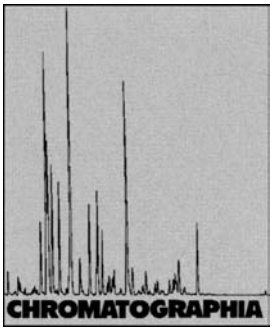
Stability-Indicating HPLC Method for the Determination of Metadoxine as Bulk Drug and in Pharmaceutical Dosage Form



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Abstract

A sensitive and reproducible method is described for the quantitative determination of metadoxine in the presence of its degradation products. The method was based on high performance liquid chromatographic separation of the drug from its degradation products on the reversed phase, kromasil column [C_{18} (5-micron, 25 cm × 4.6 mm, i.d.)] at ambient temperature using a mobile phase consisting of methanol and water (50: 50, *v/v*). Flow rate was 1.0 mL min^{-1} with an average operating pressure of 180 kg cm^{-2} and t_R was found to be 2.85 ± 0.05 min. Quantitation was achieved with UV detection at 286 nm based on peak area with linear calibration curves at concentration range 10–100 μg mL^{-1}. This method has been successively applied to pharmaceutical formulation. No chromatographic interference from the tablet excipients was found. The method was validated in terms of precision, robustness, recovery and limits of detection and quantitation. Drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, wet heat treatment and photo and UV degradation. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability indicating one. Moreover, the proposed HPLC method was utilized to investigate the kinetics of the acidic, alkaline and oxidative degradation processes at different temperatures and their respective apparent pseudo first order rare constant, half-life and activation energy was calculated with the help of Arrhenius plot. In addition the pH-rate profile of degradation of metadoxine in constant ionic strength buffer solutions with in the pH range 2–11 was studied.

Keywords

Column liquid chromatography
Stability testing
Bulk drug analysis
Metadoxine
Kinetics of degradation

Introduction

Metadoxine, pyridoxine L-2-pyrrolidone-5-carboxylate (Fig. 1) is an ion pair that combines pyridoxine (vitamin B6) and pyrrolidone carboxylate (or pyroglutamate, that is intermediate in the synthesis/degradation of glutathione). Both pyridoxine and pyroglutamate are well-known agents that are safe, naturally occurring substances, present in food, and are useful in the treatment of alcoholics and alcoholic liver disease [1–4]. Metadoxine exerts several actions that are beneficial to patients with alcoholic liver disease. It increases the clearance of alcohol and acetaldehyde, reduces the damaging effect of free radicals, restores ATP and glutathione levels, reduces steatosis and prevents liver fibrosis [5–10]. After thorough survey of literature there is not a single method reported for the analysis of metadoxine.

To our knowledge, no article related to the stability indicating chromatographic determination of metadoxine in pharmaceutical dosage form has been reported in literature. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [11]. Acidic, alkaline, oxidative and photolytic stability are required. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves

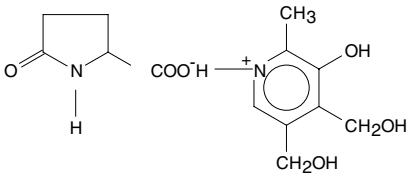

Fig. 1. Chemical structure of metadoxine

its degradation products. Consequently, the implementation of an analytic methodology to determine metadoxine in pharmaceutical dosage form in presence of its degradation products is a pending challenge of the pharmaceutical analysis. Therefore, it was thought necessary to study the stability of metadoxine towards acidic, alkaline, oxidative, UV and photo-degradation processes. The aim of this work was to develop stability-indicating HPLC method for determination of metadoxine in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its bulk dosage form. The proposed method is simple, accurate, specific, repeatable, stability indicating, reduces the duration of the analysis and suitable for routine determination of metadoxine in tablet dosage form. The proposed method was validated in compliance with ICH guidelines [12, 13] and its updated international convention [14]. Furthermore, the developed HPLC method was used to investigate the kinetics of the acidic, alkaline and oxidative degradation processes by quantitation of drug at different temperatures, and to calculate the activation energy and half-life for metadoxine degradation. The proposed HPLC method was also utilized for pH-rate profile study of degradation of metadoxine in constant ionic strength buffer solutions with in the pH range 2–11.

Experimental

Materials

Pharmaceutical grade of metadoxine (batch no: S 02-614) was kindly supplied as a gift sample by Sun Pharma Ltd, Baroda, India, used without further purification and certified to contain 99.72% (*w*/*w*) on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a pump (model jasco PU 1580, intelligent HPLC pump) with auto injecting facility (AS-1555 sampler) programmed at 20 µl capacity per injection was used. The detector consisted of a UV/ V is (Jasco UV 1575) model operated at a wavelength of 286 nm. The software used was jasco borwin version 1.5, LC-Net II/ADC system. The columns used were Kromasil C-18 (250 mm × 4.6 mm, 5.0 µ) Flexit Jour Laborarories Pvt Ltd Pune, India and Finepak SIL-5, C-18 (250 mm × 4.6 mm, 5.0 µ) Jasco Corporation, Japan. Different mobile phases were tested in order to find the best conditions for separation of metadoxine in presence of its degradation products. The optimal composition of the mobile phase was determined to be methanol: water (50: 50, *v*/*v*). The flow rate was set to 1.0 mL min^{-1} and UV detection was carried out at 286 nm. The mobile phase and samples were filtered using 0.45 µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

Standard Solutions and Calibration Graphs

Stock standard solution was prepared by dissolving 100 mg of metadoxine in 100 mL methanol (1000 µg mL^{-1}). The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 10–100 µg mL^{-1} for metadoxine. Triplicate 20 µl injections were made six times for each concentration and chromatographed under the conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample Preparation

