Original article

Pharmaceutically equivalent parenteral depot suspension of methyl prednisolone acetate

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Abstract:

The aim of the present study was to formulate and evaluate pharmaceutically equivalent injectable aqueous suspension for parenteral depot of methyl prednisolone acetate (MPA). Various aqueous suspensions were prepared by rapid stirring and colloid milling method. The prepared aqueous suspensions were subjected to particle size determination, sedimentation study, *in vitro* release studies (pH dependent dissolution study) and stability studies. The optimized formulation consisted of 4% w/w of MPA, 2.91% w/w of PEG-3350, 0.19% w/w of injection grade Tween®80, 0.68% w/w of monobasic sodium phosphate, 0.15% w/w of dibasic sodium phosphate, 0.91% w/w of benzyl alcohol and 0.32% w/w sodium meta bisulphate. The f_2 value was calculated for innovator (DepoMedrol®) and optimized formulation at pH 6.8 and pH 7.4 phosphate buffers. The f_2 values of 62.94 and 54.84 were obtained at pH 6.8 and pH 7.4 phosphate buffers, respectively. The mean particle size of innovator and test product was found to be 23.13 and 27.02 µm respectively. Stability studies revealed that no significant changes occurred in physiochemical properties of the optimized formulation (F4) at various storage temperatures for a period of three months. The shelf life of the test product was found to be 232.71 days.

Keywords: DepoMedrol®; Methyl prednisolone injectable suspension; Pharmaceutical equivalent

Introduction

Advances have been made in the area of oral controlled release drug delivery systems. However, there are number of possible loopholes in this area of research which includes difficulty in establishing a relationship between *in vivo* and *in vitro* data, unpredictable performance of oral controlled release systems under different dietary conditions, thereby rendering accurate pharmacokinetic prediction difficult and often unpredictable absorption characteristics in different regions of the gastrointestinal tract [GIT] [1]. Due to these problems, parenteral controlled release systems have been investigated [1]. Methyl prednisolone acetate (MPA), a steroidal anti-inflammatory drug is widely used in musculoskeletal disorders such as arthritis, allergic and dysmenorrhea for symptomatic relief of pain and inflammation [2]. Injectable suspensions are heterogonous system, containing solid dispersed phase. They are limited to either subcutaneous or intramuscular routes of administration. Intravenous administration may result in vasoocclusion [3]. For parenteral routes, acetate salt of methyl prednisolone is used. The major drawback of the use of this drug orally is that it undergoes extensive hepatic first pass metabolism and thus only about 50% of the administered dose reaches systemic circulation [2]. In order to avoid this degradation alternative routes have been used and amongst them parenteral route promises significant advantages over the oral route [3]. The parenteral routes are preferred when a rapid and predictable drug response is desired as in an emergency situation, when patient is uncooperative, unconscious, or unable to take drug via an enteral route and when localized drug therapy is required [1-3]. MPA is a good carrier for depot (long time action) delivery as it has long biological half life (approx. 46-990 h), undergoes substantial hepatic first-pass metabolism, is poorly bioavailability (50-60%), has low molecular weight (416.51) and C_{max} 10-28.5 ng/ml. Injectable, aqueous suspension of MPA has been prepared and studied extensively and it has been concluded that MPA can be administered successfully through the parenteral route [1]. Therefore, the aim of present study was to compare particle size, *in vitro* release profile and f_2 value of prepared parenteral depot suspension formulation with innovator (DepoMedrol®) product. The purpose was to provide the delivery of drug at a controlled rate by intramuscular or subcutaneous route for a longer period of time.

Materials and Methods

Materials

MPA was obtained from Lupin Ltd. Pune (India) as a gift sample. Polyethylene glycol-3350 (PEG-3350) was purchased from Sigma Aldrich, USA. Tween®80 was purchased from S.D. Fine Chemicals, Mumbai (India). Monobasic sodium phosphate, dibasic sodium phosphate, benzyl alcohol and sodium metabisulphite were purchased from Qualigens Chemicals, Mumbai (India). All other chemicals and reagents used were of analytical reagent (AR) grade.

