STUDY OF DEGRADATION KINETIC OF MAGNESIUM OROTATE DIHYDRATE BY SPECTROSCOPIC METHOD

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In present work we developed and validated five spectrophotometric methods for determination of magnesium orotate dihydrate in distilled water, pH 1.2, 4.5, 6.8, and 7.4 buffer systems. These media were selected to extend the applicability of the developed methods from the development of dosage forms to regular quality control. In all media, linear correlation was observed over the concentration range of 1 to 25 μg/mL. The developed methods were found to be selective and specific to the determination of magnesium orotate dihydrate in the presence of various excipients, as well as accurate, precise, and robust (the percent relative standard deviation was found to be less than 2%, and the percent recoveries were between 98 to 102%). The developed methods were also found to be highly sensitive, since the detection and quantification limits were found to be less than 1 μg/mL. The degradation kinetic studies reveal the stability of magnesium orotate dihydrate against hydrolysis (acidic or basic), oxidation, photolysis, and thermal treatment.

Keywords: *magnesium orotate dihydrate, spectrophotometry, ICH guidelines, degradation kinetics.*

ИЗУЧЕНИЕ КИНЕТИКИ РАЗЛОЖЕНИЯ ДИГИДРАТА ОРОТАТА МАГНИЯ СПЕКТРОСКОПИЧЕСКИМ МЕТОДОМ

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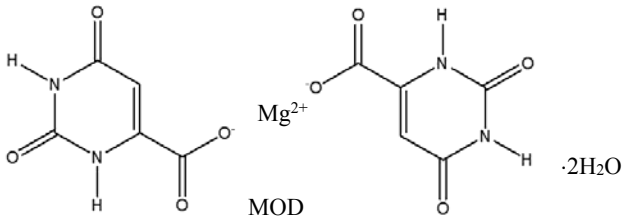
Разработаны и апробированы пять спектрофотометрических методов определения дигидрата оротата магния в дистиллированной воде и буферных системах с pH 1.2, 4.5, 6.8 и 7.4. Среды выбраны для расширения применимости разработанных методов от создания лекарственных форм до регулярного контроля качества. Во всех средах наблюдалась линейная корреляция в диапазоне концентраций 1—25 мкг/мл. Разработанные методы оказались селективными и специфичными при определении дигидрата оротата магния в присутствии различных вспомогательных веществ, а также точными, прецизионными и надежными (относительное стандартное отклонение <2%, степень извлечения 98—102%). Установлены пределы обнаружения и количественного определения разработанных методов <1 мкг/мл. Кинетические исследования деградации показывают устойчивость дигидрата оротата магния к гидролизу (кислотному или основному), окислению, фотолизу и термической обработке.

Ключевые слова: *дигидрат оротата магния, спектрофотометрия, рекомендации Международной конференции по гармонизации, кинетика деградации.*

Introduction. Magnesium orotate dihydrate (MOD) is magnesium 2,4-dioxo-1*H*-pyrimidine-6-carboxylate dihydrate



MOD

MOD is reported to be a synergistic combination of one magnesium ion with two ions of orotic acid. Orotic acid in this complex is mentioned as a "magnesium fixating agent." Orotic acid acts as a carrier to transfer the magnesium ion inside the cell [1].

In enormous preclinical and clinical studies, MOD is proven to have a beneficial effect in the management of heart diseases. It is proven to provide protection against hypercholesterolemia-induced atherosclerosis lesions. It is also reported to improve functioning of heart in rehabilitating patients suffering from acute myocardial infraction and coronary heart diseases by improving the left ventricular systolic and diastolic end volume, increasing cardiac output, increasing stroke volume, and increasing ejection fraction [2–8]. MOD improves neuronal function by renewing or replacing severely damaged or dead cells, improving the membrane electrical conductivity and preventing the oxidative damage to cells [9]. It is also proven to have lipid lowering effects and is reported to increase the effect of fenofibrate when administered concomitantly [10, 11]. Due to these enormous clinical benefits, multiple products of MOD exist in the market. Despite the tremendous usage of MOD in clinical practice, to the best of our knowledge, there is no UV spectrophotometric method available in the literature for determining MOD in bulk and in pharmaceutical dosage forms for regular quality control.

