European Journal of Chemistry 4 (3) (2013) 220-225

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Kinetic fluorimetric determination of Mesna (Sodium-2-mercaptoethane sulfonate) in drug products through oxidation with cerium(IV)

Mohamed Rizk [a], Elham Anwer Taha [b], Shereen Mowaka [c,*] and Youmna Mosaad Abdallah [b]

[a] *Department of Analytical Chemistry, Faculty of Pharmacy, Helwan University, Cairo, 11795, Egypt*
[b] *National Organization for Drug Control and Research, El Agoza, Giza, 12311, Egypt*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy, British University in Egypt, Cairo, 11837, Egypt*

**Corresponding author at: Department of Analytical Chemistry, Faculty of Pharmacy, British University in Egypt, Cairo, 11837, Egypt. Tel.: +2.02.26890000; fax: +2.02.26300010. E-mail address: magshx@yahoo.com (S. Mowaka).*

ARTICLE INFORMATION

Received: 01 April 2013
Received in revised form: 26 April 2013
Accepted: 01 May 2013
Online: 30 September 2013

KEYWORDS

Mesna
Uromes
Oxidation
Cerium(IV)
Quantum yield
Kinetic fluorimetric determination

ABSTRACT

A simple and sensitive kinetic spectrofluorimetric method was developed for the determination of mesna in drug products. The method is based on oxidation of the studied drug with cerium(IV) ammonium sulphate in acidic medium. The fluorescence of the produced Ce(III) was measured at 359 nm after excitation at 259 nm. The method involves determination of mesna by kinetic study of its oxidation at 100 °C for a fixed time of 20 min. The different experimental parameters affecting development and stability of reaction product were carefully studied and optimized. The fluorescence-concentration plot was rectilinear over the concentration range of 0.50-4.00 ng/mL, with a minimum detectability of 0.17 ng/mL. The method was successfully applied for the determination of mesna in its drug product. The results obtained were found to be in good agreement with those obtained with an official method of the investigated drug. The validity of the method was assessed according to USP guidelines.

1. Introduction

Mesna is an effective compound used for the prophylaxis of urothelial toxicity (uroprotector) in patients being treated with ifosfamide and cyclophosphamide. Unchanged mesna has free thiol group that reacts with ifosfamide and cyclophosphamide including acrolein considered to be responsible for 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 determination of studied drug, the reported method for mesna includes High Performance Liquid Chromatography (HPLC-UV) [2], HPLC with electrochemical detection [3] Vibrational Spectroscopic studies [4], HPLC-with fluorescence detection [5], Spectrophotometric method [6, 7] Kinetic Spectrophotometric method [8].

The proposed method depends on the use of Ce(IV) that is a strong oxidizing agent, for determination of mesna. Cerium was used before for determination of many pharmaceutical compounds, such as captopril [9], mucolytic drugs [10], antivirals [11], some psychoactive drugs [12], aztreonam [13], isoxsuprine hydrochloride [14] and calcium channel blockers using kinetic fluorimetric technique [15]. The suggested fluorimetric method is based on the oxidation of mesna with Ce(IV) in presence of sulphuric acid and the relative fluorescence intensity of the reduced Ce(III) was measured at 359 nm after excitation at 259 nm.

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

Kinetic-based analytical methods for analysis are not widely applied, although they offer the advantage of eliminating additive interference, which probably affect other methods such as titrations and spectrophotometric ones.

The proposed method offers the advantages of simplicity, and high sensitivity for the determination of the studied drug in drug substance and drug product.

2. Experimental

2.1. Instrumentation

The fluorescence spectra and measurements were recorded using JASCO FP -6200- Spectrofluorimeter equipped with a 150 W xenon lamp and using 1.0 cm quartz cells. Instrument excitation and emission slits both adjusted to 5 nm.

