Research Article

Kinetic Spectrophotometric Determination of Mesna in Drug Substance and Drug Product Using Alkaline Potassium Permanganate

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Abstract

A Simple and sensitive kinetic spectrophotometric method was established for the determination of the uroprotector mesna in drug substance and drug product using alkaline potassium permanganate as an oxidizing agent. The method involves determination of mesna by kinetic study of its oxidation at room temperature for a fixed time of 30 minutes. The absorbance of the colored manganate ion was measured at 610 nm. The absorbance-concentration plot was rectilinear over the concentration range of 1-10 µg/mL, with a minimum detectability of 0.151 µg/mL. The activation parameters such as E_a, ΔH, ΔS and ΔG were also evaluated for the reaction and found to be 105.77 KJ mol^{-1}, 108.29 KJ mol^{-1}, 252.26 JK^{-1} mol^{-1} and -32.99 KJ mol^{-1}, respectively. The method was successfully applied for the determination of mesna in its dosage form. The results obtained were in good agreement with those obtained with the reference method of the investigated drug.

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Keywords: Mesna ; Kinetic spectrophotometric determination ; Potassium permanganate; Activation parameters.

Introduction

Mesna (sodium-2-mercaptoethane sulfonate) is an effective compound used for the prophylaxis of urothelial toxicity (uroprotector) in patient being treated with ifosfamide and cyclophosphamide, unchanged mesna in urine has free thiol group that react with ifosfamide and cyclophosphamide including acrolein considered to be responsible for the toxic effect on bladder, In addition, mesna has been used for a long time as a mucolytic agent that breaks disulfide bonds of mucous polypeptide chains [1].

Some analytical methods have been reported for the determination of the studied drug, the reported methods for mesna include High Performance Liquid Chromatography-Ultraviolet detection (HPLC-UV) [2], HPLC- with electrochemical detection [3] HPLC- with fluorescence detection [4], Vibrational Spectroscopic studies [5], and a Spectro-photometric method [6].

To the best of our knowledge, no kinetic spectrophotometric methods have been reported for the analysis of mesna up till now.

The aim of the present work is to study the reaction between mesna and potassium permanganate in alkaline medium kinetically in an attempt to evaluate it in its dosage form. The proposed method is simple and did not need sophisticated instruments or special skills, sensitive, rapid and readily adaptable to both drug substance and drug product.

Experimental

Apparatus

JASCO V-630 BIO spectrophotometer (S/N B206561148) equipped with kinetic accessory provided with temperature controlled cell (EHCS - 760) thermo-electric temperature. Recording range, 0-1; wavelength, 610 nm; factor 1; number of cell, 1; reaction time 30 min.; cycle time, 0.2 min.

Reagents and materials

All chemicals used were of analytical reagent grade and all solvents were of spectroscopic grade. Potassium permanganate (Merck, Darmstadt, Germany): $3x10^{-2}$M aqueous solutions, freshly prepared. Sodium hydroxide (BDH, UK): 0.5M aqueous solution is prepared. Mesna (batch no. ME-10037, purity 99.90 %, manufactured by Emic united company) and the pharmaceutical

preparation Uromitexan 100 mg/mL (batch no. 0J019, manufactured by Baxter Co.)

Standard solutions

Stock solution of mesna was prepared by dissolving 10.0 mg of mesna in 100 mL distilled water (0.1 mg/mL), other concentrations were prepared by further dilution with water. These solutions were found to be stable for five days if kept in a refrigerator.

General Procedure

Construction of the kinetic calibration graphs

Aliquot solutions of the stock solution equivalent to 10-100 µg of mesna solution were transferred into a series of 10 mL volumetric flasks, 1mL of 0.5M NaOH was added followed by 1.8mL of $3x10^{-2}$ M $KMnO_4$. The mixture was well mixed and completed to volume with distilled water. The increase in absorbance at 610 nm was scanned during 30min at ambient temperature (25°C) against an appropriate blank prepared simultaneously. The reaction order was obtained by plotting log reaction rate ($\Delta A/\Delta t$) over the specified time period *versus* log concentration of the drug. The calibration graph and the regression equations were obtained by plotting absorbance (A) at specified time *versus* concentration of the drug in µg/mL.