To determine the content of metadoxine in conventional tablets (label claim: 500 mg metadoxine per tablet), the twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 500 mg metadoxine was weighed. Then equivalent weight of the drug was transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, milford, MA). The above stock solution was further diluted to get sample solutions at three different concentrations of 20, 40 and 60 µg mL^{-1} respectively. A 20-µL volume of each sample solution was injected into HPLC, six times, under the conditions described above. The peak area of the spots were measured at 286 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Method Validation

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of metadoxine was accurately weighed and assayed. System repeatability was determined by six replicate applications and six times measurement of a sample solution at the analytical concentration. The repeatability of sample injection and measurement of peak area for active compound were expressed in terms of % RSD (relative standard deviation) and SE (standard error) and found to be less than 1%. Method repeatability was obtained from RSD value by repeating the assay six times in same day for intra-day precision. Intermediate precision was assessed by the assay of two, six sample sets on different days (inter-day precision). The intra-day and inter-day variation for determination of metadoxine was carried out at three different concentration levels 20, 40, 60 µg mL^{-1} respectively.

Robustness of the Method

To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation of C_{18} columns from different manufacturers, flow rate, percentage of methanol in the mobile phase, column temperature and methanol of different lots. Two analytical columns, Kromasil C 18 column from Pune, India and Finepak C 18 column from Japan, were used during the experiment. Robustness of the method was done at three different concentration

levels 20, 40, 60 μg mL^{-1} for metadoxine respectively.

Limit of Detection and Limit of Quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at a signal to noise ratio (S/N) of 3 and 10. LOD and LOQ were experimentally verified by diluting known concentrations of metadoxine until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Specificity

The specificity of the HPLC method was determined by the complete separation of metadoxine in presence of its degradation products along with other parameters like retention time (t_r), capacity factor (k), tailing or asymmetrical factor (T) etc.

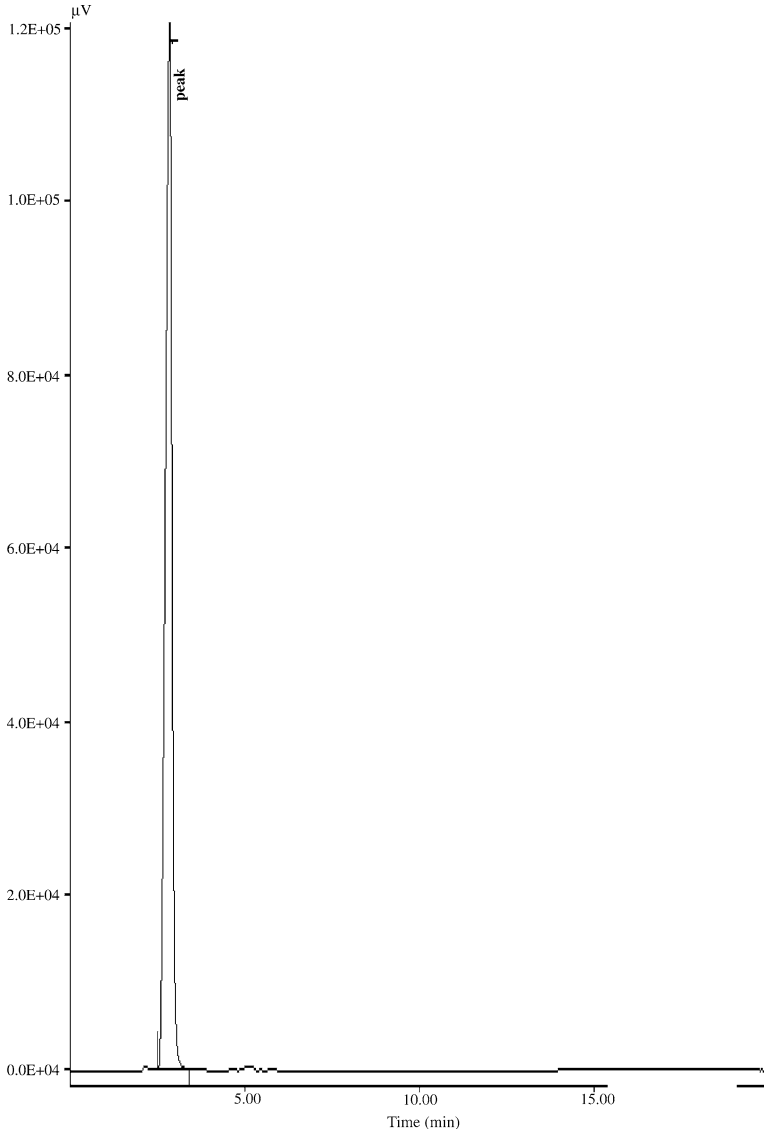

Fig. 2. Chromatogram of standard metadoxine (50 μg mL^{-1}); (t_R: 2.85 ± 0.05) measured at 286 nm, mobile phase: methanol and water (50: 50, v/v)

Recovery Studies

The recovery studies was carried out by applying the method to drug sample to which known amount of metadoxine corresponding to 80, 100 and 120% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

Forced Degradation of Metadoxine

A stock solution containing 100 mg metadoxine in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the proposed method. In all degradation studies the average peak area of metadoxine after application (100 μg mL^{-1}) of seven replicates was obtained.

Preparation of Acid and Base Induced-Degradation Product

To 10 mL of methanolic stock solution, 10 mL each of conc. HCl and 10.0 N NaOH were added separately. These mixtures were refluxed for 6.0 h at 70 °C. To study the degradation of drug in phosphate buffer pH 7.4, and 9.0, 10 mL of buffer solution was added to 10 mL of methanolic stock solution. It was refluxed at 70 °C for 2.0 h.The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. The resultant solution was diluted to obtain 100 μg mL^{-1} solution and 20 μL were injected into the system.