Hence, the objective of this research is to develop and validate simple, accurate, precise and economical methods for estimating MOD in bulk and pharmaceutical dosage forms. Five spectrophotometric methods were developed for use in distilled water, pH 1.2, 4.5, 6.8, and 7.4 buffer systems. These media were selected in order to increase the scope of developed methods from regular dissolution and assay tests for quality control purposess (for example, methods developed in distilled water) to pH gradient dissolution test to assist the product development (as the developed methods cover the major absorptive site pH of the gastrointestinal tract, as well as the pH of blood). MOD was found to have absorption maxima λ_{max} at 278 nm (in distilled water), 282 nm (in pH 1.2 buffer), and 279 nm (in pH 4.5, 6.8, and 7.4 buffer). The developed analytical methods were validated as per ICH Q2 (R2) 2005 guidelines [12]. Since knowledge of the degradation kinetics of the drug molecule under various stress conditions helps in the selection of suitable excipients, as well as in selection of a suitable pharmaceutical process for dosage form development, we also aim to study the degradation kinetics of MOD under various stress conditions as described in the literature [13].

Experimental. *Instrument.* In the present work a double-beam ultraviolet-visible spectrophotometer Pharma Spec UV-1700 (Shimadzu, Japan) with quartz cells of 1 cm path length was used. The instrument had an automatic wavelength check with an accuracy of 0.1 nm.

Chemicals and regents. MOD was purchased from ShanPar Industries Pvt. Ltd, Vadodara, India. Aerosil was purchased from Wacker Chemie AG, Germany. Magnesium stearate was purchased from Nitika Pharmaceuticals Specialities Pvt. Ltd, India. Talcum was purchased from Golcha Associated, India. Sodium lauryl sulfate was purchased from Aarti Industries Ltd, India. Polyvinyl pyrollidone K-30 was purchased from Boai Pharmaceuticals Ltd., China. Mannitol was purchased from Shijiszhuang Hua Xu Pharmaceutical Co. Ltd., China. Lactose monohydrate was purchased from Saputo Ingredients, Canada. All other regents were of analytical grade.

Procedure for calibration curve. Accurately weighed MOD was transferred to a 100 mL precalibrated volumetric flask to generate 1000 μg/mL (for distilled water and pH 7.4 buffer) and 100 μg/mL (for pH 1.2, 4.5, and 6.8 buffer) stock solutions in the selected media by ultra-sonication for 10 min. Five sample solutions were prepared from each respective stock solution over the concentration range 1 to 25 μg/mL using the respective solvents, and their absorbance values were recorded at a previously specified λ_{max}. Calibration plots were constructed by plotting concentration values (on the *x*-axis) against observed absorbance values (on the *y*-axis) [14].

Sample preparation. Twenty in-house prepared tablets of MOD were powdered in a mortar pestle. A quantity of powdered mixture equivalent to 10 mg of MOD was taken and transferred to a 100 mL precalibrated volumetric flask and extracted with 50 mL of individual media by sonication for 15 min. After sonication, the volume was adjusted to the mark with the respective media, and the prepared solution was filtered through a nylon filter (0.45 μm pore size). The filtered solution was suitability diluted to get a 10 μg/mL concentration in each media. The absorbance of the prepared solutions was recorded at a specified wavelength in individual media, and the results were calculated (n = 6).