2.2. Reagents and materials

All chemicals used were of analytical reagent grade and all solvents were of Analytical Reagent Grade. Cerium(IV) ammonium sulphate (Merck, Darmstadt, Germany): 2.30 x10^{-3} M aqueous solution, prepared in 1.00 M sulphuric acid, the solution was stable for 5 days if kept at 4 °C. Sulphuric acid (BDH, Poole Dorset, UK): 5 M aqueous solution. Distilled water from "Aquatron" Automotive water Still A 4000 (bibby Sterillin Ltd, Staffordshire, UK). Mesna (Batch no. ME-10037, purity was found to be 99.90 % according to official method of analysis [16], manufactured ad kindly supplied by EMIC United Pharmaceuticals, Cairo, Egypt and the pharmaceutical preparation Uromes 400 mg/4 mL (Batch no. 310166, manufactured by EMIC United Pharmaceuticals).

Stock solution of mesna was prepared by dissolving 10.00 mg of mesna in 100 mL distilled water (0.10 mg/mL), working solution was prepared daily by dilution of 0.10 mL from stock solution to 100 mL distilled water, other concentrations were

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.4.3.220-225.785

prepared by further dilution with water. The stock solution was found to be stable for 5 days if kept in a refrigerator.

2.3. General procedures

2.3.1. Construction of the calibration graph

Aliquot volumes of the drug working solution equivalent to 5.00 - 40.00 ng of mesna were transferred into a series of 10 mL volumetric flask, 2 mL of 5M H_2SO_4 was added followed by 0.30 mL of 2.30x10^{-3} M Ce(IV). The flasks were heated in a thermostatically controlled water-bath at 100 ^{o}C for 20 min. The solution was well mixed, cooled and then completed to volume with distilled water. A blank experiment was performed simultaneously. The relative fluorescence intensity (FI) of the solution was measured at 359 nm after excitation at 259 nm. The reaction order was obtained by plotting log reaction rate ($\Delta FI/\Delta t$) over the specified time period *versus* log concentration of drug. The calibration graph and the regression equations were obtained by plotting relative fluorescence intensity (FI) at specified time *versus* concentration of drug in µg/mL.

2.3.2. Determination of mesna in drug product

About 5 ampoules of sample injection solution, Uromes, were mixed. 0.10 mL of sample injection solution equivalent to 10.00 mg of mesna was accurately transferred into 100 mL volumetric flask. The content of the flask was completed to 100 mL with water, then 0.10 mL was diluted to 100 mL with water. Then 1.00 mL 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 regression equation.

3. Results and discussion

Ce(IV) is a very stable oxidizing agent, its high oxidation potential (E^{o}) and excellent solution stability promoted us to use this reagent in determination of mesna. Mesna is a saturated aliphatic sulphonate, HS-CH_2-CH_2-SO_3 Na, possesses low molar absorbitivity, therefore, in this manuscript a fluorimetric method was suggested for its determination via oxidation. As the fluorescence intensity of the formed Ce(III) increases with the time, this fact was used as a basis for a useful kinetic method for quantitative determination of mesna, since mesna, as all thiols, was susceptible to oxidation by many oxidizing agents, the nature of the oxidation product depends on the oxidizing agent used [17]. In the present study, oxidation of the studied drug with Ce(IV) in acid medium yields an equivalent amount of fluorescent Ce(III) which exhibits maximum fluorescence at 359 nm after excitation at 259 nm. Figure 1 illustrates the resulting fluorescence spectra of the produced Ce(III) in acidic medium. The oxidation product was found to be non fluorescent product. This confirmed the fluorescence induced in the oxidation of investigated drug with Ce(IV) was not attributed to their oxidation products; however; it was mainly due to the formation of Ce(III).

3.1. Study of experimental parameters

The different experimental parameters affecting the formation of Ce(III) were studied such as the Ce(IV) concentration, type of acid and its concentration, heating time, temperature and diluting solvents.

3.1.1. Effect of Ce (IV) concentration

The effect of Ce(IV) concentration was studied using increasing volumes of 2.30 x 10^{-3} M Ce(IV). It was found that the maximum and constant fluorescence intensity was attained upon using 0.30 mL of 2.30 x 10^{-3} M Ce(IV), as shown in Figure 2. As at lower concentration the fluoresecence intensity decreases due to the insufficient concentration, on the other hand, higher concentration of Ce(IV) was reported to probably quench fluorescence thus decreases the detected fluorescence intensity [9,18-21].