Preparation of Hydrogen Peroxide Induced-Degradation Product

To 10 mL of methanolic stock solution, 10 mL of 50% w/v of hydrogen peroxide was added. The solution was refluxed for 8.0 h at 70 °C and then heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide. The resultant solution was diluted to

Table 1. Linear regression data for the calibration curves[a]

Linearity range (ng spot^{-1})	r^2 ± S.D	Slope ± S.D	Confidence limit of slope[b]	Intercept ± S.D	Confidence of intercept[b]
10–60	0.9998 ± 0.65	0.96 ± 0.14	0.85–1.07	3.35 ± 1.41	2.22–4.48
10–100	0.9992 ± 1.26	1.57 ± 0.24	1.38–1.76	4.49 ± 1.50	3.29–5.69

[a]$n = 6$
[b]95% confidence limit.

Table 2. Two-way ANOVA test of slopes in six independent samples in duplicate for two sets of standard calibration curves.

S.No.	Set 1		Set 2	
	1st Sample	2nd Sample	1st Sample	2nd Sample
1	1.02	0.99	0.92	1.03
2	1.00	0.94	0.99	0.91
3	0.94	1.10	1.01	0.93
4	0.96	1.03	0.95	1.10
5	0.98	0.91	1.00	0.92
6	0.93	0.95	0.98	1.02

ANOVA: Two-Factor With Replication

SUMMARY	Set 1	Set 2	Total
Sample 1			
Count	6	6	12
Sum	5.83	5.85	11.68
Average	0.9716666	0.975	0.973333333
Variance	0.0012166	0.00115	0.001078788
Sample 2			
Count	6	6	12
Sum	5.92	5.91	11.83
Average	0.9866666	0.985	0.985833333
variance	0.0048266	0.00587	0.004862879
Total			
Count	12	12	
Sum	11.75	11.76	
Average	0.9791666	0.98	
Variance	0.0028083	0.0032181	

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.0009375	1	0.0009375	0.287063026	0.598017391	4.351250027
Columns	4.166E-06	1	4.16667E-06	0.001275836	0.971860651	4.351250027
Interaction	3.75E-05	1	3.75E-05	0.011482521	0.915732439	4.351250027
Within	0.0653166	20	0.003265833			
Total	0.0662958	23				

[a]$F_{stat} < F_{crit}$.

obtain 100 μg mL^{-1} solution and 20 μL were injected into the system.

Dry Heat and Wet Heat Degradation Product

The standard drug was placed in oven at 100 °C for 4.0 h to study dry heat degradation and the stock solution was refluxed for 2.0 h on boiling water bath for wet heat degradation.

Photochemical and UV degradation product

The photochemical stability of the drug was also studied by exposing the stock solution (1 mg mL^{-1}) to direct sunlight for 4 days on a wooden plank and kept on terrace. For UV degradation study, the solution was exposed to UV radiation for 8 days. The solution was diluted to 100 μg mL^{-1} and then 20 μL of the solution was injected into the system.

Neutral Hydrolysis

To 10 mL of methanolic stock solution, 10 mL double distilled water was added and the mixture was refluxed for 2.0 h at 70 °C to study the degradation under neutral conditions.

Detection of the Related Impurities

To determine the related unknown impurity associated with metadoxine using HPLC, triplicate 20 μL of sample solution (500 μg mL^{-1}) and standard solution (5 μg mL^{-1}) were injected and their respective areas were correlated.

Kinetic Investigation

Accurately weighed 100 mg of drug was dissolved in 100 mL methanol. Separate 15 mL aliquots of this standard solution were transferred into separate 100 mL of double neck round bottom flask and mixed respectively with 15.0 mL of 10.0 N NaOH, conc. HCl and 50% *w*/*v* hydrogen peroxide to get final concentration of 500 μg mL^{-1}. The flasks were refluxed at different temperatures (40, 50, 60, 70, 80 and 90 °C) for acidic, basic and oxidative degradation for different time intervals. At the specified time the contents of the flask were neutralized to pH 7.0 using predetermined volumes of 10.0 N HCl and 10.0 N NaOH and for oxidative degradation the excess of hydrogen peroxide was removed by heating on water bath. The contents of the flasks (1.0 mL each) were quantitatively transferred to 10 mL volumetric flasks with the help of microsyringe and appropriately diluted to volume with methanol to obtain the concentration of 50 μg mL^{-1} and estimated by HPLC method by one point standardization using external standard. Each experiment was repeated three times at each temperature and time interval. Aliquots of 20 μL of each solution were chromatographed under the conditions described above and the concentration of the remaining metadoxine was calculated at each temperature and at time interval for the three replicates. Data was further processed and degradation kinetics constants were calculated.

pH Rate Profile

Accurately weighed 100 mg of metadoxine were transferred into 100 mL volumetric flask and diluted to volume with constant ionic strength buffer solutions prepared as described in Indian Pharmacopoeia [15], to obtain concentration of 1000 μg mL^{-1}. The pH-values of buffer solutions used for the measurement of the pH-rate profile of the degradation of metadoxine were as follows., pH 1.8, 2.8, 3.8, 4.6, 5.7, 6.8, 8.0, 9.2, 9.7 and 10.8. The pH values of these buffer solutions were checked before and after the reaction and were unchanged. The ionic strength of these buffer solutions was adjusted with sodium chloride. Separate 20 mL aliquots of the buffer solution containing metadoxine were transferred into separate stoppered round-bottomed flasks. The flasks were then refluxed at 70 °C for different time intervals. At the specified time interval the contents of the flasks were neutralized to pH 7.0 using 5.0 N sodium hydroxide or 5.0 N hydrochloric acid solutions. The contents of the flasks (2.0 mL each) were transferred into 50 mL volumetric flasks and diluted to volume with mobile phase to obtain the concentration of 40 μg mL^{-1}. Aliquots of 20 μL of each solution were chromatographed under the conditions described above and the concentration of the remaining metadoxine was calculated at each pH value and time interval.