Analytical method validation. *Specificity and selectivity.* Three separate solutions containing 5, 10, and 15 μg/mL of MOD were prepared in each media along with and without the common excipients used in the tablet preparation (mannitol, lactose monohydrate, polyvinyl pyrrolidone from 400 to 200 nm) and analyzed for any change and/or shift in the absorption maxima of MOD in each media. All measurements were performed in replicate of six (n = 6), and both the percent relative standard deviation (% RSD) and the percent recovery (% recovery) were calculated in each case.

Accuracy. The accuracy of the proposed methods was evaluated by preparing three concentration levels, viz. low concentration (LC) 5 μg/mL, intermediate concentration (IC) 10 μg/mL, and high concentration (HC) 15 μg/ mL, in each media. Each solution was analyzed at the previously specified wavelength in six replicates (n = 6). The accuracy was accessed based on the standard deviation and % RSD at each level in each media. To further confirm the accuracy of the developed methods, the standard addition method was used. In each media, a known amount of MOD (10 μg/mL) was added to the pre-analyzed sample solution of MOD (IC), and the total concentration was calculated. The percent recovery of the additionally added MOD was calculated in each case (n = 6).

Precision. The repeatability of the developed methods was checked by preparing LC, IC, and HC (as mentioned in the accuracy studies) in each medium from an independent stock solution, and the prepared solutions were analyzed for their respective absorbance at the previously specified wavelengths (n = 6). Intermediate precision was checked by performing intra-day and interday precision measurements. Three concentration levels of MOD (LC, IC, and HC) were prepared in each medium at three different times of the day, and their absorbance was recorded to assess the intraday precision. The same procedure was adopted for three consecutive days to assess the interday precision of the developed methods. All measurements were performed in six replicates (n = 6). The % RSD associated measurements were taken as a measure of precision.

Linearity. To establish the linearity of the developed methods, five different concentrations of MOD (1 to 25 μg/ mL) were prepared in each medium (as used for constructing the calibration plot) from independent stock solutions, and their absorbance was recorded. A least square regression analysis was performed, and the regression coefficient (R^2) was taken as a measure of linearity [15].

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations mentioned in ICH Q2 (R1), 2005:

$$\mathrm{LOD} = 3.3\sigma/S, \quad (1)$$

$$\mathrm{LOQ} = 10\sigma/S, \quad (2)$$

where σ is the standard deviation of the response; S is the slope of the calibration curve.

Robustness. The robustness of the proposed methods was checked by: (a) changing pH of the media by ± 0.1 units, and (b) assessing the stability of the solutions by storing them for 24 h. All measurements were performed at three concentration levels (LC, IC, and HC) in each medium in six replicates (n = 6). The percent recovery was determined in each case and was taken as a measure of robustness.

Degradation kinetic studies. *Acid hydrolysis*. The MOD solutions were prepared in 0.1 M hydrochloric acid (HCl) at LC, IC, and HC levels and were exposed to the stressor for 24 h. The samples were withdrawn at specific time intervals (at baseline, after 6, 12, and 24 h) and were immediately neutralized with a 0.1 M sodium hydroxide (NaOH) solution and analyzed using the developed methods. The percent recovery at each time point and the associated % RSD were calculated. Each measurement was performed in threes replicates (n = 3). Degradation was said to be significant if the % recovery was found to be less than 90% [13]. The degradation reaction order and the reaction rate constant were determined using the established graphical method after applying suitable mathematical transformations [16].

Base hydrolysis. The procedure adopted for analyzing the effect of 0.1 M NaOH as a stressor on the stability of MOD was similar to that of the acid hydrolysis except that initial solutions of MOD (at LC, IC, and HC levels) were prepared in 0.1M NaOH and the samples taken at a specific time point were neutralized with 0.1M HCl.

Oxidative degradation kinetics. The solutions of MOD at LC, IC, and HC levels were exposed to a 3% v/v hydrogen peroxide (H_2O_2) solution for a period of 24 h. At specified time intervals (baseline, after 6, 12, and 24 h), the sample was removed and assayed for MOD using the developed methods. The percent recovery and % RSD were calculated in each case. All measurements were performed in three replicates of (n = 3). Degradation was considered significant if the % recovery was less than 90% [13]. The degradation reaction order and reaction rate constant were determined using the established graphical method after applying suitable mathematical transformations [16].