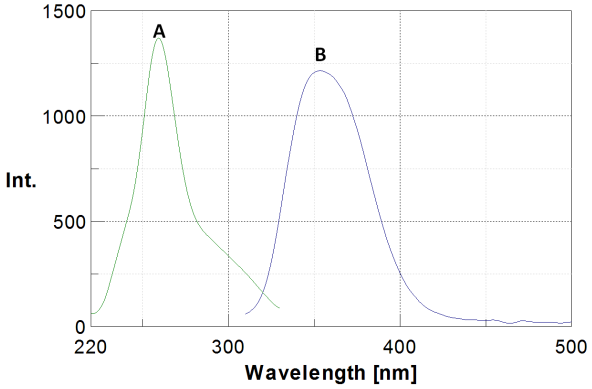

Figure 1. Excitation and emission spectra of induced Ce(III) by oxidation of 4 ng/mL mesna with 0.3 mL of 2.3x10^{-3} M Ce(IV) at 100 ^{o}C for 20 min. using water as solvent, where: (A) Excitation spectrum when λ_{Em} was 359 nm; (B) Emission spectrum when λ_{Ex} was 259 nm, and slit width was 5 nm.

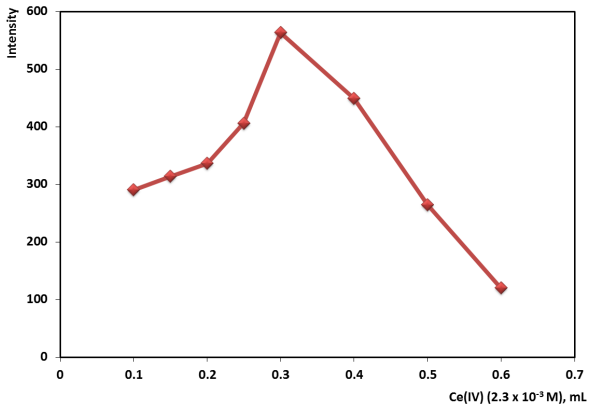

Figure 2. Effect of volume of Ce(IV) (2.30 x 10^{-3} M) on the fluorescence intensity from oxidation of mesna 4.00 ng/mL by Ce(IV).

3.1.2. Effect of acid type and its concentration

The oxidation reaction of Ce(IV) have to be performed in acid medium to prevent precipitation of $Ce(OH)_3$. Different acids such as, sulphuric acid, hydrochloric acid, nitric acid and acetic acid were tested. As shown in Table 1, nitric acid gave lowest fluorescence intensity; this was attributed to the inhibitory effect of nitrate ions on fluorescence of Ce(III) [22]. In the presence of hydrochloric acid and sulphuric acid, the reaction rate and the fluorescence of Ce(III) were found to be high. However, hydrochloric acid gave high blank readings, so sulphuric acid was selected for this study. The effect of sulphuric acid concentration on the fluorescence intensity was studied using conentration range from 0.50-15.00 M sulphuric acid as shown in Figure 3 and Table 1.

3.1.3. Effect of temperature and heating time

In order to accelerate the reaction, oxidation of the studied drug with Ce(IV) was carried out at different temperature setting, using a thermostatically controlled water bath, ranged

from ambient temperature, 40, 60, 80 °C and boiling water bath for the periods of time ranging from 5 to 60 min, as shown in Figure 4. The results revealed that the optimum temperature was at 100 °C and the optimum heating time was 20 min as prolonged heating time has no effect on the measured fluorescence intensity. Therefore, heating for 20 min at 100 °C in a thermostatically controlled water bath was selected for full fluorescence development, followed by cooling to ambient temperature before measurement.

Table 1. Effect of type of acid medium and diluting solvent on the fluorescence intensity induced from oxidation of mesna by Ce(IV) [a].

Parameter	Fluorescence intensity
Acid medium [b]	
Sulphuric	610
Hydrochloric	333
Acetic	139
Nitric	19
Diluting solvents	
Water	610
Methanol	325
Ethanol	301
Acetonitrile	187

[a] The concentration of the drug was 4.00 ng/mL and that of Ce(IV) was 2.30×10^{-3} M.
[b] The concentration of acids were 5 M.