Solubility studies

The solubility of MPA was determined by adding an excess amount of drug in phosphate buffers pH 6.8 and pH 7.4. The flasks were kept on a water bath shaker for 72 h at 37 °C. After 72 h, solutions were filtered through 0.45 µm membrane filter and aliquots were suitably diluted for estimation of MPA spectrophotometrically at 244 nm.

Drug excipient interaction studies

The drug excipient interactions studies were performed using thin layer chromatography (TLC) and UV spectroscopy. Samples of pure drug and different formulations (F1-F4) were kept at room temperature (25 °C), refrigerator temperature (4 °C), incubator (37 °C) and oven (50 °C). After a period of one month, the mixtures were withdrawn and evaluated for appearance, color, odour, gas formation and degradation. The drug excipient mixtures were analyzed spectrophotometrically and by TLC method.

Preparation of aqueous suspension

Aqueous suspension of MPA containing 40 mg/ml MPA was prepared by dissolving accurately weighed quantity of PEG-3350, Tween® 80, mono-or di-basic sodium phosphate, benzyl alcohol and sodium metabisulphite in milli-Q water by continuous stirring. The drug was added during stirring condition in rapid stirrer, at least for half an hour. The formula for MPA suspension is given in Table 1.

Particle size determination

Various samples like active pharmaceutical ingredient (API), prepared formulation and innovator product (DepoMedrol®) were suspended in milli-Q water and sonicated to form a smooth and uniform dispersion [4-6]. The sample was added till obscuration range was within 10-20%. The particle size of different formulation was obtained using a Malvern particle size analyzer (Mastersizer-2000, UK). The pump speed was 2,600 rpm. The mean particle size distributions of the various products were compared.

In vitro release studies (pH dependent dissolution study)

Release of MPA from various aqueous suspension formulations were studied using dissolution apparatus USP II [7-9]. 2 ml of suspension (containing MPA 40 mg/ml) was taken in the flask having phosphate buffer pH 6.8 and phosphate buffer pH 7.4 as medium. Speed was adjusted to 50 rpm and temperature was maintained at 37 ± 2 °C throughout the study. 20 ml of aliquots were withdrawn at predetermined time intervals for a period of 8 days and each time an equal volume was replaced with the respective media. The drug content was estimated using a HPLC at wavelength of 254 nm. The cumulative percent drug releases (CPR) were plotted against time for test and innovator (DepoMedrol®) formulations.

Determination of f_2 value for innovator and optimized formulation

The f_2 value for innovator (DepoMedrol®) and optimized formulation was determined at pH 6.8 and 7.4. The following formula was used for calculation of f_2 value [10]:

$$f_2 = 50 \log \left[(1 + 1/n \sum_{t=1}^{n} W_t (R_t - T_t)^2)^{-0.5} \times 100\right]$$

Where f_2 is similarity factor, W_t is the optional weight factor, n is products over all time points (n = 8), R_t is percent drug release of reference product and T_t is the percent drug release of test product.

Table 1 Description of various formulations prepared

Ingredients (mg/100 ml)	Formulations			
	F1	F2	F3	F4
*MPA (% w/w)	4.00	4.00	4.00	4.00
PEG3350 (% w/w)	2.91	2.84	3.10	2.91
Tween®80 (% w/w)	0.19	0.21	0.19	0.19
Mono-Na-Phosphate (% w/w)	0.68	0.62	0.42	0.68
Di-basic-Na-phosphate (% w/w)	0.11	0.14	0.14	0.15
Benzyl alcohol (% w/w)	0.91	0.59	0.91	0.91
Sodium meta bisulphate (% w/w)	0.32	0.32	0.32	0.32
Water for injection (% w/w)	q.s to 100	q.s to 100	q.s to 100	q.s to 100

*MPA = Methyl prednisolone acetate

Sedimentation study of optimized formulation

In sedimentation study, the suspension was transferred to a stoppered measuring cylinder and was stored at room temperature (25 ± 1 °C) for 72 h. The volume of sediment formed was noted at regular interval of time. The sedimentation volume, ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the suspension (i.e. Hu/Ho) was calculated.