Photolytic degradation kinetics. MOD in solid form was exposed to sunlight for 10 days, and suitable controls were exposed to darkness. At appropriate time intervals (viz. baseline, after 3, 5, and 10 days), 100 mg of MOD was weighed and a 1000 μg/mL solution was prepared in distilled water after sonication. Appropriate dilutions were made to obtain LC, IC, and HC levels. The absorbance was recorded, and the concentration of MOD was calculated. The percent recovery was taken as a measure of degradation, and % RSD was taken as a measure of accuracy. All measurements were done in replicate of three (n = 3). Student's t-test at a 95% confidence interval was used to compare the stability of MOD under sunlight and in darkness. Degradation was said to be significant if the % recovery was found to be less than 90% [13]. The degradation reaction order and reaction rate constant were determined using the established graphical method after applying suitable mathematical transformations [16].

Thermal degradation kinetics. In this work, the thermal stability of MOD in both solid and liquid form was explored. MOD in solid form was exposed to both dry heat (at 25, 45, 60, and 80°C) and wet heat (40°C +75%R_H, 60°C +75%R_H, and 80°C +75%R_H) for 5 days. Similarly, MOD solutions were exposed to dry heat at 25, 45, 60, and 80°C for 5 days. The samples were withdrawn at specified time intervals (viz. at baseline, after day 1, 3, and 5). Assay of the remaining MOD was done at LC, IC, and HC levels. Degradation was said to be significant if % recovery was found to be less than 90% [13]. The degradation reaction order and reaction rate constant were determined using the established graphical method after applying suitable mathematical transformations [16]. The Arrhenius equation [16] was used to construct the Arrhenius plot taking log k on the x-axis and $1/T$ on the y-axis. From the negative slope of the Arrhenius plot, the activation energy of the degradation reaction was calculated in each case:

$$\log k = \log A - [E_a/2.303RT], \quad (3)$$

where k is reaction rate constant, A is Arrhenius factor, E_a is activation energy, R is gas constant, and T is absolute temperature.

Results and discussion. To develop an optimum UV spectrophotometric method, it is essential that the compound have good absorption, as well as a clear peak shape in selected media. In this work the selection of media was carried out based on the sensitivity of the developed method, the cost of solvents, ease of preparation, as well as on the wide applicability of the developed methods (from regular dissolution and assay tests for quality control to the pH gradient dissolution test to assist product development). In the present work we tried to meet all these needs. We observed that in all media MOD showed good absorption and peak shape (Fig. 1). We also observed a slight bathochromic shift in the absorption spectra of MOD (from 282 to 278 nm) when one moves from acidic to alkaline pH.

A linear correlation was observed in the absorbance vs concentration plot of MOD in all media. The linear regression equations and R^2 of MOD in different media are shown in Fig. 2. The value of R^2 was close to 1 in all the media (>0.999), depicting the good linearity of the developed methods. When the developed methods were applied to determine MOD in the in-house prepared tablets, the percent assay was found to range from 99.21 to 101.54% in all the media, showing good applicability of the developed methods.

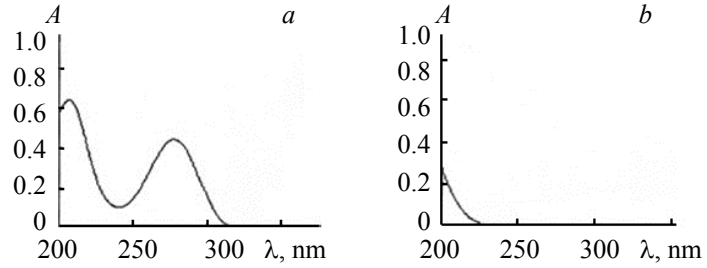


Fig. 1. UV absorption spectra of (a) MOD and (b) placebo solution (in all media similar spectrum was observed).

