Kinetics study of metaxalone degradation under hydrolytic, oxidative and thermal stress conditions using stability-indicating HPLC method

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Abstract An isocratic stability indicating RP-HPLC–UV method is presented for the determination of metaxalone (MET) in the presence of its degradation products. The method uses Dr. Maisch C18 column (250 mm × 4.6 mm, 5 μm) with mobile phase consisting of acetonitrile–potassium dihydrogen orthophosphate buffer with 4 mL of 0.4% triethyl amine (pH 3.0; 10 mM) (58:42, v/v) at a flow rate of 1.0 mL/min. pH of the buffer was adjusted with o-phosphoric acid. UV detection was performed at 225 nm. The method was validated for specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness. The calibration plot was linear over the concentration range of 1–100 μg/mL having a correlation coefficient ($r^2$) of 0.999. Limits of detection and quantification were 0.3 and 1 μg/mL, respectively. Intra-day and inter-day precision (% RSD) was 0.65 and 0.79 respectively. The proposed method was used to investigate the degradation kinetics of MET under different stress conditions employed. Degradation of MET followed a pseudo-first-order kinetics, and rate constant ($K$), time left for 50% potency ($t_{1/2}$), and time left for 90% potency ($t_{90}$) were calculated.

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1. Introduction

Metaxalone (MET) is a muscle relaxant used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions. Chemically, it is 5-(3,5-dimethylphenoxy methyl)-2-oxazolidinone (Fig. 1) [1]. The first published report about the use of MET as a skeletal muscle relaxant appeared in the 1960s [2]. The mechanism of action of MET in humans has not been established, but may be due to general central...
nervous system depression. MET has no direct action on the contractile mechanism of striated muscle, the motor end plate, or the nerve fiber. MET is metabolized by the liver and excreted in the urine as unidentified metabolites. Hepatic cytochrome P450 enzymes play a role in the metabolism of MET [3].

Literature survey revealed that, RP-HPLC [4–8], LC-MS [9], HPTLC [10] and UV spectrophotometric methods [11,12] are available for the estimation of MET alone or in combination with diclofenac sodium. Among them two methods are stability indicating methods, in which limited stress conditions were applied. In the modern analytical laboratory, there is always need for significant stability-indicating methods (SIMs) of analysis. Environmental factors, such as temperature, pH, buffer species, ionic strength, light, oxygen, moisture, additives and excipients, can play an important role in the stability of drug substances. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances. With the advent of the International Conference on Harmonization (ICH) guidelines [14], requirements for the establishment of SIMs have become more clearly mandated. Moreover, kinetic studies and accelerated stability experiments are important to solve problems encountered in quality control and to predict the expiry dates of pharmaceutical products. The scientific novelty of the present work is that the suggested method represents the first kinetics study of MET degradation using HPLC. In addition, it is more efficient and accurate than the reported HPLC methods. Suitability of the proposed method for determination of diclofenac potassium (which is found in combination drug products) is also tested.

2. Experimental

2.1. Chemicals and reagents

Pharmaceutical grade metaxalone was gifted by Dr. Reddy’s Laboratories Ltd. (Hyderabad, India). Methanol and acetonitrile (HPLC grade) were purchased from Merck Chemical Company (India). Potassium dihydrogen orthophosphate, o-phosphoric acid, hydrochloric acid, sodium hydroxide and 30% hydrogen peroxide used were of analytical grade and purchased from S D Fine Chem. Ltd. (Mumbai, India). Buffer was prepared by dissolving 1360 mg (10 mM) of potassium dihydrogen orthophosphate in 980 mL of HPLC grade water, and 4 mL of triethylamine (0.4%) was added, pH was adjusted to 3.0 with o-phosphoric acid and made up to 1 L with HPLC grade water.

2.2. HPLC instrumentation and chromatographic conditions

The HPLC system consisted of two pumps (Analytical Technologies P2230 HPLC pump), a manual injector with 20 μL capacity per injection, and a temperature-controlled column oven. The UV–vis detector (Analytical Technologies UV 2230) was operated at a wavelength of 225 nm. The software used was chromatography workstation A-2000, version 1.6. Columns used were Lichrospher C 18, 250 mm × 4.6 mm, 5.0 μm (Merck, Germany), Atlantis C 18, 250 mm × 4.6 mm, 5.0 μm (Waters Corporation, USA) and Dr. Maisch C-18, 250 mm × 4.6 mm, 5.0 μm (GMBH, Germany).