Results and Discussion

Optimization of Procedures

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Pure drug along with its degraded products were injected and run in different solvent systems. Initially methanol and water in different ratios were tried. It was found that methanol: water in ratio of 50: 50, *v*/*v* gave acceptable retention time (t_R = 2.85 min) at the flow rate of 1.0 mL min^{-1} and drug showed typical peak nature at 286 nm (Fig. 2). Tailing factor for metadoxine peak was less than 2% and the resolution of standard in presence of degradation products was satisfactory. Ultimately mobile phase consisting of methanol and water (50: 50, *v*/*v*) was selected for validation purpose and stability studies.

Table 3. Robustness evaluation[a] of the HPLC method (n = 6)

Chromatographic changes		t_r[c]	k[d]	T[e]
Factor[b]	Level			
A: Flow Rate (ml min^{-1})				
0.90	−1	2.95	2.18	1.41
1.00	0	2.85	2.15	1.38
1.10	1	2.75	2.12	1.26
Mean ± S.D (n = 6)		2.85 ± 0.10	2.15 ± 0.03	1.35 ± 0.07
B: %age of methanol in the mobile phase (*v*/*v*)				
48	−1	2.91	2.12	1.41
50	0	2.85	2.15	1.38
52	−1	2.78	2.17	1.24
Mean ± S.D (n = 6)		2.85 ± 0.07	2.15 ± 0.03	1.34 ± 0.09
C: Temperature				
24	−1	2.87	2.19	1.43
25	0	2.85	2.15	1.38
26	1	2.81	2.11	1.36
Mean ± S.D (n = 6)		2.84 ± 0.03	2.15 ± 0.04	1.39 ± 0.04
D: Columns from different Manufacturers				
Kromasil		2.85	2.15	1.38
Finepak		2.83	2.17	1.37
Mean ± S.D (n = 6)		2.84 ± 0.01	2.16 ± 0.01	1.38 ± 0.01
E: Solvents of different lots				
First lot		2.85	2.15	1.38
Second Lot		2.83	2.18	1.34
Mean ± S.D (n = 6)		2.84 ± 0.01	2.16 ± 0.02	1.36 ± 0.03

[a]average of three concentrations 20, 40, 60 μg mL^{-1}.
[b]Four factors were slightly changed at three levels (1, 0, −1); each time a factor was changed from level (0) the other factors remained at level (0).
[c]Retention time.
[d]Retention factor.
[e]Tailing factor.

Linearity

Metadoxine showed good correlation coefficient in concentration range of 10–100 μg mL^{-1} (r^2 = 0.9992 ± 1.26). Linearity was evaluated by determining six standard working solutions containing 10-60 μg mL^{-1} twice in triplicate (Table 1). The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient and the S.D. for intercept value was less than 2%. For the proposed method no significant difference was observed in the slopes of standard curves (ANOVA; $p < 0.05$) (Table 2).

Precision

The within-run precision and between-run precision of the proposed HPLC method were determined by assaying the tablets in six times per day for consecutive six days and expressed as % RSD. The intra-day and inter-day precision has been found to be 0.97 and 1.25 respectively.

Robustness of the Method

Each factor selected (except columns from different manufacturers and solvents of different lots) to examine were charged at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of six chromatographic parameters (factors). Results, presented in Table 3 indicate that the selected factors remained unaffected by small variations of these parameters. The results from the two columns indicated that there is no significant difference between the results from the two columns. It was also found that methanol of different lots from the same manufacturer had no significant influence on the determination. Insignificant differences in peak areas and less variability in retention time were observed.