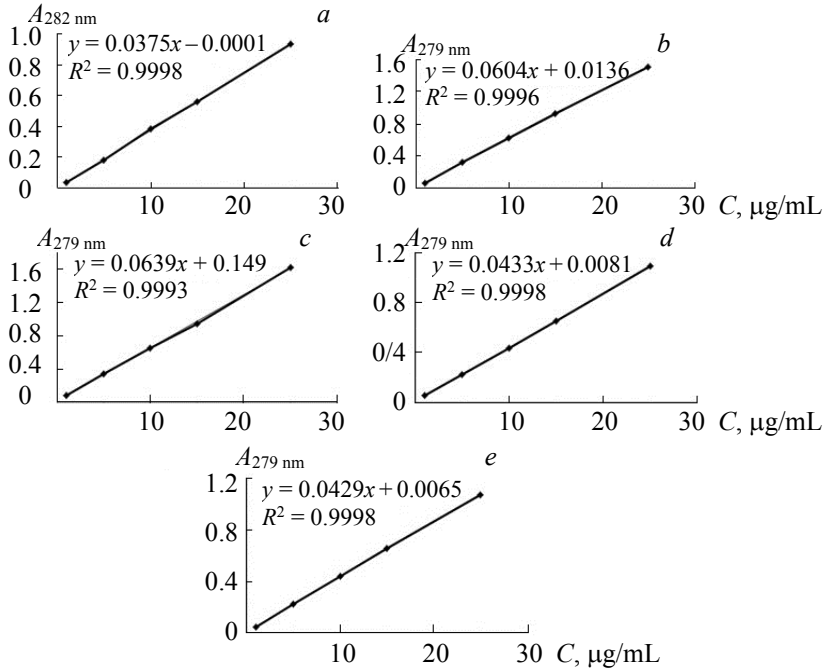


Fig. 2. Calibration plot of MOD in (a) pH 1.2 buffer system, (b) pH 4.5 buffer system, (c) pH 6.8 buffer system, (d) pH 7.4 buffer system, and (e) distilled water.

As shown in Fig. 1, no absorption interference was observed due to any of the excipients at the selected wavelength used for determining MOD in all the media. The calculated *t* values were found to be less than the tabulated values at $p < 0.05$, which depicts no interference of the excipients in the determination of MOD. These results were further supported by a good % recovery of MOD (between 98 to 102%) in the samples with or without excipients (Table 1). Therefore, the developed methods are highly selective and specific for MOD.

TABLE 1. Specificity and Selectivity Data of the Developed Methods for Quantification MOD in the Presence of Various Excipients ($n = 6$)

Media	Concentration level	With excipients		Without excipients		*t*-value*
		% Recovery	% RSD	% Recovery	% RSD	
Distilled water	LC	101.010	0.650	100.777	0.367	0.0022
	IC	100.699	0.191	100.544	0.172	
	HC	100.958	0.150	100.958	0.150	
pH 1.2 buffer	LC	98.631	1.050	98.276	1.348	0.02956
	IC	101.000	0.595	101.013	0.783	
	HC	101.381	1.106	100.077	0.774	
pH 4.5 buffer	LC	100.464	1.230	101.892	0.977	0.00797
	IC	101.805	0.387	100.121	0.777	
	HC	101.552	0.231	101.571	0.871	
pH 6.8 buffer	LC	101.388	1.521	101.388	1.599	0.00896
	IC	100.668	0.556	99.390	0.567	
	HC	99.506	0.432	101.106	0.241	
pH 7.4 buffer	LC	99.540	1.637	100.493	0.871	0.01908
	IC	99.515	0.815	98.245	0.987	
	HC	99.831	0.526	98.825	1.054	

N o t e. HC – high concentration (15 μg/mL), IC – intermediate concentration (10 μg/mL), LC – low concenration (5 μg/mL), %RSD – percent relative standard deviation.

