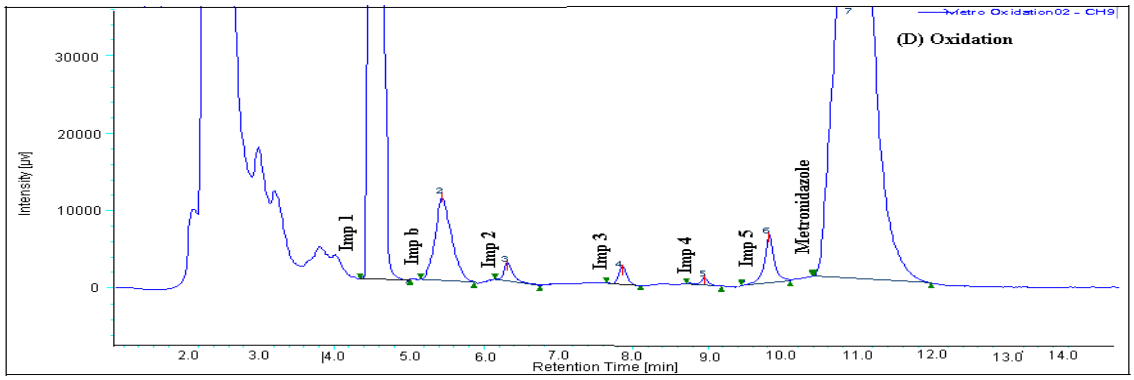

Fig. 3: (d) Sample degraded 3% H_2O_2

CONCLUSION

A novel liquid chromatographic method for the determination of related components in Metronidazole was developed after subjecting the samples to stress testing under ICH recommended conditions using Solvent-X as a mobile phase component. The RP-LC method developed for quantitative and related components determination of Metronidazole is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was found "specific" to the drug and for the dosage form, as the peaks of the degradation products did not interfere with the drug peak. Hence, the proposed method can be employed for assessing the stability of Metronidazole as a bulk drug and also for its dosage form.

Thus, it can be concluded that Solvent-X is an Ecofriendly replacement for ACN in RP-HPLC. Due to the various advantages of PC over ACN, viz. low vapour pressure and low clinical toxicity, cases of accidental fires and occupational hazards would be minimized if Solvent-X is used instead of ACN. Its high biodegradability would help industries in saving money spent on effluent treatment without any concern about environmental pollution. Thus, this work is expected to be of interest to scientists who are working in the field of developing cleaner and greener chemical technologies which is definitely the need of the hour.

REFERENCES

1. Baveja S.K., Khosla H.K. Decomposition of Metronidazole in Aqueous Solutions. Indian Journal Technology, 1975; 13: 528.
2. Akarim E.I., Ibrahim K.E., Adam, M.E. Studies on the Photochemical Decomposition of Metronidazole. International Journal Pharmaceutics, 1991; 6: 261-264.
3. Barnes A. R., Sudgen J.K. Assay of Photolysed Metronidazole Solution. Journal of Pharmacy and Pharmacology, 1985; 37 (supplement):10P.
4. Godfrey R., Edwards R. A Chromatographic and Spectroscopic Study of Photodegraded Metronidazole in Aqueous Solution. *Journal* of Pharmaceutical Science, 1991; 80: 212-218.
5. Habib M. J., Asker A.F. Complex Formation between Metronidazole and Sodium Urate: Effect of Photodegradation of Metronidazole. Pharmaceutical Research, 1989; 6: 58-61.
6. Marcinie B., Bugaj A., Kedziora W. Kinetic Studies of the Photodegradation of Nitroimidazole Derivatives in the solid state. Pharmazie, 1997; 52: 220-223.
7. Kendall A.T., Starck E., Sugden J.K. Effect of Hydroxyl Radicals on the stability of Metronidazole in Buffer Solution at pH 9.2, International Journal of Pharmaceutics, 1989; 57: 217-211.
8. Wilkins B. J., Grainsford G.J. Moore D.E.; Photolytic Rearrangement of Metronidazole to N-(2-Hydroxy ethyl)-5-methyl-1, 2, 4-oxadiazole-3-carboxamide. Crystal Structure of its 4-Nitrobenzoate Derivative. Journal of the Chemical Society, 1987; 8: 1817-1820.
9. Moore D.E., Wilkins, B.J. Common Products from Gamma-Radiolysis and Ultraviolet Photolysis of Metronidazole. Radiation Physics and Chemistry, 1990; 36: 547-550.
10. Devani M.B. Rapid Method of Analysis of Metronidazole and Tinidazole in Tablets. Indian Journal of Pharmaceutical Science, (1981); 43: 151-152.
11. Fabayo A.B., and Grudzinski S.K. Quality Control of Drug III. Quatative Determination of Metronidazole in Tablet by UV-Absorption Spectrophotometry. Acta Poloniae Pharmaceutical, (1985); 42: 49-54.
12. Patel Y. Separation and Estimation of Five Imidazoles by Packed Column Super-critical Fluid Chromatography. Analytica Chimica Acta, 1998; 362: 271-277.
13. Vega E. Validation of a Reversed Phase LC Method for Quantitative Analysis of Intravenous Admixtures of Ciprofloxacin and Metronidazole. Journal Pharmaceutical and Biomedical Analysis, 1999; 21: 1003-1009.
14. Ali M.S., Chaudhary R.S., Takeiddin M.A. Simultaneous Determination of Metronidazole Benzoate, Methylparaben, and

Propylparaben By High-Performance Liquid Chromatography Drug Development and Industrial Pharmacy, 1999; 25:1143-1147.

15. Sa'sa S. I., Khalil H.S., Jalal I.M. Determination of Metronidazole Benzoate in Liquid Preparations by Reversed Phase HPLC. Journal of Liquid Chromatography 1986; 9: 3617-3631.
16. Pashankov P.P., Kostova L.L. Reversed Phase High-Performance Liquid Chromatography of Metronidazole Benzoate in Suspension Dosage Form, Journal of Chromatography,1987; 394: 382-387.
17. Baveja S.K., and Rao A.V.R. Kinetics of Metronidazole Hydrolysis. Indian Journal of Technology, (1973); 11: 311-312.
18. BP (Bristish Pharmacopoeial Commission, Middlesex, 2002), 2318-2320 : pp. 1159-1160,.
19. Ebel S., Ledermann M., and Mummler B. Analytical Procedures in Stability Testing of Solutions of Metronidazole. Archives of Pharmacal Research, 1990; 323:195-200.
20. Mathew M., Das Gupta V., Bethea C. Stability of Metronidazole benzoate in suspensions, Journal of Clinical Pharmacy & Therapeutics, 1994; 19: 31-34.
21. Rovers J.P. Use of Stability-Indicating Assays to Determine the In Vitro Compatility and Stability of Metronidazole/Gentamicin Admixtures. The Candian Journal of Hospital Pharmacy, 1989; 42: 143-146.
22. Gupta V.D. Quantiation of Metronidazole in Pharmaceutical Dosage Forms Using High-Performance Liquid Chromatography. Journal of Pharmaceutical Science, 1984; 73: 1331-1333.
23. Verma P., Namboodiry N., Ashok B., and Suvarna, B. Significance of Propylene Carbonate as a Mobile Phase Component in Estimation of Aspirin and its Impurities Using RP-HPLC. International Journal of Research in Pharmacy and Science, 2011; 1(3):29-40.
24. Cione A. P., Tonhi E., and Silva P. Stability Indicating Method; Quality Control of Herbal Medicines and Related Areas, 25-36