Accelerated stability studies

Accelerated stability studies were performed on optimized MPA depot suspension. Three batches of test formulation were packed in glass vial and kept in stability chamber maintained at 40 °C, 30 °C, 25 °C and 5 °C for 90 days [11]. The suspensions were observed for their crystallization, particle size, potency and pH. Samples were withdrawn at regular interval of 0, 30, 60 and 90 days. The amount of drug degraded and the amount remaining at each time interval was calculated. The log of drug remaining was plotted against time (days). Slope of each line was obtained and degradation rate constant was calculated by the formula:

Slope = -k/2.303

Where k is the degradation rate constant. The effect of temperature on the degradation was studied by plotting log k v/s 1/T (Arrhenius plot). Degradation rate constant at 25 °C (k_{25}) was calculated by extrapolation of Arrhenius plot then shelf life was calculated.

Results and Discussion

The acetate form of methyl prednisolone was chosen because of its insolubility in water, as depot (long time) action was required [2]. The mean solubility of MPA in phosphate buffer pH 6.8 and pH 7.4 was found to be 2.23 ± 0.16 mg/ml and 1.84 ± 0.12 mg/ml respectively. Phosphate buffer (pH 6.8 and 7.4) was chosen as the *in vitro* release media. The muscle tissue pH was simulated by phosphate buffer pH 6.8 and physiological tissue pH was simulated by phosphate buffer pH 7.4 [7]. TLC and UV studies were performed to assess any interaction between the drug and the excipients in the formulations. The data obtained suggested that there was no interaction between the drug and the excipients because the R_f values of both pure drug and drug-excipients present in different formulations were nearly similar (Table 2). There were no significant changes observed in λ_{max} of drug and drug-excipient present in different formulations (Table 3). Moreover there was no color change, gas formation or any sign of degradation. In particle size analysis, mean particle size of drug (API), innovator (DepoMedrol®) and test were found to be 7.14, 23.13 and 27.02 μm, respectively (Table 4). The polydispersity index (PI) of innovator and different test formulations ranged from 0.100-0.162. This indicated uniform size distribution in innovator and test formulations. The PI value was lowest for innovator product (0.100) but it was not significantly lower than formulation F4 (0.103).

Table 2 Drug-excipient interaction by TLC method at different temperatures

Temperature	R_f values (Mean ± SD, n = 3)				
	Pure drug	F1	F2	F3	F4
Refrigerator temperature (4 °C)	0.893 ± 0.013	0.915 ± 0.021	0.898 ± 0.013	0.854 ± 0.021	0.798 ± 0.011
Room temperature (25 °C)	0.894 ± 0.016	0.886 ± 0.017	0.895 ± 0.014	0.873 ± 0.024	0.796 ± 0.010
37 °C	0.887 ± 0.011	0.892 ± 0.018	0.902 ± 0.023	0.815 ± 0.016	0.764 ± 0.012
50 °C	0.883 ± 0.010	0.854 ± 0.012	0.887 ± 0.024	0.912 ± 0.028	0.782 ± 0.014

Table 3 Drug-excipient interaction by UV spectrophotometer at different temperatures