* at $p<0.05$, nonsignificant.

The study accuracy was ensured by good % recoveries (between 98 to 102%) and lower % RSD values (<2%) in all the media (Table 2). The reliability and validity of these results were further ensured by the "standard addition method." The values of % recovery (%RSD) were found to be 101.642 (1.156), 101.322 (1.844), 100.049 (0.294), 98.334 (1.460), and 100.217 (0.158) in the pH 1.2, 4.5, 6.8, and 7.4 buffer systems and in distilled water, respectively.

Precision was determined by repeatability and intermediate precision. The results associated with them are reported in Table 3. Repeatability indicates the precision of the developed method during repetitive analysis. Since the % RSD associated with developed methods was less than 2%, this indicates that the method gives a precise measurement during repetitive analysis. Intermediate precision was determined by intraday and interday precision to indicate the precision of the method when it is carried out at different times or days in the same laboratory. The developed methods were found to be precise in the interday and intr-day precision studies as the % RSD was found to be less than 2% in each case.

TABLE 2. Accuracy Data of the Developed Methods for Quantification of MOD (n = 6)

Media	Concentration level	%Recovery	SD	%RSD	Standard addition method (using IC)		
					% Recovery	SD	% RSD
Distilled water	LC	100.932	0.0028	0.802	100.217	0.0008	0.158
	IC	100.233	0.0029	0.676			
	HC	100.803	0.0022	0.340			
pH 1.2 buffer	LC	98.720	0.0031	1.675	101.642	0.0052	1.156
	IC	101.872	0.0038	0.989			
	HC	100.610	0.0035	0.617			
pH 4.5 buffer	LC	99.525	0.0037	1.181	101.322	0.0137	1.844
	IC	100.232	0.0039	0.622			
	HC	100.302	0.0048	0.512			
pH 6.8 buffer	LC	101.127	0.0027	0.794	100.049	0.0023	0.294
	IC	99.077	0.0051	0.781			
	HC	100.828	0.0031	0.320			
pH 7.4 buffer	LC	100.955	0.0035	1.545	98.334	0.0079	1.460
	IC	99.785	0.0029	0.665			
	HC	99.856	0.0029	0.483			

N o t e. As in Table 1.

TABLE 3. Precision Data of the Developed Methods for Quantification of MOD (n = 6)

Media	Concentration level	Repeatability		Intra-day precision		Inter-day precision	
		%Recovery	%RSD	%Recovery (overall)	%RSD (overall)	%Recovery (overall)	%RSD (overall)
Distilled water	LC	101.088	0.542	101.476	1.033	100.389	0.963
	IC	100.350	0.709	99.961	0.736	100.078	0.551
	HC	100.984	0.124	100.777	0.273	100.622	0.321
pH 1.2 buffer	LC	98.098	0.937	98.342	1.779	98.987	0.818
	IC	101.077	0.930	101.804	0.271	101.026	0.489
	HC	100.670	0.343	100.196	0.460	100.343	0.414
pH 4.5 buffer	LC	100.629	0.711	100.408	0.675	100.574	0.737
	IC	100.673	0.390	101.225	0.485	100.370	0.514
	HC	101.000	0.847	100.320	0.327	100.504	0.251
pH 6.8 buffer	LC	101.439	1.469	101.127	0.265	99.979	0.646
	IC	99.624	0.867	99.025	0.465	99.285	0.813
	HC	100.619	0.286	100.410	0.210	100.776	0.100
pH 7.4 buffer	LC	101.263	1.065	100.108	1.446	101.647	0.937
	IC	100.554	0.584	100.939	0.299	100.439	0.742
	HC	99.446	0.362	99.574	0.263	99.574	0.367

N o t e. As in Table 1.