Procedure for the Determination of the Mesna in Dosage Form

Mix about 5 ampoule of sample injection solution. Transfer accurately 1 mL of sample injection solution equivalent to 100 mg of mesna into 100 mL volumetric flask. The content of the flask was completed to 100 mL with distilled water, then dilute 1 mL of the solution to 10 mL with distilled water. An aliquot of the cited solution was taken and the above procedure was applied. The nominal content was calculated either from a previously plotted calibration graph or using the corresponding regression equation.

Results and Discussion

Mesna is poorly absorbing entity at its λ_{max} (210 nm) which is far from common spectrometric range, so it is difficult to undergo quantitative analysis of this compound by direct measurement in UV. As the reported spectrophotometric methods depend on the formation of colored derivatives through chemical reaction with either primary amines or thiols, this studied kinetic spectrophotometric method appears as a new additional procedure that based on the oxidation of mesna with $KMnO_4$ in alkaline medium, in an attempt to develop simple and precise method for its determination in drug substance and drug product.

Oxidation of the studied drug (Fig. 1) with $KMnO_4$ was carried out in the presence of NaOH. Trials were made to determine the drug through oxidation with $KMnO_4$ in neutral medium but a brown ppt was observed. Potassium permanganate in alkaline medium oxidized the drug and yielded the green colored manganate radical, which absorbs maximally at 610 nm (Fig. 2). The intensity of the color was increased with time, and so, a kinetic method was developed for determination of studied drug at 610 nm.

$HS\text{-}CH_2\text{-}CH_2\text{-}SO_3\ Na$

Figure 1 The structure of mesna.

Other oxidants were tested to determine the studied drug, such as 10% H_2O_2, sodium persulfate in neutral and alkaline medium but all failed to give satisfactory results.

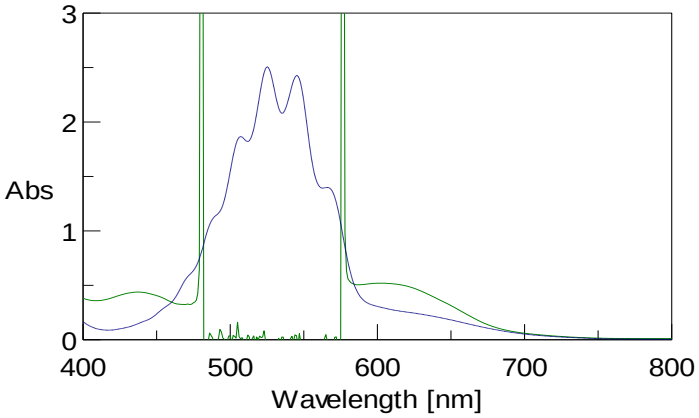


Figure 2 Absorption spectra of the studied drug after reaction with $KMnO_4$/ NaOH system. **(a)** $KMnO_4$ ($3x10^{-3}$M); **(b)** The produced manganate ion after the reaction of $KMnO_4$ with mesna (10µg/mL).

Study of Experimental Parameters

The different experimental parameters affecting the formation of oxidation product were studied. Variables were optimized by changing each in turn while keeping all others constant. Namely, reagent concentration, alkalinity, temperature and effect of time.

Effect of $KMnO_4$ Concentration

The influence of $KMnO_4$ concentration on the absorbance of the reaction product was studied using different volumes (0.2-2.5mL) of $3x10^{-2}$M $KMnO_4$, it is noticed that, increasing the volume of $3x10^{-2}$M $KMnO_4$ will result in subsequent increase in the absorbance of the reaction product up to 1.8mL which is adequate for the maximum absorbance of mesna, as shown in Fig. 3