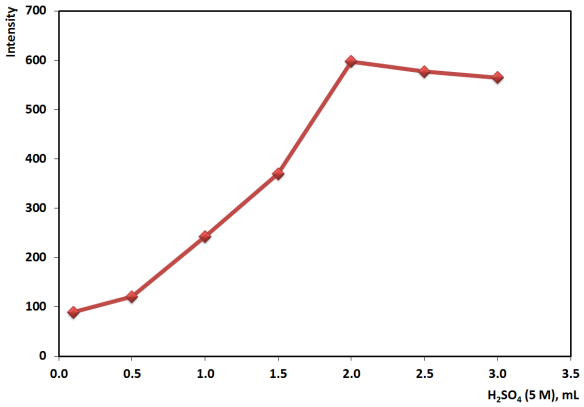

Figure 3. Effect of volume of 5.00 M H_2SO_4 on the fluorescence intensity from oxidation of mesna 4.00 ng/mL by Ce(IV).

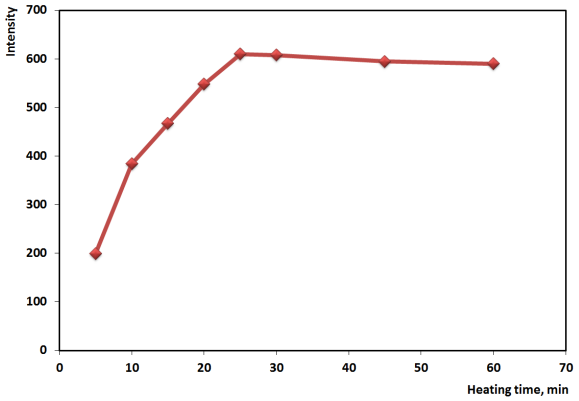

Figure 4. Effect of heating time at 100 °C on the fluorescence intensity from oxidation of mesna 4.00 ng/mL by Ce(IV).

3.1.4. Effect of diluting solvents

Dilution with different solvents such as water, methanol, ethanol, acetonitrile was attempted. It was found that, water was the best solvent for dilution as it gave the highest fluorescence intensities and the lowest blank reading, moreover its choice adds to advantages of the proposed method. Distinct and sharp decrease in the fluorescence intensities was attained upon using methanol, ethanol and acetonitrile as shown in Table 1.

3.2. Evaluation of the kinetic parameters

The rate of the reaction was found to be dependent on the concentration of the studied drug. The rate was followed with various concentrations in the range of 0.50-4.00 ng/mL keeping Ce(IV) and H_2SO_4 acid concentrations constant at the recommended levels mentioned before. The rate of the reaction obeys the equation (1).

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

where K` is the pseudo-order rate constant, C is the drug concentration and n is the order of the reaction.

The rate of the reaction may be estimated by variable time measurement [23] where FI is the fluorescence intensity and t is the time in seconds, taking logarithms of rates and drug concentrations, the equation (1) it transformed into:

$$\text{Log } V = \log \Delta FI/\Delta t = \log K` + n \log C \quad (2)$$

where V is the reaction rate. Plot of log reaction rate versus log drug concentration is shown in Figure 5 at 359 nm after excitation at 259 nm, where the slop (n) is the order of the reaction and intercept (Log K`) is the logarithm of the rate constant.

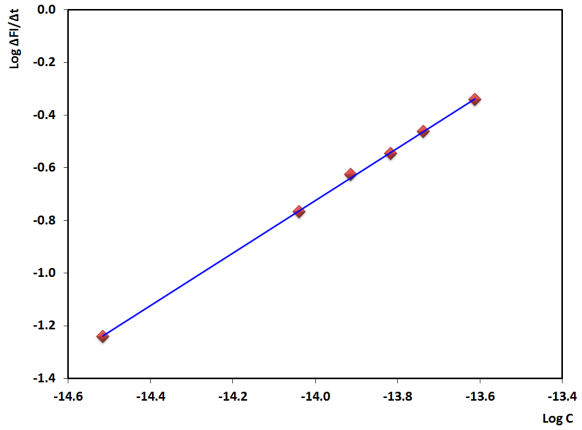

Figure 5. Plot of Log reaction rate (Log ΔFI/Δt) versus Log concentration (log C, M) of mesna.

A plot of log reaction rate versus log drug concentration resulted in a pseudo order rate constant and first order of the reaction as stated in the following regression equation (3).

$$\text{Log } V = 13.24 + 0.997 \log C \ (r = 0.9998) \quad (3)$$

hence $K` = 1.73 \times 10^{13}$ and the reaction is first order (n = 0.997).