LOQ was found to be 0.906, 0.678, 0.638, 0.916, and 0.227 μg/mL in the buffer with pH 1.2, 4.5, 6.8, 7.4, and in distilled water, respectively. It is evident from the results that the developed methods can quantify even a minute amount of MOD in selected media. LOD was found to be 0.299, 0.224, 0.211, 0.302, and 0.075 μg/mL in the buffer with pH 1.2, 4.5, 6.8, 7.4, and in distilled water, respectively. It is evident from the results that the developed methods are highly sensitive and can detect even a small variation in the concentration of MOD. We observed a slightly greater sensitivity of the method in distilled water, as compared to other media.

Slight variations in the media pH was not found to affect the results of the developed methods as % recovery was found to be ranging between 98–102% (Table 4). The MOD solutions in the selected media were found to be stable upon 24 h storage as % recoveries were found to range between 98–102% (Table 4). We also did not observe any MOD spectral changes upon 24 h storage. Hence, the developed methods are robust, and the results do not change under selected experimental variations.

TABLE 4. Robustness Data of the Methods Developed for Quantification of MOD (n = 6)

Media	% Recovery		
	LC (5 μg/mL)	IC (10 μg/mL)	HC (15 μg/mL)
Variation in pH ±0.1 unit			
Distilled water	101.476	99.922	100.622
pH 1.2 buffer	98.009	101.582	100.670
pH 4.5 buffer	101.899	100.508	100.706
pH 6.8 buffer	101.231	98.685	98.828
pH 7.4 buffer	101.340	98.245	99.882
Storage at room temperature for 24 h			
Distilled water	100.778	100.078	100.466
pH 1.2 buffer	98.098	101.271	101.499
pH 4.5 buffer	101.347	100.894	100.835
pH 6.8 buffer	100.918	99.546	99.993
pH 7.4 buffer	101.647	99.746	99.523

N o t e. HC – high concentration, IC – intermediate concentration, LC – low concentration.

The degradation kinetics of MOD was found to follow first-order rate kinetics under various stress conditions because the values of the reaction rate constant at the LC, IC, and HC levels were found to be nearly constant when the log % recovery of MOD was plotted against time in each case (Table 5). The percent recovery in each case was found to be >90%, which means MOD is quite stable against hydrolysis (both acidic and basic), oxidation, photolysis, and thermal stress (Table 5). During kinetic studies of photolytic degradation, while comparing the results of samples exposed to sunlight to that of the control kept in dark using Student's t test, the t value was found to be –1.0298, which was less than the tabulated value at $p < 0.05$. Therefore, the presence of sunlight does not have a catalytic effect on the degradation rate of MOD, and the processing of MOD can be carried out in the presence of light. During studies of thermal stability under dry heating, the values of E_a for solid MOD and MOD solution were found to be 3.750 and 1.484 kcal/mol, respectively. On the other hand, solid MOD was found to have E_a of 2.300 kcal/mol during wet heat thermal studies (Fig. 3). Since the % recovery in each case of the thermal stability study was >90%, drying of MOD can be done at high temperature, for example, up to 80°C (as in case of an aqueous wet granulation process).

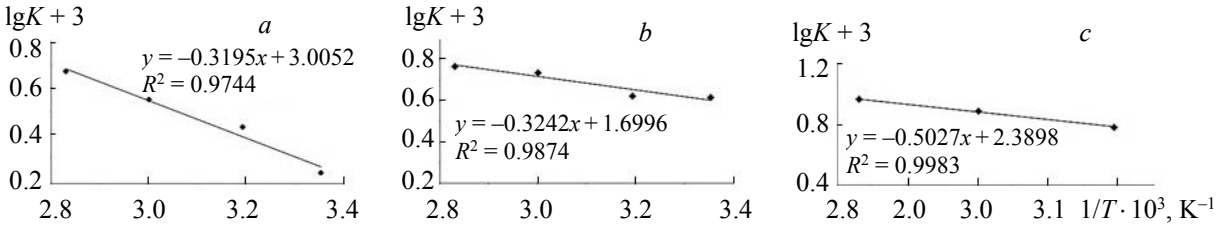


Fig. 3. Arrhenius plot of MOD (a) dry heat-solid, (b) dry heat-liquid, and (c) wet heat.