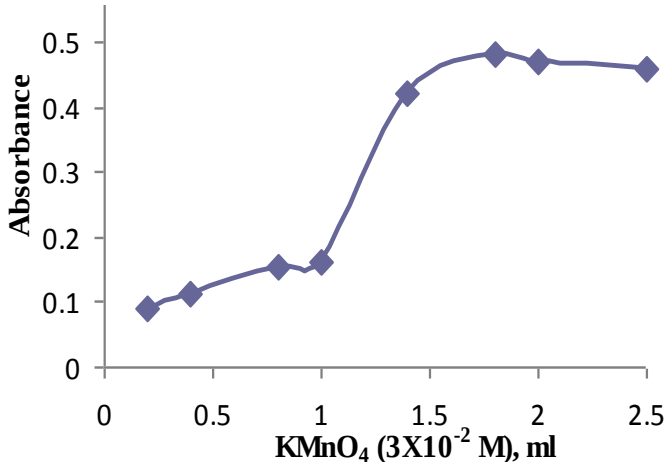


Figure 3 Effect of $KMnO_4$ (3 x 10^{-2} M)on the absorbance intensity of mesna 10 µg/mL at 610 nm.

Effect of NaOH concentration

The influence of NaOH volume on the absorbance of the reaction product was studied using different volumes (0.2–2mL) of 0.5M NaOH. It was found that increasing the volume of 0.5M NaOH would increase the absorbance of the reaction product up to 1mL after which further increase in the volume will produce no change, as shown in Fig.4.

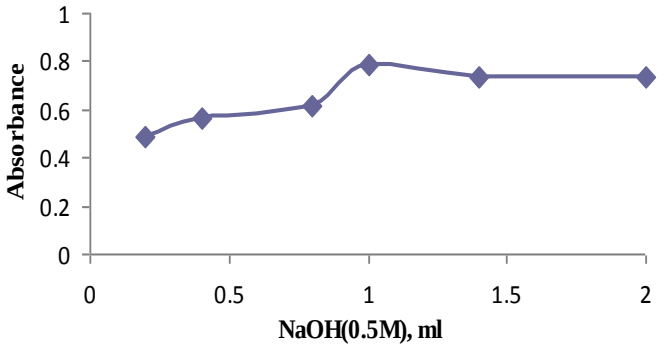


Figure 4 Effect of NaOH (0.5 M) on the absorbance intensity of mesna 10 µg/mL at 610 nm.

Effect of temperature

The effect of temperature on the reaction rate was studied, it was found that, permanganate was reduced to manganate radical at room temperature (20±5°C) while at higher temperatures, reaction rate is increased but manganese dioxide was precipitated. Therefore, room temperature was selected as the optimum temperature.

Effect of time

The effect of time on the reaction between KMnO4 and the studied drug was investigated. The absorbance of the reaction mixture was increased with time and never reach maximum in a reasonable time. Therefore, a fixed time of 30 min was selected giving the highest slope and the best correlation coefficient (Fig.5).

Evaluation of the Kinetic Parameters

The rate of the reaction was followed at room temperature with various concentrations of the drug in the rang of 1-10 µg/mL keeping $KMnO_4$ and NaOH concentrations constant at the recommended levels mentioned above.

The rate of the reaction obeys the following equation:

$$\text{Rate} = \Delta A/\Delta t = K` [\text{drug}]^n \quad \textbf{(1)}$$

Where K` is the pseudo-order rate constant, n is the order of the reaction.

The rate of the reaction may be estimated by variable time measurement [7], where A is the absorbance, t is the time in seconds. Taking logarithms of rates and drug concentrations, the previous equation is transformed into:

$$\text{Log (rate)} = \log \Delta A/\Delta t = \log K` + n \log [\text{drug}] \quad \textbf{(2)}$$

Plot of log reaction rate versus log drug concentration at 610 nm gave the regression equation, correlation coefficient, pseudo order rate constant and order of the reaction which are indicated in Table 1. These results indicate that the reaction is pseudo first order reaction in the drug concentration.

Table 1 Logarithm of the rate for different concentrations of Mesna at room temperature at 610 nm

Log ΔA/Δt	-5.26 -4.936 -4.378 -4.134 -3.941
log [Mesna]	-5.215 -4.913 -4.516 -4.312 -4.215
Regression equation	Log rate=1.55+1.312logC
Correlation coefficient	0.9979
Rate constant	35.48
Order of reaction	1.31