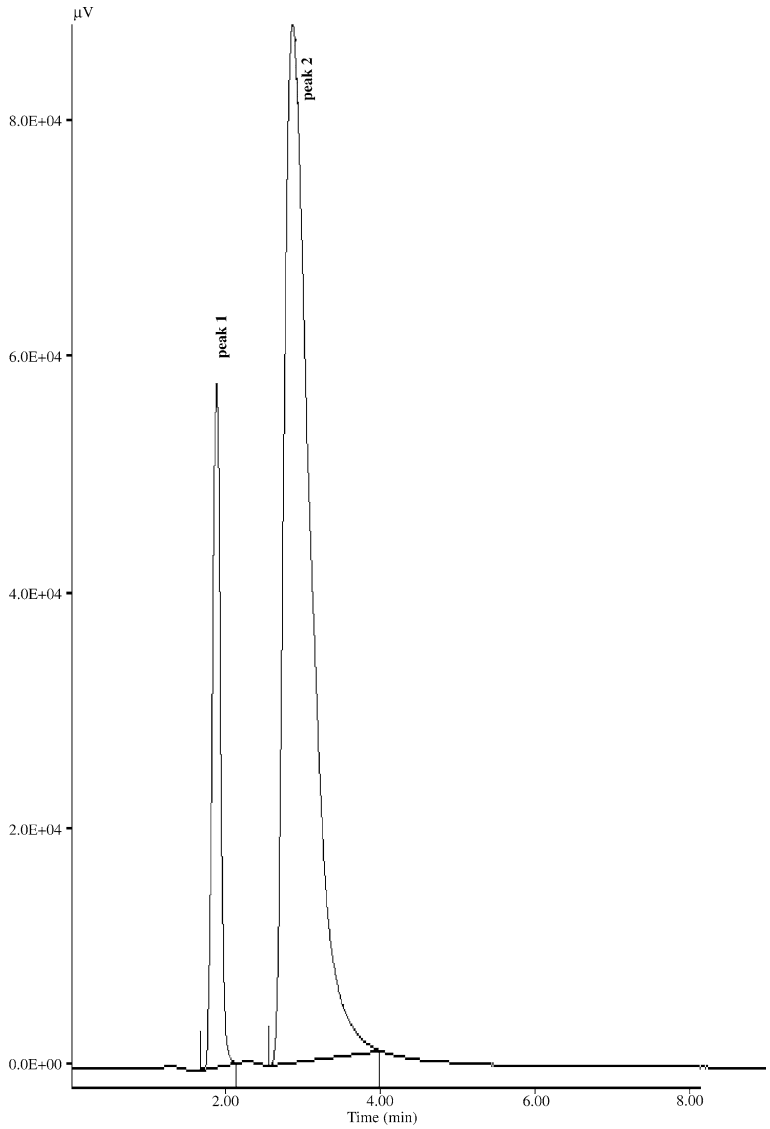

Fig. 3. Chromatogram of buffer (pH 9.0, reflux for 2.0 h, temp 70 °C) treated metadoxine (100 µg mL^{-1}). Peaks: *1* (degraded) (t_R: 1.90 min), *2* (metadoxine) (t_R: 2.85 min)

Table 4. Summary of validation parameters

Parameter	Data
Linearity range	10–60 µg mL^{-1}
Correlation coefficient	0.9998 ± 0.65
Limit of detection	0.15 µg mL^{-1}
Limit of quantitation	0.40 µg mL^{-1}
Recovery (n = 6)	99.45 ± 1.12
Precision (% RSD)	
Inter-day (n = 6)	1.25
Intra-day (n = 6)	0.97
Robustness	Robust
Specificity	Specific

LOD and LOQ

The signal to noise ratios 3:1 and 10:1 were considered as LOD and LOQ respectively. The LOD and LOQ were found to be 0.15 µg mL^{-1} and 0.40 µg mL^{-1} respectively for metadoxine.

Specificity

The specificity of the HPLC method is illustrated in Figs. 3 and 4 where complete separation of metadoxine in presence of its degradation products was noticed. The average retention time ± 0.05, for six replicates. The peaks obtained were sharp and have clear baseline separation.

Recovery Studies

The proposed method when used for extraction and subsequent estimation of metadoxine from pharmaceutical dosage form after spiking with additional drug afforded recovery between 98–102% and mean recovery for metadoxine from the marketed formulation was found to be 99.45 ± 1.12.

Stability in Sample Solution

Three different concentrations of metadoxine, 20, 40 and 60 µg mL^{-1} were prepared from sample solution and stored at room temperature for 3 days. They were then injected into the HPLC system and no additional peak was found in the chromatogram indicating the stability of metadoxine in the sample solution. The mean areas with % RSD at 20, 40 and 60 µg mL^{-1} were found to be 310175.75 (1.05), 622133.57 (1.41) and 939527.04 (1.23) respectively

Analysis of the Marketed Formulation

The peaks at t_R 2.85 min for metadoxine was observed in the chromatogram of the drug samples extracted from tablets. Experimental results of the amount of metadoxine 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. The drug content was found to be 99.75% ± 1.41 (% RSD of 1.24) for metadoxine.

The data of summary of validation parameters are listed in Table 4.

Stability Indicating Property

Acid and Base Induced-Degradation Product

The chromatograms of the acid and base degraded sample showed one additional peak at t_r 4.50 and 1.80 min respectively. The chromatogram of the phosphate buffer degraded sample showed additional peak at t_R 1.90 min for pH 7.4 and 9.0 respectively (Fig. 3). The concentration of the drug was found to be changing from the initial concentration indicating that metadoxine undergoes degradation under acidic and basic conditions.

Hydrogen Peroxide Induced-Degradation Product

The sample degraded with 50.0% w/v hydrogen peroxide showed three additional peaks at t_r 1.85, 2.50, 3.66 min.

Dry Heat and Wet Heat Degradation Product

The samples degraded under dry heat condition showed additional peak at t_R 1.93 min. The samples degraded under wet heat conditions showed additional peak at t_R 1.82 min The peak of degraded products were well resolved from the standard peak.

Photochemical and UV Degradation Product

The photo and UV degraded sample showed one additional peaks at t_R 1.89, 2.36 min when drug solution was left in day light for 4 and 8 days respectively.

Neutral Degradation

The HPLC chromatogram for neutral degradation showed decrease in peak area of standard with corresponding rise in new peak at t_R 1.78 min.

This indicates that the drug is susceptible to acid-base hydrolysis, oxidation dry and wet heat degradation and photo degradation. The results are listed in Table 5.

Table 5. Degradation of metadoxine

Condition	Time (h)	%Recovery	t_R (min) value of degradation products
Acid conc. HCl, ref* (70 °C)	6.0	8.03	4.50
Base 10.0 N NaOH, ref	6.0	18.99	1.80
Phosphate buffer (pH 7.4), ref	2.0	96.19	1.90
Phosphate buffer (pH 9.0), ref	2.0	84.34	1.90
H_2O_2 50.0% *w/v*, ref	8.0	1.39	1.85, 2.50, 3.66
Dry Heat (100 °C)	4.0	99.79	1.93
Wet Heat, ref (100 °C)	2.0	99.41	1.82
Day light (25 °C)	96.0	0.0	1.89, 2.36
UV light	192.0	99.13	1.89, 2.36
Neutral (70 °C), ref	2.0	99.44	1.78

*refluxed.