These results indicate that the reaction is pseudo first order reaction, depending on the drug concentration.

3.3. Selection of the best kinetic method

Several kinetic techniques were adopted for the selection of the best method. Rate constant, fixed fluorescence and fixed time methods [12] were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, i.e. the slope of calibration graph and the correlation coefficient (*r*).

3.3.1. Rate constant method

Graphs of log fluorescence intensity versus time for mesna concentration in the range 1.21 $x10^{-14}$- $1.82x10^{-14}$ M were plotted and all appeared to be rectilinear. Pseudo-first order rate constant (K`) corresponding to different drug concentrations were calculated from the slopes multiplied by -2.303 [24] and are presented in Table 2.

Regression of (C) versus K` gave equation (4).

$$K` = -145.3 + 3x10^{15}\ C \quad (r = 0.9990) \qquad (4)$$

where C is the molar concentration of the drug.

Table 2. Application of the rate constant method in the quantification of the studied drug by proposed method.

C	K` (s^{-1}) *
$1.21x10^{-14}$	-110.45
$1.52x10^{-14}$	-102.09
$1.83x10^{-14}$	-93.05

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

3.3.2. Fixed fluorescence method

Reaction times required to reach specific fluorescence of redox reaction for different concentrations of mesna in the range of $6.09x10^{-15}$ -1.82 x 10^{-14} M. A preselected value of fluorescence intensity 180 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 equation (5) was obtained.

$$1/t = -5.0x10^{-4} + 11x10^{10}\ C \quad (r = 0.9960) \quad (5)$$

where C is the molar concentration of the drug and t is time in second.

Table 3. Application of the fixed fluorescence method in the quantification of the studied drug by proposed fluorimetric method.

[Drug]	1/t (s^{-1})
$6.09x10^{-15}$	$1.30x10^{-3}$
$1.22x10^{-14}$	$1.93x10^{-3}$
$1.52x10^{-14}$	$2.31x10^{-3}$
$1.83x10^{-14}$	$2.71x10^{-3}$

3.3.3. Fixed time method

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

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

As a conclusion, the fixed time method was chosen for quantification because it gave the best correlation coefficient in a reasonable time of 20 min.

Table 4. Application of the fixed time method in the quantification of the studied drug by the proposed fluorimetric method.

Time (min)	Regression equation	Correlation coefficient
5	FI= 0.006+2762C	0.9924
10	FI= 3.865+4271C	0.9939
15	FI= 7.790+4737C	0.9949
20	FI= 11.10+5003C	0.9994
25	FI= 14.08+4933C	0.9984

3.4. Validation criteria

Validation of the proposed method was assessed according to USP guidelines [25] for linearity and range, accuracy, precision, detection limit, quantitation limit and robustness.

3.4.1. Linearity range

The fluorescence-concentration plot for the studied drug was linear over the range of 0.50-4.00 ng/mL (Table 5). Linear regression analysis of the data gave the equation (6).

$$FI = 11.10+5003C \quad (r = 0.9994) \qquad (6)$$

where FI is the fluorescence intensity, C is the concentration of mesna in ng/mL and r is correlation coefficient.

The statistical analysis of regression line regarding standard deviation of the residual ($S_{y/x}$), intercept (S_a) and slope (S_b) were evaluated. The small values of the figures point out to the low scattering of the points around the calibration curve.

The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation, the results are abridged in Table 5.

Table 5. Analytical performance data of the proposed method.

Parameters *	Results of the proposed method
Linearity range (ng/mL)	0.50- 4.00
Limit of detection (ng/mL)	0.17
Limit of quantification	0.50
Correlation coefficient	0.9994
Slope	5003.6
Intercept	11.100
$S_{y/x}$	0.178
S_a	0.257
S_b	76.120
(%RSD)	0.516
%Er	0.211

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

3.4.2. Precision and accuracy

The intra-day precision was evaluated through replicate analysis of drug sample of 1.00, 1.50 and 4.00 ng/mL. Each concentration was analyzed three times. The mean percentage recoveries are shown in Table 6. The repeatability and reproducibility of the proposed method are good as indicated by small value of standard deviation (SD).