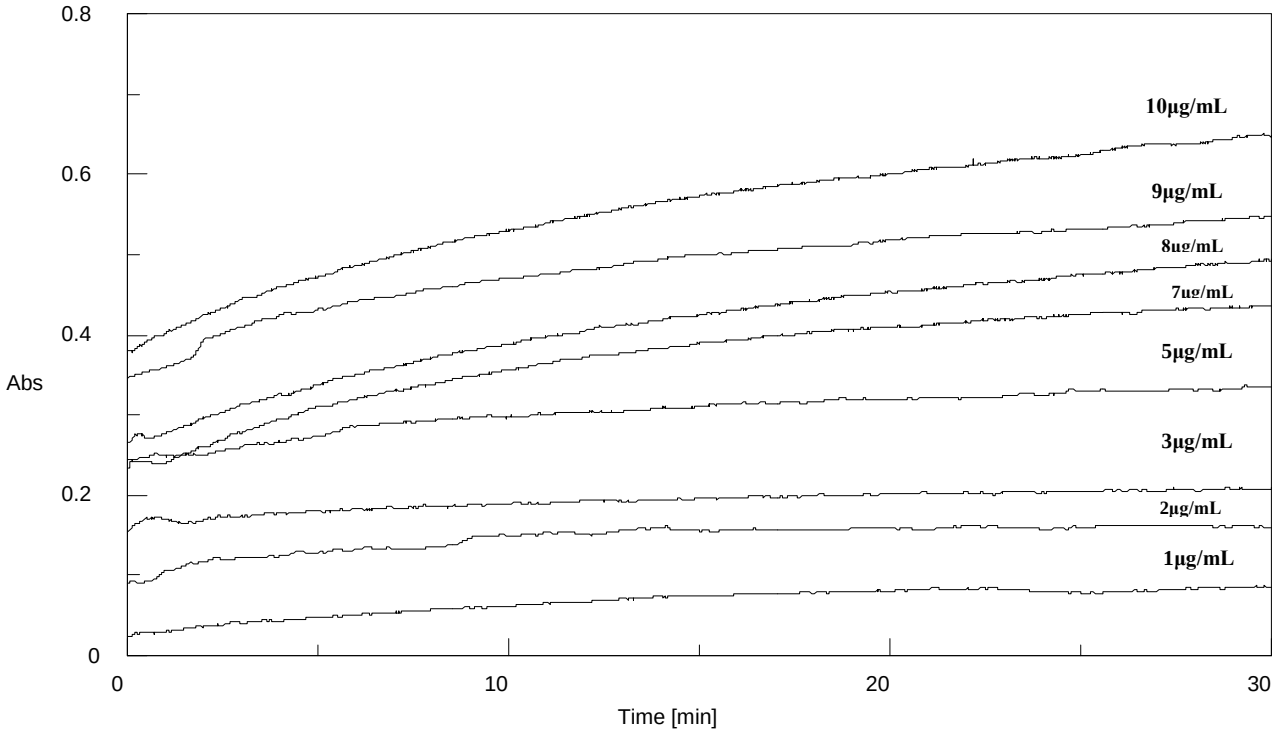


Figure 5. Absorption versus time graphs for the reaction between mesna and $KMnO_4$ at 610 nm.

Selection of the best kinetic method

Several kinetic techniques were adopted for the selection of the best method. Rate constant, fixed absorbance and fixed time methods [7] were tried and the most suitable analytical method was selected considering the applicability, the sensitivity, i.e. the slope of calibration graph and the correlation coefficient (r).

Rate constant method

Graphs of log absorbance versus time for mesna concentration in the range $4.26x10^{-5} – 6.09x10^{-5}$ M were plotted and all appeared to be rectilinear. Pseudo-first order rate constant (K`) corresponding to different drug concentrations was calculated from the slopes multiplied by -2.303 and are presented in Table 2.

Regression of (C) versus K` gave equation:

K` = -0.0004 + 4.912 C (r = 0.966)

Where C is the molar concentration of the drug.

Fixed absorbance method

Reaction rates were recorded for different mesna concentrations in the range of $3.045x10^{-5}$ - $5.482x10^{-5}$ M. A preselected value of absorbance (0.4) was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus initial concentration of drug was plotted. Table 3 and the following equation were obtained :

$1/t = -6x10^{-4} + 37.841$ C (r = 0.9984)

Where C is the molar concentration of the drug.