	λ_{max} values (nm) (Mean ± SD, n = 3)				
Temperature	**Pure drug**	**F1**	**F2**	**F3**	**F4**
Refrigerator temperature (4 °C)	244.50 ± 1.45	244.00 ± 1.88	243.50 ± 1.97	244.00 ± 1.13	243.50 ± 1.77
Room temperature (25 °C)	244.00 ± 1.32	244.50 ± 1.12	244.00 ± 1.54	244.00 ± 1.99	244.00 ± 1.59
37 °C	245.00 ± 1.67	245.50 ± 1.18	245.00 ± 1.39	246.00 ± 1.43	246.00 ± 1.78
50 °C	245.50 ± 1.75	245.00 ± 1.98	243.50 ± 1.53	244.00 ± 1.67	244.50 ± 1.33

Table 4 Particle size analysis of pure drug, innovator product and formulations (F1 to F4)

Sample	**Source**	**Bulk lot**	**Obscuration**	**Mean size (μm)[a]**	**PI**
*MPA (API)	Pure drug	BK-031	18.74	7.14 ± 1.10	0.154
**Depo Medrol®	Pharmacia	LK-099	13.58	22.98 ± 2.30	0.100
**Depo Medrol®	Pharmacia	LK-034	13.06	23.13 ± 3.50	0.151
Formulation-F1	In-house	AFT-F1	13.26	38.06 ± 6.20	0.162
Formulation-F2	In-house	AFT-F3	16.51	62.52 ± 7.90	0.126
Formulation-F3	In-house	AFT-F3	13.83	53.67 ± 7.60	0.141
Formulation-F4	In-house	AFT-F4	13.68	27.02 ± 2.80	0.103

*MPA (API) = methyl prednisolone acetate (active pharmaceutical ingredient)

**Depo Medrol® = Innovator sample, PI = Polydispersity index, [a]Mean ± SD, n = 3

In vitro release studies are important for ensuring the depot action performance and the reproducibility of the product. The drug release was carried out in alkaline phosphate buffer pH 6.8 and pH 7.4 at 37 ± 2 °C (7-9). The optimized formulation with Type F4 exhibited better depot action at phosphate buffer pH 6.8 (Figure 1) as compared with phosphate buffer pH 7.4 (Figure 2). From *in vitro* drug release study, it was revealed that formulation F4 exhibited best release when compared with other formulations. Cumulative percent drug release (CPR) in 192 h (8 days) from innovator (DepoMedrol®) and test product were 93.3 and 90.0% at pH 6.8 and 80.2 and 94.2% at pH 7.4 phosphate buffer respectively. The f_2 value is a measurement of the similarity between the dissolution profiles of two true profiles (test and innovator). The f_2 value was found to be 62.94 and 54.84 at pH 6.8 (Table 5) and pH 7.4 (Table 6) respectively. The value showed a similarity between the dissolution profiles of optimized formulation (F4) with the innovator (DepoMedrol®) product. The sedimentation volume is the ratio of ultimate height (Hu) of the sediment to the final height (Ho) of the suspension. The ratio was found to be 0.72 to 0.50 for a period of 72 h (Table 7). Stability studies of optimized formulation revealed that no significant changes occurred in physiochemical properties like crystal growth, particle size and pH of the optimized formulation at various storage temperatures for a period of three months (Table 8). Order of degradation was determined by graphical method at each temperature. The order of degradation was found to be first order (Figure 3). The correlation coefficients were significant at each temperature as shown in Figure 3 ($p < 0.05$). Therefore for first order degradation, Log% of drug remaining was plotted against time (days) and degradation rate constant (k) was calculated from the slope of the curve at each temperature.

Table 5 f_2 value calculation at pH 6.8 phosphate buffer (Mean CPR)*

f_2 value of optimized formulation (F4)									
Time (t)	n_1	n_2	n_3	n_4	n_5	n_6	n_7	n_8	Sum
Reference	26.9	28.8	29.2	32.1	34.5	42.7	66.5	8.4	-
Test	31.9	34.6	34.9	36.1	40.1	46.8	73.9	93.5	-
R_t-T_t	-5.0	-5.7	-5.7	-4.0	-5.6	-4.1	-7.4	-5.1	-
$(R_t-T_t)^2$	25.0	32.5	32.5	16	31.4	16.8	54.8	26.0	234.9
f_2 = 50*log{[1+(1/nsum(R_t-T_t)^2]-0.5}*100					62.94066786				