TABLE 5. Degradation Kinetic Data of MOD under Various Stress Conditions

Concentration level	%Recovery*	%RSD*	Reaction rate constant (k)×10^{-4}
Acid hydrolysis (0.1 M HCl)			
LC	98.579	0.513	4.606 h^{-1}
IC	99.025	0.606	
HC	100.030	0.302	
Base hydrolysis (0.1 M NaOH)			
LC	99.240	0.976	2.303 h^{-1}
IC	99.543	1.199	
HC	99.798	0.411	
Oxidation (3%v/v H_2O_2)			
LC	100.155	1.881	4.606 h^{-1}
IC	99.262	1.110	
HC	100.000	0.000	
Photolysis (sunlight exposure)			
Test			
LC	99.223	1.182	6.909 day^{-1}
IC	99.106	1.512	
HC	99.534	0.413	
Control			
LC	99.378	0.271	
IC	99.308	1.220	
HC	99.068	0.415	
Dry heat exposure – Solid MOD			
At 25°C			16.889 day^{-1}
LC	99.223	1.435	
IC	99.495	0.488	
HC	99.845	0.412	
At 40°C			26.868 day^{-1}
LC	99.378	0.271	
IC	99.184	0.470	
HC	99.637	0.501	
At 60°C			35.313 day^{-1}
LC	98.135	1.257	
IC	98.019	1.037	
HC	98.032	0.183	
At 80°C			46.828 day^{-1}
LC	97.203	1.439	
IC	97.319	0.864	
HC	97.565	0.487	
Dry heat exposure – Liquid solution of MOD			
At 25°C			41.454 day^{-1}
LC	98.601	1.061	
IC	98.718	0.625	
HC	98.653	0.637	
At 40°C			42.222 day^{-1}
LC	98.291	0.548	
IC	98.019	1.559	
HC	98.135	0.317	
At 60°C			54.504 day^{-1}
LC	97.358	1.813	
IC	97.552	0.717	
HC	97.669	0.574	

Continue Table 5

Concentration level	%Recovery*	%RSD*	Reaction rate constant (k)×10^{-4}
At 80°C			58.727 day^{-1}
LC	97.047	1.544	
IC	97.319	0.830	
HC	97.151	0.462	
Wet heat exposure – Solid MOD at 75% R_H			
At 40°C			60.646 day^{-1}
LC	97.203	1.091	
IC	97.164	0.367	
HC	97.721	0.459	
At 60°C			76.767 day^{-1}
LC	96.892	0.735	
IC	96.232	0.504	
HC	96.633	0.609	
At 80°C			92.120 day^{-1}
LC	95.649	0.563	
IC	95.998	1.823	
HC	96.093	0.374	

*at last sampling point, HC – high concentration (15 μg/mL), IC – intermediate concentration (10 μg/mL), LC – low concentration (5μg/mL), %RSD – percent relative standard deviation, v/v – volume by volume, R_H – relative humidity.

Conclusions. Five spectrophotometric methods were developed for determining MOD in distilled water and pH 1.2, 4.5, 6.8, and 7.4 buffer systems. The developed method was also applied to quantify MOD in in-house prepared tablets. The percent recovery in each case was found to be between 98 to 102%. The developed methods were validated as per ICH guidelines for their accuracy, precision, sensitivity, specificity, and selectivity. They were also found to be robust under selected experimental conditions and hence can be used for regular quality control purposes, as well as for dosage form development. The degradation kinetic studies revealed the stability of MOD against hydrolysis (acidic or basic), oxidation, photolysis, and thermal treatment.

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