Chromatographic separation of MET was achieved at temperature 25 ± 2 °C using a Dr. Maisch RP C18 (250 mm × 4.6 mm, 5 μm) analytical column; the mobile phase consisted of acetonitrile–potassium dihydrogen orthophosphate buffer with 4 mL of triethyl amine (0.4%; pH 3.0; 10 mM; 58:42, v/v) at a flow rate of 1.0 mL/min. pH of buffer was adjusted with o-phosphoric acid. Before use, the mobile phase was filtered through a 0.22 μm nylon membrane filter and sonicated for 15 min. Injection volume was 20 μL, and the optimum wavelength selected for quantification was 225 nm.

2.3. Construction of the calibration curve

Standard stock solution of MET was prepared in methanol at a concentration of 10 mg/mL and further diluted with the mobile phase to furnish working standard stock solution of 100 μg/mL. The working standard stock solution was diluted with the mobile phase to prepare calibration samples in the concentration range of 1–100 μg/mL. Triplicate injections of 20 μL were made for each calibration sample and chromatographed under the specified HPLC conditions described previously. Peak areas were plotted against the corresponding concentration to obtain the calibration curve.

2.4. Forced degradation of MET

2.4.1. Hydrolytic conditions: acid, alkali and water induced degradation

One milliliter of the standard stock solution was transferred to each of three 10 mL volumetric flasks and the volume was made up to the mark with 5 M HCl, 0.01 M NaOH, and water separately. These were kept at 60 °C on a thermostatic water bath for 6 days.

2.4.2. Oxidizing conditions: hydrogen peroxide-induced degradation

One milliliter of the standard stock solution was transferred to a 10 mL volumetric flask and the volume was made up to the mark with 10% H₂O₂. This was kept at room temperature for 6 days.

2.4.3. Thermal conditions: dry heat induced degradation

One milliliter of the standard stock solution was transferred to a 10 mL volumetric flask and the volume was made up to the mark with methanol. This was kept at 60 °C in an oven for 6 days.

2.5. Sample collection, storage and preparation

Before collecting samples, the volume was made up to the mark with respective solvent. 200 μL of the sample was collected at 0th, 1st, 2nd, 3rd, 4th, 5th and 6th day. The samples from acid and base induced degradation were neutralized by adding 200 μL of appropriate strength of NaOH and HCl. All samples were stored at 2–8 °C in the refrigerator. On the day of analysis samples were diluted with the mobile
phase up to 10 mL, filtered with a 0.22 μm membrane syringe filter and injected three times for each sample into HPLC.

3. Results and discussion

3.1. HPLC method development and optimization

The UV absorption spectrum of MET shows absorption maxima at 219 and 279 nm; the response at 219 nm was higher than that of 279 nm, but detection wavelength of 225 nm was selected for chromatographic monitoring to reduce the base-line noise at 219 nm and to get the better response than from monitoring at 279 nm. For HPLC method development initially the mobile phase used was acetonitrile and water at different proportions, but the peak shape was not good, so water was replaced by phosphate buffer. Different molar concentrations of the phosphate buffers were prepared in the range of 10–25 mM and tested; there was no difference in the chromatographic behavior of MET in the tested range and the tailing factor observed was 2.57. pH of the buffer was also varied between 2.5 and 6.5 and peak shape was improved at pH 3.0, so that it was selected. For further improvement of peak shape, triethyl amine was tested at different concentration levels (0.1–0.5%) and it was found that 4 mL of 0.4% of triethyl amine was optimum, which resulted in a tailing factor of 1.62. Finally the optimized mobile phase composition for MET was acetonitrile–potassium dihydrogen orthophosphate buffer with 4 mL of triethyl amine (0.4%; pH 3.0; 10 mM; 58:42, v/v) at a flow rate of 1.0 mL/min. A model chromatogram for the standard is shown in Fig. 2A.

3.2. Solution stability

The stability of MET in the mobile phase was investigated by analyzing the standard of MET (20 μg/mL) at 0, 3, 6, 9, 12 and 24 h. No significant variation in the peak area of standard solution was observed (RSD=1.52%) and also no additional peaks were found in the chromatogram, indicating that the MET was stable in the mobile phase.

3.3. Method validation

To confirm the suitability of the method for its intended purpose, the method was validated in accordance with ICH guidelines [15], for system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, specificity and robustness.

3.3.1. System suitability

System-suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. Retention time (Rt), capacity factor (k), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 20 μg/mL. The results presented in Table 1 are within the acceptable limits.