The inter-day precision was evaluated through replicate analysis of 1 ng/mL mesna on three successive days. The percentage recoveries based on average of three separate determinations are abridged in Table 6.

The accuracy of the proposed method was evaluated by analyzing standard solution of studied drug. The results obtained by this proposed method were compared with those given by the official method [16].

3.4.3. Detection limit and quantitation limit

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to USP 34 recommendations, below which the calibration graph is non linear and was found to be 0.50 ng/mL. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected and was found to be 0.17 ng/mL. LOQ and LOD were calculated according to the equations (7) and (8).

$$LOQ = 10\ ó\ /S \qquad (7)$$

$$LOD = 3.3\ ó\ /S \qquad (8)$$

where ó is the standard deviation of the intercept of regression line and S is the slope of the calibration curve.

Table 6. Precision and accuracy of the proposed method for the spectrofluorimetric determination of pure mesna *.

Parameters	Amount taken (ng/mL)	Recovery (%)
Intra-day "repeatability"		
	1.0	101.50
		102.60
		100.80
x±S.D.		101.75±1.04
	1.5	98.30
		98.50
		98.60
x±S.D.		98.48±0.15
	4.0	101.10
		101.00
		100.30
x±S.D.		100.80±0.47
Inter-day "ruggedness"		
1st day	1.0	102.60
2nd day		99.10
3rd day		101.80
x±S.D		101.20±1.91

* Each result is the average of three different separate determinations.

Table 7. Application of the proposed method for the determination of mesna in its drug substance.

Parameters	Amount taken (ng/mL)	Proposed method		Official method [16]
		Amount found (ng/mL)	Recovery [a] (%)	Recovery (%)
	1.00	1.01	101.75	
	1.50	1.47	98.48	
	4.00	4.03	100.84	
x±S.D.			100.30±1.68	100.90±1.50
t			0.406 (2.77) [b]	
F			1.16 (19.00) [b]	

[a] Average of three different separate determinations.
[b] Figures in parentheses are the tabulated t and F values, respectively at $p = 0.05$ [27].

Table 8. Application of the proposed method for the determination of the studied drug in its pharmaceutical product.

Parameters	Amount taken (ng/mL)	Proposed method		Comparison method [26]
		Amount found (ng/mL)	Recovery [a] (%)	Recovery (%)
Uromes 100 mg/mL	1.00	1.01	100.96	
	1.50	1.48	98.87	
	2.00	1.99	99.96	
x±S.D.			99.93±1.04	98.26±1.1
t			0.88 (2.77) [b]	
F			1.01 (19.00) [b]	

[a] Average of three different separate determinations.
[b] Figures in parentheses are the tabulated t and F values, respectively at $p = 0.05$ [27].

3.4.4. Robustness of the method

The robustness of the method adopted in the proposed method is demonstrated by the constancy of the fluorescence intensity with minor changes in experimental parameters such as 2.30×10^{-3} M Ce(IV) volume, 0.3±0.02 mL and change in the concentration of sulphuric acid, 2±0.2 M. These minor changes didn't affect the fluorescence intensity.

3.5. Pharmaceutical application

The proposed method was successfully applied for the determination of mesna in its drug substance, to prove the accurate analytical performance of the method, and drug product (Table 7 and 8). The specificity of the method was investigated by observing any interference encountered from the common excipients, such as disodium edetate, sodium hydroxide. These exicipients didn`t interfere with the proposed method (Table 8). The results obtained were in a good agreement with those of the official and manufacturer methods [16,26].

Statistical analysis of the obtained results revealed no significant difference regarding the accuracy and precision as indicated by the student t-test and F-test [17], as shown in Table 8.