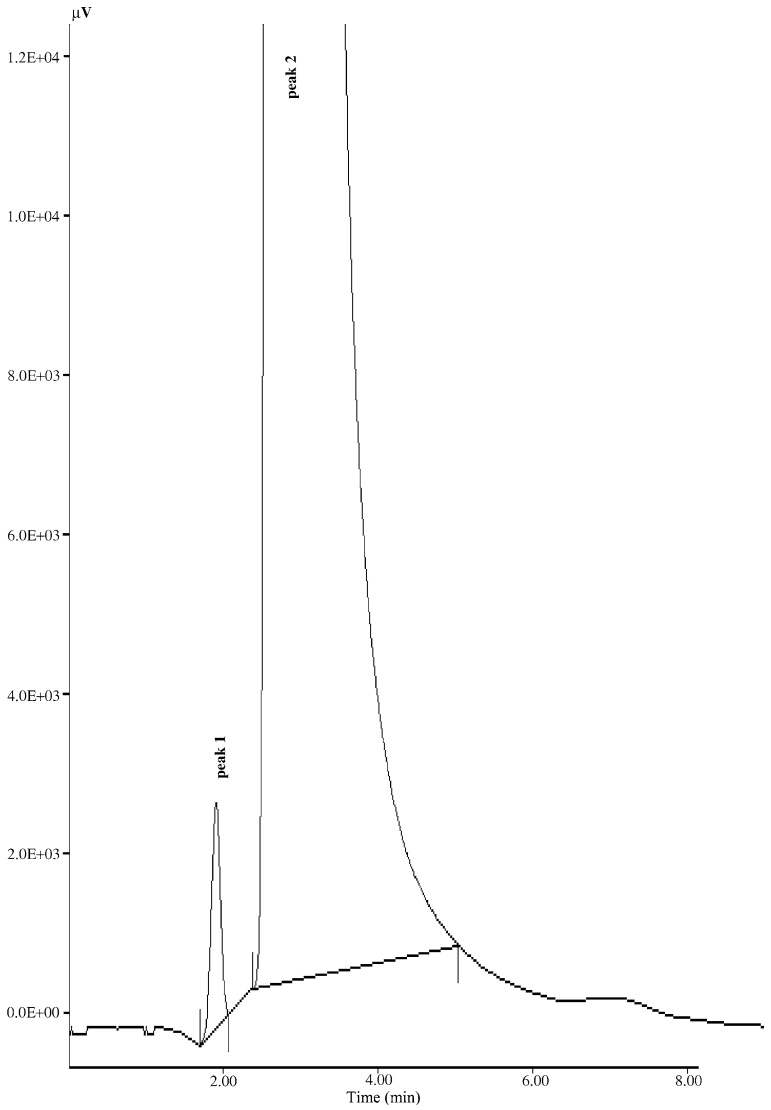

Fig. 4. Chromatogram of metadoxine and its unknown impurity (500 µg mL^{-1}). Peaks: *1* (impurity) (t_R: 1.90 min), *2* (metadoxine) (t_R: 2.85 min)

Detection of the Related Impurities

While injecting higher concentration of standard metadoxine drug solution (500 µg mL^{-1}) in triplicate, an additional peak was observed at t_R 1.90 min, which was considered as unknown impurity associated with metadoxine (Fig. 4). The area of the additional peak (peak area = 21461.50) was found to be much less as compared to the standard solution (103210.83). The amount of impurity was found to be 0.14%.

From Table 5, it can be observed that the t_R of phosphate buffer pH 7.4 and 9.0, dry heat and first component of peroxide, sunlight and UV degraded product matches with the t_R of unknown impurity. Therefore it might be possible that during processing, transaction or storage the drug may have undergone exposure to hydrolysis or oxidation.

Degradation Kinetics

The kinetic of degradation of metadoxine was investigated in 10.0 N NaOH, conc. HCl and 50.0% *w/v* hydrogen peroxide, since the decomposition rate of metadoxine at lower strength of NaOH, HCl and hydrogen peroxide was too slow to obtain reliable kinetic data. Each experiment was repeated three times at each temperature and time interval. The mean concentration of metadoxine was calculated for each experiment. A regular decrease in the concentration of meta-

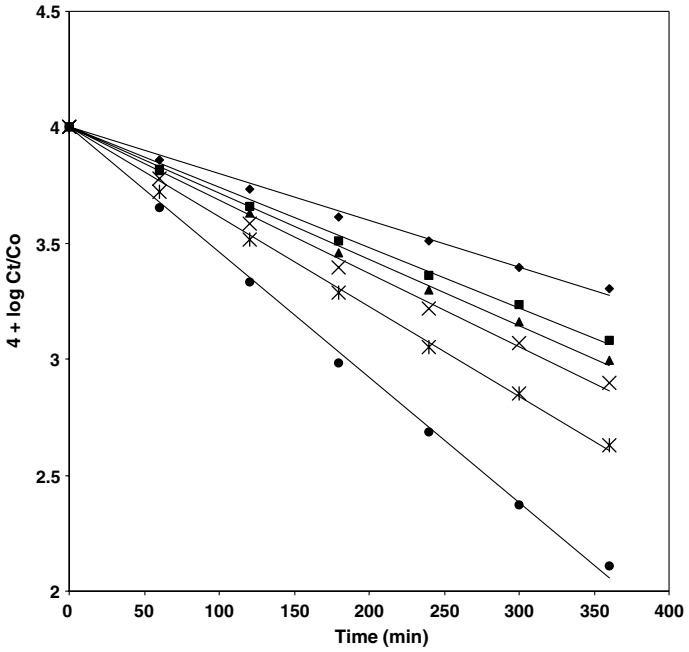

Fig. 5. Pseudo first-order plots for the degradation of metadoxine with conc. HCl at various temperatures using HPLC method. Key: 90 °C (•), 80 °C (*), 70 °C (×), 60 °C (■), 50 °C (▲), 40 °C (♦), C_t = concentration at time t; C_0 = concentration at time zero