3.3.2. Linearity

Linearity of the proposed method was evaluated according to the ICH guidelines [15]. MET showed linearity in the concentration range of 1–100 μg/mL. The regression equation obtained was \( Y = 30.43X + 8.991 \), where Y is peak area and X is concentration of MET (μg/mL). This equation was used to determine the amount of MET present in the stress induced samples.

3.3.3. Limits of detection and quantification

The LOD was defined as the lowest concentration of MET resulting in a signal-to-noise ratio of 3:1 and LOQ was expressed as a signal-to-noise ratio of 10:1. Due to the difference in detector response, different concentrations ranging from 0.01 to 2 μg/mL were prepared and analyzed. The LOD and LOQ obtained were 0.3 and 1 μg/mL, respectively.

3.3.4. Accuracy

Accuracy of the method was determined by performing the recovery experiments. Known amount of the standard at 80%, 100% and 120% levels was fortified to the degradation sample. Peak area of the standard was calculated by the difference of peak area between fortified and unfortified samples. Three replicate samples of each concentration level were prepared and the percentage recovery at each level (n=3) was determined (Table 2). For MET, the results obtained are in good agreement with the added amounts.

| Property               | Mean ± SD (n=6) | RSD (%) | Required limits |
|------------------------|-----------------|---------|-----------------|
| Retention time \( R_t \) | 5.05 ± 0.01    | 0.20    | RSD ≤ 2%        |
| Capacity factor \( k \) | 1.190 ± 0.004  | 0.33    | –               |
| Theoretical plates \( N \) | 28570 ± 240  | 0.84    | \( N > 2000 \)  |
| Tailing factor \( T \)   | 1.62 ± 0.03    | 1.85    | \( T ≤ 2 \)     |

Figure 2  Representative chromatograms of MET standard (20 μg/mL) [A] and stress samples under acidic (5 M HCl) [B], basic (0.01 M NaOH) [C], neutral [D], oxidative (10% H₂O₂) [E] and dry heat [F] conditions.
The HPLC method is illustrated in Fig. 2, where complete separation of the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The specificity of the analyte was established, having good correlation coefficients (Fig. 3). The kinetic parameters in its concentration is negligible compared with the change in concentration of the other reactant (drug). The kinetic parameters during the experiment, one from Germany (Lichrospher C 18, column) and the other from USA (Atlantis C 18 column).}

Each of the three examined factors (pH, flow rate, and acetonitrile percentage) selected was changed one at a time to estimate the effect. Replicate injections (n = 6) of standard solution (20 μg/mL) were performed under small changes of chromatographic parameters (factors). Flow rate was varied by 1 ± 0.1 mL/min; level of acetonitrile in the mobile phase was varied by 58 ± 2% (v/v), while pH was varied by 3.0 ± 0.1. Results obtained are presented in Table 4, indicating that the results 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.

### Table 2 Recovery of the standard from the stress sample by standard addition method.

| Level of standard added (%) | Amount of standard added (μg) | Peak area (mean ± SD) (n = 3) | Amount of standard found (μg) |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|
| 80                          | 40                            | 442 ± 3                       | 1667 ± 8                     | 1225 ± 6                     |
| 100                         | 50                            | 349 ± 5                       | 1884 ± 9                     | 1535 ± 4                     |
| 120                         | 60                            | 240 ± 3                       | 2075 ± 4                     | 1835 ± 5                     |

Values are expressed as mean ± SD (n = 6).

### Table 3 Results for the analysis of intra-day and inter-day precision.

| Concentration (μg/mL) | Intra-day precision | Inter-day precision |
|------------------------|----------------------|---------------------|
|                        | Peak area (%)        | RSD (%)             | Peak area (%)        | RSD (%)             |
| 10                     | 324 ± 2              | 0.65                | 325 ± 2              | 0.53                |
| 30                     | 978 ± 6              | 0.64                | 975 ± 8              | 0.79                |
| 50                     | 1583 ± 9             | 0.57                | 1585 ± 11            | 0.71                |
| 80                     | 2550 ± 12            | 0.49                | 2549 ± 13            | 0.50                |

### 3.3.5. Intra-day and inter-day precision

Intra-day and inter-day precision was evaluated by injecting four different concentrations (10, 30, 50, and 80 μg/mL) of MET. For intra-day variation, sets of six replicates of the four concentrations were analyzed on the same day; for inter-day variation, six replicates were analyzed on six different days. The intra-day and inter-day precision (% RSD) was found to be less than 2% (Table 3), indicating that the method was precise.