*CPR = Cumulative percent release; n_1-n_8 = Time points; n = Mean time points

Table 6 f_2 value calculation at pH 7.4 phosphate buffer (Mean CPR)*

f_2 value of optimized formulation (F4)									
Time (t)	n_1	n_2	n_3	n_4	n_5	n_6	n_7	n_8	Sum
Reference	23.0	33.4	43.9	47.7	68.9	82.7	86.1	96.6	-
Test (T)	28.1	33.9	36.4	49.6	50.6	73.7	87.6	97.0	-
R_t-T_t	-5.1	-0.5	7.5	-1.9	18.3	9.0	-1.5	-0.4	-
$(R_t-T_t)^2$	26.0	0.3	56.3	3.6	334.9	81.0	22.5	0.2	504.4
f_2 = 50*log{[1+(1/nsum(R_t-T_t)^2]-0.5}*100						54.83659787			

*CPR = Cumulative percent release; n_1-n_8 = Time points; n = Mean time points

Table 7 Sedimentation study of optimized formulation F4

Time (hrs.)	Sedimentation volume (Hu/Ho) (Mean ± SD, n = 3)
0.5	0.72 ± 0.009
12	0.70 ± 0.010
24	0.68 ± 0.011
30	0.60 ± 0.014
48	0.59 ± 0.012
54	0.50 ± 0.009
72	0.50 ± 0.009

Table 8 Changes in crystal formation, pH and particle size during stability studies of optimized formulation (F4)

Time point	Storage temp	Crystal formation	pH ± SD (n = 3)	Particle size (μm) (Mean ± SD, n = 3)
0 day	-	Nil	6.34 ± 0.14	23.13 ± 0.78
30 days	40 °C	Nil	6.26 ± 0.12	22.56 ± 0.67
	30 °C	Nil	6.31 ± 0.19	23.48 ± 1.11
	25 °C	Nil	6.20 ± 0.22	21.63 ± 1.21
	5 °C	Nil	6.19 ± 0.21	20.31 ± 0.89
60 days	40 °C	Nil	6.14 ± 0.23	24.95 ± 0.92
	30 °C	Nil	6.25 ± 0.24	23.14 ± 1.28
	25 °C	Nil	6.19 ± 0.29	26.53 ± 1.09
	5 °C	Nil	6.13 ± 0.30	21.37 ± 0.74
90 days	40 °C	Nil	6.08 ± 0.31	25.48 ± 1.52
	30 °C	Nil	6.12 ± 0.33	26.31 ± 1.16
	25 °C	Nil	6.21 ± 0.31	22.14 ± 1.13
	5 °C	Nil	6.15 ± 0.26	23.92 ± 1.18