Table 6. Degradation rate constant (K_{obs}), half-life ($t_{1/2}$) and t_{90} for metadoxine in presence of conc. HCl, 10.0 N NaOH and 50% w/v H_2O_2 determined by HPLC method

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)
In Conc. Hydrochloric acid			
40	0.0046	2.51	0.38
50	0.0057	2.03	0.31
60	0.0067	1.72	0.26
70	0.0080	1.44	0.22
80	0.0095	1.22	0.18
90	0.0118	0.98	0.15
In 10.0 N Sodium hydroxide			
40	0.0032	3.61	0.54
50	0.0041	2.82	0.42
60	0.0052	2.22	0.33
70	0.0064	1.80	0.27
80	0.0075	1.54	0.23
90	0.0089	1.29	0.19
In 50% w/v Hydrogen peroxide			
40	0.0021	5.50	0.83
50	0.0027	4.28	0.65
60	0.0035	3.30	0.50
70	0.0053	2.18	0.33
80	0.0069	1.67	0.25
90	0.010	1.16	0.18

doxine with increasing time intervals was observed. At the selected temperatures (40, 50, 60, 70, 80 and 90 °C for acidic, alkaline and oxidative degradation) the degradation process followed pseudo-first order kinetic (Fig. 5–7). From the slopes of the straight lines it was possible to calculate apparent first degradation rate constant, half life ($t_{1/2}$) and t_{90} (i.e., time where 90% of original concentration of the drug is left) at each temperature for acidic, alkaline and oxidative degradation processes determined by HPLC method (Table 6). Data obtained from first order kinetics treatment was further subjected to fitting in Arrhenius equation;

$$Log\ K = Log\ A - E_a/2.303RT \qquad (1)$$

Where K is rate constant, A is frequency factor, E_a is energy of activation (Cal mol^{-1}), R is gas constant (1.987 Calories deg^{-1} mol^{-1}) and T is absolute temperature (K). A plot of (2 + log K_{obs}) values versus ($1/T \times 10^3$), the Arrhenius plot was obtained (Fig. 8), which was found to be linear in the temp range 40 °C to 90 °C. The activation energy the Arrhenius frequency factor was calculated respectively for acidic, alkaline and oxidative degradation processes determined by HPLC method. The method of accelerating testing of pharmaceutical products based on principles of chemical kinetics was used to obtain a measure of the stability of the drug under said conditions [16, 17]. The degradation rate constant at room temperature (K_{25}) is obtained by extrapolating to 25 °C (where $1000/T = 3.356$) by inserting this into equation 1 and $t_{1/2}$ and t_{90} are calculated respectively for different stress conditions (Table 7).

Table 7. Summary of degradation kinetic data at 25 °C using HPLC method

Parameters	In conc. HCl	In 10.0 N NaOH	In 50 % w/v H_2O_2
E_a(Kcal deg^{-1} mol^{-1})[a]	4.14×10^{-3}	4.69×10^{-3}	7.11×10^{-3}
K_{25} (h^{-1})[b]	2.07×10^{-2}	2.19×10^{-2}	1.08×10^{-2}
$t_{1/2}$ (h)[c]	33.47	31.60	64.46
t_{90} (h)[d]	5.07	4.79	9.67
A[e]	374.50	16.74	488.65

a = Activation energy
b = Degradation rate constant
c = Half life
d = Time left for 90 % potency
e = Arrhenius frequency factor

The pH-rate profile of degradation of metadoxine in constant ionic strength buffer solutions was studied at 70 °C using HPLC method (Fig. 9). The apparent first order degradation rate constant and the half-life were calculated for each pH value (Table 8). The pH-rate profile study shows that the metadoxine was found to be most stable at pH of 6.8.

Table 8. Degradation rate constant (K_{obs}), half-life ($t_{1/2}$) and t_{90} for metadoxine in constant ionic strength buffer at different pH values and a temperature of 70 °C

pH	K_{obs} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)
1.8	0.0032	3.61	0.55
2.8	0.0039	2.96	0.45
3.8	0.0027	4.28	0.64
4.6	0.0025	4.62	0.70
5.7	0.0029	3.98	0.60
6.8	0.0021	5.50	0.83
8.0	0.0032	3.61	0.55
9.2	0.0036	3.21	0.48
9.7	0.0029	3.98	0.60
10.8	0.0041	2.81	0.43

Conclusion

The proposed HPLC method provide simple, accurate, reproducible and stability indicating for quantitative analysis for determination of metadoxine in pharmaceutical tablets, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative and photolytic degradation products. The chromatographic method was

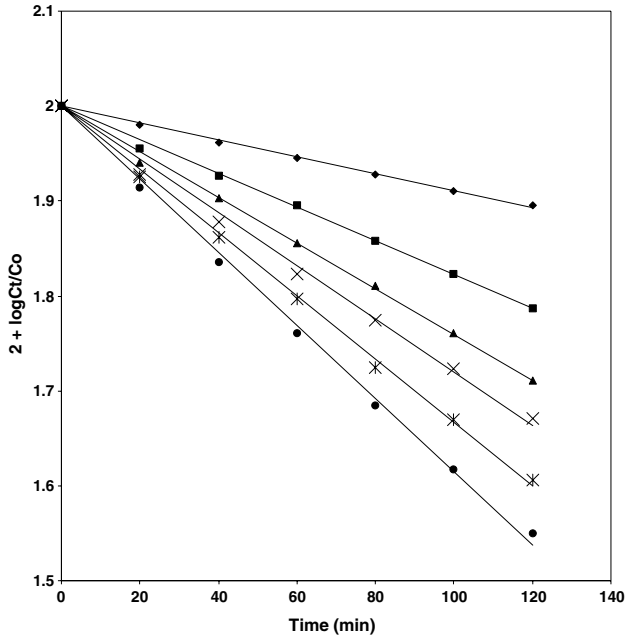

Fig. 6. Pseudo first–order plots for the degradation of metadoxine with 10.0 N NaOH at various temperatures using HPLC method. Key: 90 °C (●), 80 °C (*), 70 °C (x), 60 °C (▲), 50 °C (■), 40 °C (♦), C_t, concentration at time t; C_0, concentration at time zero