Table 2 Application of the rate constant method in the quantification of Mesna through oxidation with $KMnO_4$

[Mesna]	**K` (S^{-1})**
$4.26x10^{-5}$	$-2.30x10^{-4}$
$4.87x10^{-5}$	$-1.84x10^{-4}$
$5.48x10^{-5}$	$-1.61x10^{-4}$
$6.09x10^{-5}$	$-1.38x10^{-4}$

K**',** the pseudo-first order rate constant.

Table 3 Application of the fixed absorbance method in the quantification of Mesna through oxidation with $KMnO_4$ at 610 nm.

[Mesna]	**1/t (S^{-1})**
$3.045x10^{-5}$	$5.56x10^{-4}$
$3.654x10^{-5}$	$7.79x10^{-4}$
$4.263x10^{-5}$	$9.37x10^{-4}$
$5.482x10^{-5}$	$1.43x10^{-3}$

Fixed time method

At a preselected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of the absorbance versus initial concentrations of mesna were established with the regression equation assembled in Table 4.

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) were chosen as the most suitable time interval for the measurement.

Table 4. Application of the fixed time method in the quantification of Mesna with $KMnO_4$ at 610 nm.

Time (min)	Regression Equation	Correlation Coefficient
5	A = 0.046 + 0.041C	0.9929
10	A = 0.046 + 0.047C	0.9949
15	A = 0.044 + 0.053C	0.9969
20	A = 0.040 + 0.055C	0.9979
25	A = 0.034 + 0.058C	0.9984
30	A = 0.030 + 0.061C	0.9994

As a conclusion, the fixed time method was chosen for quantification because it gave the best correlation coefficient in a reasonable time of 30 min.

Quantification and method validation

After optimizing the reaction conditions, the fixed time method was applied to the kinetic determination of 1-10 μg/mL of mesna.

Analysis of data gave the following regression equation:

$$A = 0.030 + 0.061\ C \qquad (r = 0.9994)$$

Where A is the absorbance and C is the concentration of mesna in μg/mL.

The method was validated according to USP guidelines for linearity, accuracy, precision, LOD, LOQ as shown in Table 5 [8]. These small values point out to the high precision of the proposed method.

Statistical analysis of the results obtained by both the proposed and the reference methods [8] revealed no significant difference regarding the accuracy and precision as indicated by the student t-test and F test [9], as shown in Table 6. The reference method for mesna was a titration method using 0.1N Iodine solution and 0.1 N sodium thiosulphate [8], the data was provided by NODCAR, Egypt. The proposed method was successfully applied for the determination of mesna in its dosage form. The results obtained were in a good agreement with those of the reference method [8], as shown in Table 7. Also standard addition technique was applied and analysis of injection solution spiked with known concentrations of mesna was shown in Table 8.

Mechanism of the reaction

The data used in the optimization of $KMnO_4$ concentration and the data of calibration graphs were used to calculate the stoichiometry of the reaction adopting the limiting logarithmic method [10]. The ratio of the reaction between mesna and $KMnO_4$ in alkaline medium was calculated by dividing the slope of $KMnO_4$ curve over the slope of the drug curve (Fig. 6A & B) . It was found that the ratio was 1.422 : 0.482 pointing out to a ratio of 2.99:1 ($KMnO_4$ to drug). Based on the obtained molar reactivity, the reaction pathway is proposed to proceed as follows:

$$R\text{-}SH + 3MnO_4^- + 6\ OH^- \rightarrow R\text{-}SO_3H + 3MnO_4^{2-} + 3H_2O$$

Table 5 Validation report obtained by applying the suggested method for the determination of mesna.

Parameters	Results
Linearity range (μg/mL)	1-10
Limit of detection (μg/mL)	0.1512
Limit of quantification (μg/mL)	0.458
$S_{y/x}$	2.03×10^{-5}
S_a	1.43×10^{-3}
S_b	2.63×10^{-4}
Precision (%RSD)	
Interday	1.07
Intraday	0.845
%Er	2.01
Accuracy	102.13±1.27

$S_{y/x}$, Standard deviation of the residual; S_a, Standard deviation of intercept; S_b, Standard deviation of slope ; %RSD , relative standard deviation, *%Er*, percentage error.