### 3.3.6. Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. The specificity of the HPLC method is illustrated in Fig. 2, where complete separation of MET was noticed in the presence of degradants. The average Rt ± standard deviation for MET was found to be 5.05 ± 0.01 min, for six replicates. The peaks obtained were sharp and had clear baseline separation. Specificity of the method is further confirmed by adding diclofenac potassium to the MET solution and analyzed. There was no interference from diclofenac potassium and it was eluted at 9.4 min. This indicates that the developed method was specific and also applicable to simultaneous estimation of diclofenac potassium and MET in combination drug products.

### 3.3.7. Robustness

A method is robust if it is unaffected by small changes in operating conditions. To evaluate HPLC method robustness a few parameters were deliberately varied. The parameters included variation of C18 columns from different manufacturers, pH of the buffer, flow rate and percentage of acetonitrile in the mobile phase. Two analytical columns were used during the experiment, one from Germany (Lichrospher C 18, column) and the other from USA (Atlantis C 18 column). Each of the three examined factors (pH, flow rate, and acetonitrile percentage) selected was changed one at a time to estimate the effect. Replicate injections (n = 6) of standard solution (20 μg/mL) were performed under small changes of chromatographic parameters (factors). Flow rate was varied by 1 ± 0.1 mL/min; level of acetonitrile in the mobile phase was varied by 58 ± 2% (v/v), while pH was varied by 3.0 ± 0.1. Results obtained are presented in Table 4, indicating that the results 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.

### 3.4. Stability-indicating property

An analytical method is stability-indicating if this method can separate all the process-related impurities and all the degradation products from the major peak of the sample. The 6th day chromatograms for various stress induced samples are presented in Fig. 2 B-F. The acid degraded sample showed one additional peak and at the end around 62% of drug was degraded. Base degraded samples showed one major additional peak at Rt 2.90 min and around 85% of drug was degraded at the end. For neutral hydrolysis, oxidative and dry heat conditions, around 29%, 45% and 15% of MET was degraded at the end respectively. Among the tested conditions degradation was mild in neutral hydrolysis and thermal conditions compared to other conditions. In all above cases the degradant peaks did not interfere with the MET peak, suggesting that the method enabled specific analysis of MET in the presence of its degradation products.

### 3.5. Kinetic investigation

Treatment of MET under specified stress conditions resulted in a gradual decomposition of MET in all conditions. Since the degradation was performed with a large excess of solvent (9 mL) compared to drug solution (1 mL), the degradation of MET followed pseudo-first-order kinetics [16] as a linear relationship between log percentage of MET remaining and time was established, having good correlation coefficients (Fig. 3). Pseudo-first-order is the term used when two reactants are involved in the reaction but one of them is in such a large excess that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug). The kinetic parameters are presented in Table 5. Rate constant (K), time left for 50% potency (t1/2) and time left for 90% potency (t90) for each stress condition is calculated.
condition were calculated using Eqs. (1), (2) and (3), respectively [17]:

\[
\log \frac{C_t}{C_0} - \frac{2.303}{t_{1/2}} = \frac{\log C_0 - Kt}{2.303}
\]  

(1)

\[
t_{1/2} = \frac{0.693}{K}
\]  

(2)

\[
t_{90} = \frac{0.105}{K}
\]  

(3)

where \( K \) is the rate constant, \( [C_0] \) is the concentration of MET at time \( t = 0 \) and \( [C_t] \) is its concentration at time \( t \).

The \( K \) values per day were found to be \( 1.84 \times 10^{-1}, 3.24 \times 10^{-1}, 4.37 \times 10^{-2}, 8.75 \times 10^{-2} \) and \( 2.53 \times 10^{-2} \) for 5 M HCl, 0.01 M NaOH, neutral, 10% \( \text{H}_2\text{O}_2 \) and dry heat conditions respectively. Extensive degradation was observed in basic conditions, where \( K \) value was found to be the highest among all the tested conditions; \( t_{1/2} \) and \( t_{90} \) values for all the tested stress conditions are shown in Table 5, both \( t_{1/2} \) and \( t_{90} \) were found to be lowest (2.13 and 0.32 days) for basic condition (0.01 M NaOH) and highest (27.39 and 4.15 days) for dry heat condition.

4. Conclusion

The proposed HPLC method was proved to be simple, accurate, precise, specific and selective for quantitative analysis of MET in the presence of its degradants and also suitable for simultaneous estimation of MET and diclofenac in combination drug products. The degradation of MET was found to follow a pseudo-first-order reaction. The drug was extensively degraded under basic stress conditions. The \( t_{1/2} \) values under different stress conditions were found to decrease in the following order: dry heat > neutral > 10% \( \text{H}_2\text{O}_2 \) > 5 M HCl > 0.01 M NaOH.

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