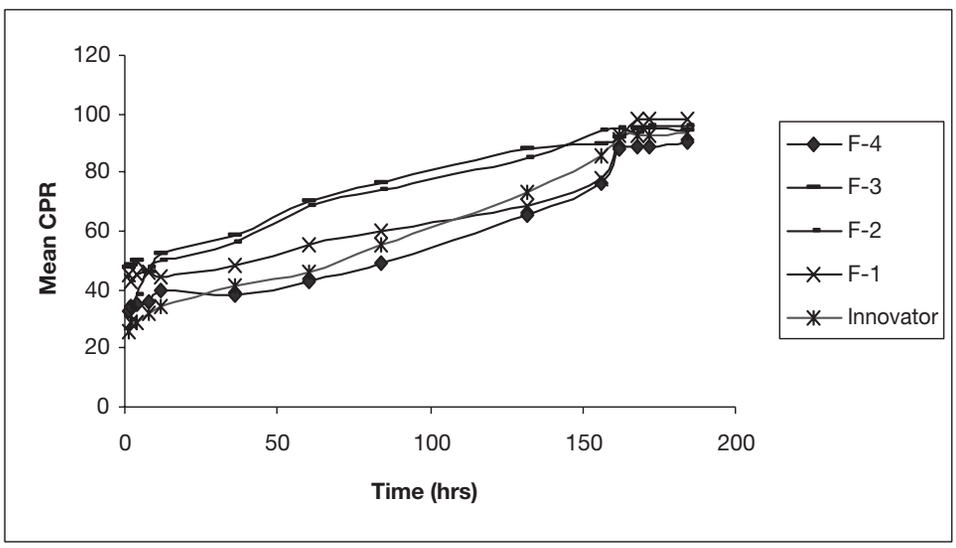

Figure 1 Comparative *in vitro* drug release profile for innovator and different test formulations (F1-F4) in phosphate buffer pH 6.8 (CPR = Cumulative percent release)

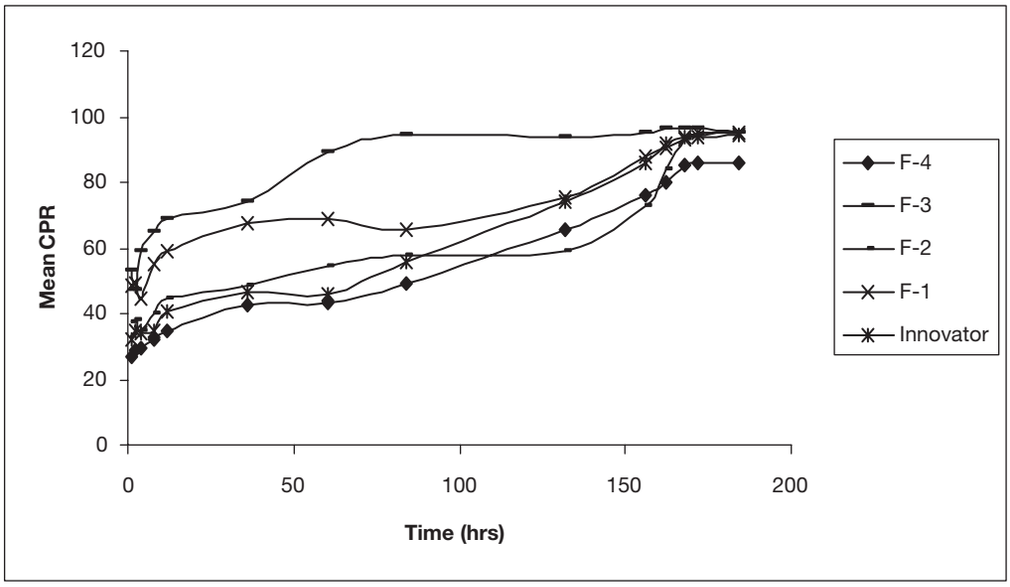

Figure 2 Comparative *in vitro* drug release profile for innovator and different test formulations (F1-F4) in phosphate buffer pH 7.4 (CPR = Cumulative percent release)