Table 6. Application of the proposed method for the determination of mesna in its raw material.

Parameters	Proposed method			Ref. method
	Taken µg/mL	Found µg/mL	Recovery (%)	Recovery (%)
	3.0	2.95	98.36	
	7.0	7.13	101.87	
	8.0	8.06	100.81	
	10.0	9.81	98.19	
X±S.D.			99.81± 1.82	99.9±1.4
t			0.33 (2.57)[a]	
F			1.81 (9.27)[a)]	

Each result is the average of three separate determinations.
[a]Figures in parentheses are the tabulated t and F values, respectively at p= 0.05[9].

Table 7. Application of the proposed method for the determination of Mesna in its pharmaceutical product.

Parameters	Proposed method			Ref. method
	Taken µg/mL	Found µg/mL	Recovery (%)	Recovery (%)
	3.0	3.09	103.2	
	7.0	5.03	100.65	
	8.0	7.12	101.7	
	10.0	9.83	98.3	
X±S.D.			101.01± 2.06	98.26±1.1
t			1.39 (2.57)[a]	
F			2.24 (9.55)[a)]	

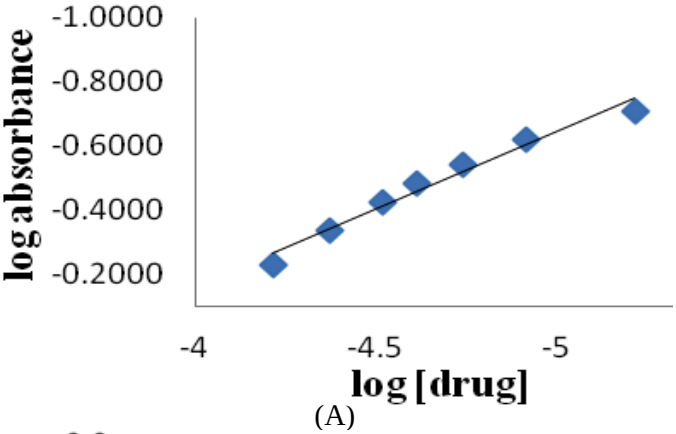
Each result is the average of three separate determinations.

[a]Figures in parentheses are the tabulated t and F values, respectively at p= 0.05[9].

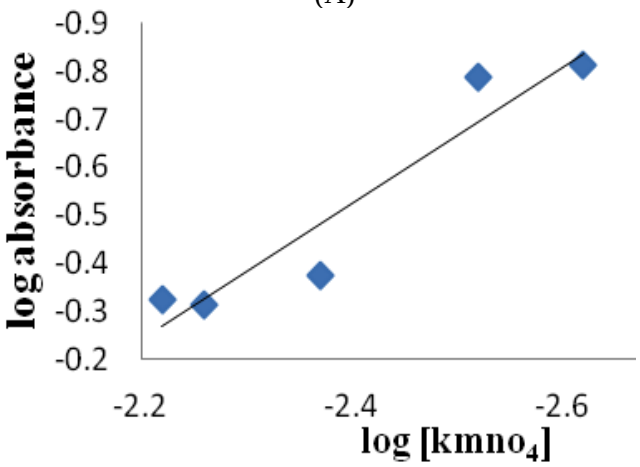
The ratio of the reaction between mesna and $KMnO_4$ in alkaline medium was calculated by dividing the slope of $KMnO_4$ curve over the slope of the drug curve (Fig. 6A & B) . It was found that the ratio was 1.422 : 0.482 pointing out to a ratio of 2.99:1 ($KMnO_4$ to drug).

Table 8. Analysis of commercial injection solution with known concentrations of mesna at 610nm.

Taken µg/mL	Mesna µg/mL	Found µg/mL	Recovery (%)
6.5	1.0	7.61	101.5
6.5	1.5	8.11	101.42
6.5	2.0	8.79	103.40
X±S.D.			102.13±1.27



(A)



(B)

Figure 6 Stoichiometry of the reaction between mesna and $KMnO_4$ adopting limiting logarithmic method [10]. (A) Variable concentrations of mesna at constant $KMnO_4$ concentration; (B) Variable concentrations of $KMnO_4$ at constant mesna concentration.