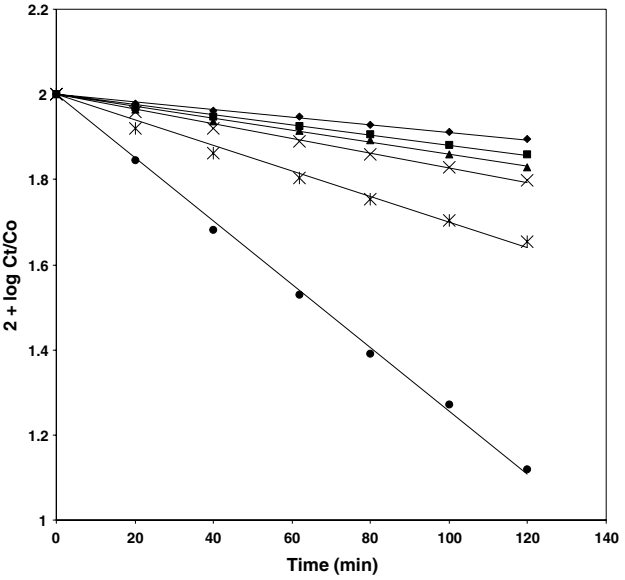

Fig. 7. Pseudo first–order plots for the degradation of metadoxine with 50.0% *w*/*v* hydrogen peroxide at various temperatures using HPLC method. Key: 90 °C (\blackcircle), 80 °C (*), 70 °C (x), 60 °C (▲), 50 °C (■), 40 °C (♦), C_t, concentration at time t; C_0, concentration at time zero

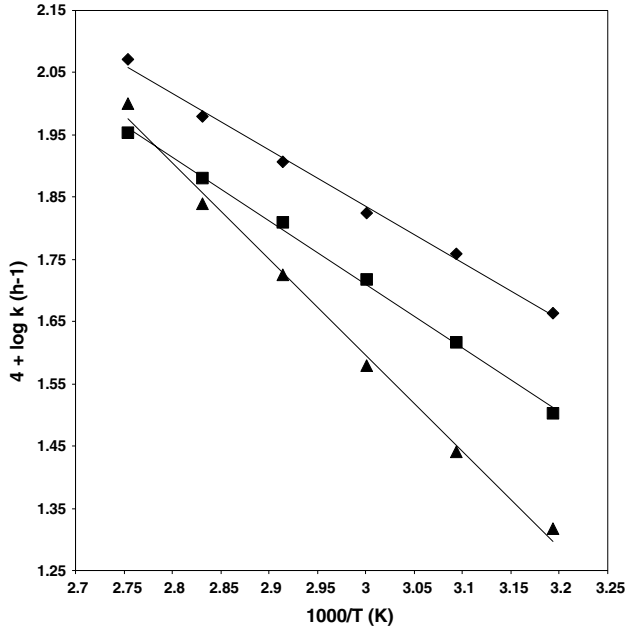

Fig. 8. Arrhenius plot for the degradation of metadoxine in presence of conc. HCl (♦), 10.0 N NaOH (■) and 50% *w*/*v* hydrogen peroxide (▲)

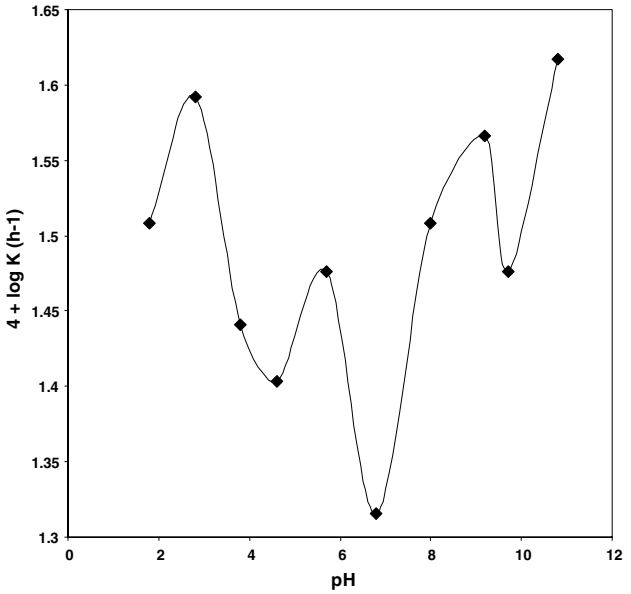

Fig. 9. pH-rate profile for the decomposition of metadoxine at constant ionic strength and 70 °C

validated as per ICH guidelines. The proposed HPLC method reduce the duration of analysis and appear to be suitable for routine determination of metadoxine in pharmaceutical formulation in quality control laboratories, where economy and time are essential. This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities and also in stability studies. It is proposed for the analysis of the drug and degradation products in stability samples in industry. The above results showed the suitability of proposed HPLC method for acid, base and peroxide induced

degradation kinetic study of metadoxine. The degradation rate constant, half-life and t_{90} of metadoxine can be predicted under different stress conditions. The stability of metadoxine was found to be at pH 6.8. It may be extended for quantitative estimation of said drug in plasma and other biological fluids. The method, however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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