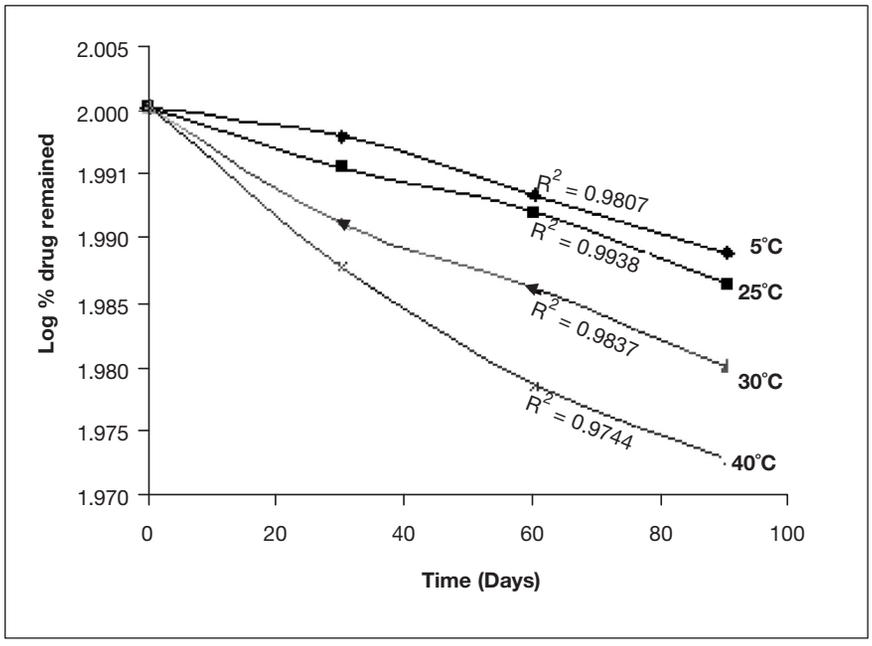

Figure 3 First order degradation kinetics plot of methyl prednisolone acetate (MPA) suspension at different temperatures

The effect of temperature on the degradation was studied by plotting log k v/s 1/T. (Figure 4). The value of k at 25 °C (k_{25}) was obtained by extrapolation of the plot and shelf life was then calculated by substituting K_{25} in the following equation:

$$T_{0.9} = \frac{0.1052}{K_{25}}$$

Where $T_{0.9}$ is the shelf life of the product. The value of k_{25} was found to be 4.53 x 10^{-4} $days^{-1}$. The shelf life of the suspension was found to be 232.71 days.

Summary

The present study was undertaken to prepare and evaluate parenteral depot dosage form of methyl prednisolone acetate. The prepared parenteral depot formulation may provide a convenient dosage form for achieving best performance regarding sustained release and particle size. From the results of *in vitro* drug release, particle size and f_2 value it was concluded that developed parenteral depot suspension was pharmaceutically equivalent to DepoMedrol®.

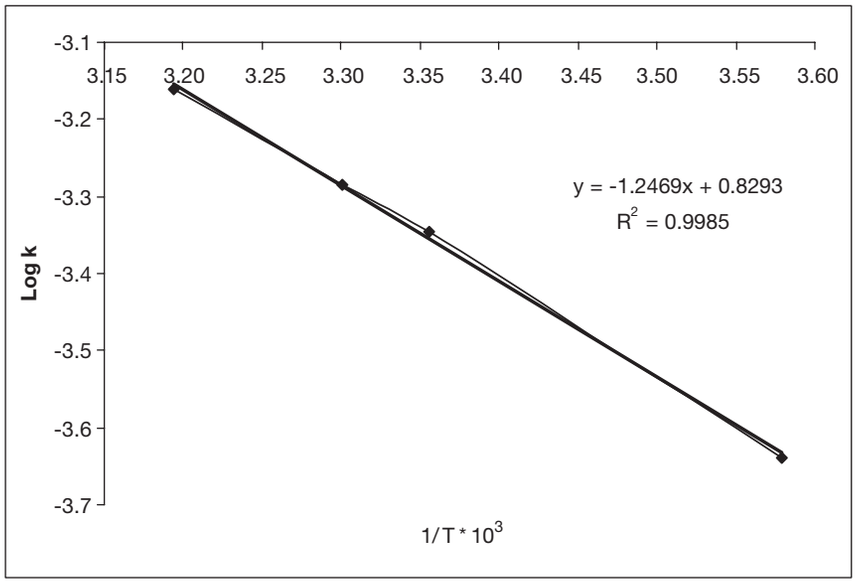

Figure 4 Arrhenius plot between Log k and 1/T for methyl prednisolone acetate (MPA) suspension

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