Activation Parameters

For the evaluation of apparent activation parameter [11], the reaction was studied at 298, 303, and 308 K at [Mesna] = 5.482×10^{-5}M, [$KMnO_4$] = 3×10^{-2} M and [NaOH] = 0.5 M.
The Arrhenius plot of ln K. versus 1/T was found to be linear with a correlation coefficient of -0.999 (Fig. 7). The Eyring plot of ln K/ T versus 1/T was linear with a correlation coefficient of -0.999 (Fig. 8). The value of Ea was evaluated from the slope (-Ea/R) of Arrhenius

plot and found to be 105.77 KJ mol^{-1}. The value of ΔH and ΔS were evaluated from the slope (-ΔH/R) and intercept [ln (kb/h) + ΔS/R] of Eyring plot and found to be 108.29 KJ mol^{-1} and 252.66 JK^{-1} mol^{-1}, respectively. The value of Gibbs free energy (ΔG) of activation of the reaction product was found to be -32.99 KJ mol^{-1}. This value indicated that the proposed reaction is a favored reaction (spontaneous) can be occurred without external supply of energy.

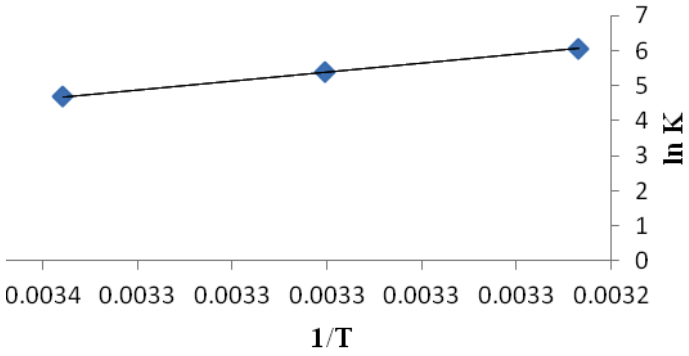


Figure 7. Arrhenius plot of log k versus 1/T at 298.0, 303.0, and 308.0 K for determination of activation energy.

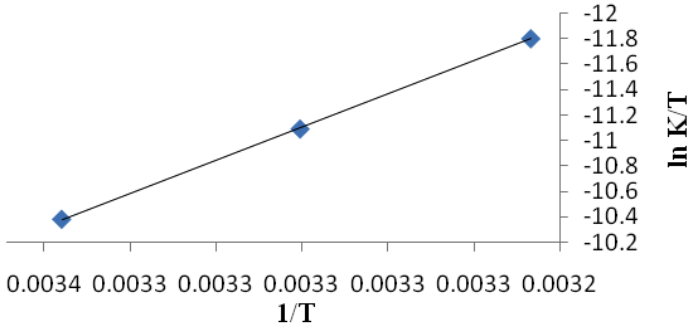


Figure 8. Eyring plot of log k/T versus 1/T at 298.0, 303.0, and 308.0 K for determination of ΔH and ΔS.

Stability indication of the method

The proposed method is based mainly on the presence of thiol group which is oxidized by MnO_4^-/OH^- system; hence the absence of thiol group by oxidation for instance will stop the reaction pathway. Previous report reveals that mesna is highly sensitive to oxidation and rapidly decomposes on contact with oxygen to form dimesna, (Fig.9) which would not be further oxidized [12]. Hence, it is concluded that, the proposed method can be considered as a stability-indicating assay of mesna.

$NaSO_3$ -CH_2 -CH_2 -S-S-CH_2 -CH_2 -SO_3 Na

Fig.9: Structure of dimesna

Conclusion

The proposed method is simple, accurate, low cost, environmentally friendly, applied successfully for the determination of mesna in its drug substance as well as in its drug product.

From the above study some specific advantages in the application of kinetic method over the other spectroscopic methods can be expected [13]:

1. Selectivity due to the measurement of the evolution of the absorbance with the time of reaction instead of the measure of a concrete absorbance value.

2. Possibility of no interference of other absorbent active compound present in the commercial product, if they are exhibiting stability in the chemical reaction conditions established for the proposed kinetic method.

3. Possibility of no interference of the colored and/or turbidity back ground of the sample.

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