3.6. Mechanism and stoichiometry of the reaction

The data used in the optimization of Ce(IV) concentration and the data of calibration graphs were used to calculate the stoichiometry of the reaction adopting the limiting logarithmic method [9] as shown in Figure 6. The ratio of the reaction between mesna and Ce(IV) in acidic medium was calculated by dividing the slope of Ce(IV) curve over the slope of the drug curve. It was found that the ratio was 0.211:0.433 pointing out to a ratio of 1:2 (Ce(IV) to drug). Based on the obtained molar reactivity, the reaction pathway is proposed to proceed as follows:

$$2\ \text{R-SH} + 2\text{Ce}^{+4} \rightarrow \text{R-S-S-R} + 2\text{Ce}^{+3} + 2\text{H}^{+} \quad (9)$$

3.7. Quantum yield (Φ)

Determination of quantum yield of produced Ce(III) was obtained applying the equation (10) [29]:

$$\Phi u = [\Phi s Fu/Fs] \times [As/Au] \quad (10)$$

where Φu and Φs referred to the fluorescence quantum yields of the drug and quinine sulphate (QS), respectively; Fu and Fs represented the integral fluorescence intensity of the drug and QS, respectively; Au and As referred to the absorbance of the drug and QS at the excitation wavelength, respectively.

Dilute solution of quinine sulfate dissolved in 0.05 M sulfuric acid with fluorescence quantum yield of 0.543 was used as reference reagent. The concentration was selected so that the absorbance was less than 0.05 to minimize error arising from inner effect [28]. The fluorescence quantum yield obtained in the present work was found to be 0.899.

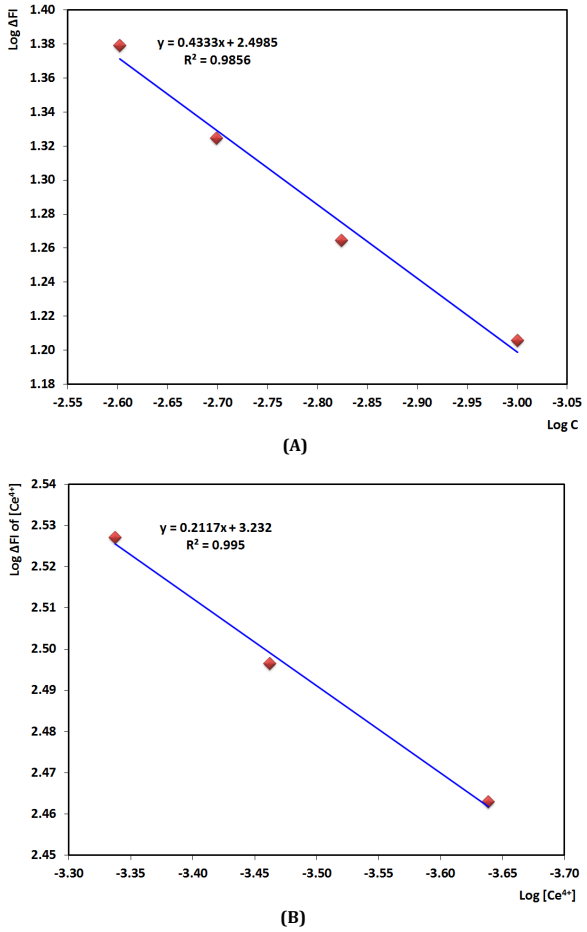

Figure 6. Stoichiometry of the reaction between mesna and Ce(IV) adopting limiting logarithmic method [28]. (A) Variable concentrations of mesna at constant Ce(IV) concentration; (B) Variable concentrations of Ce(IV) at constant mesna concentration.

4. Conclusions

The proposed method for determination of mensa is simple, accurate, and more economical with regards to solvent and reagent consumption without loss of accuracy and precision. Hence it can be routinely applied for the determination of mesna in any drug product.

References

[1]. Reynolds, J. E. F. Martindale The Extra Pharmacopoeia, 29th edition, Pharmaceutical Press, London, 1989.
[2]. Glowackia, R.; Wojcika, K.; Bald, E. *J Chromatogr. A* **2001**, *914*, 29-35.
[3]. Verschraagen, M.; Bosma, M.; Ursula, Z. T.; Torun, E.; Van Dervijgh, W. J. F. *J. Chromatogr. B* **2003**, *783,* 33-42.
[4]. El-Yazigi, A.; Ahmed, Y.; Sameer, A. R. *Ther. Drug Monit.* **1995**, *17*, 153-158.
[5]. Mare, S.; Penugonda, S.; Ercal, N. *Biomed. Chromatogr.* **2005,** *19*, 80–86.
[6]. Skowron, M.; Ciesielski, W. *Chem. Anal. -Warsaw* **2009**, *54*, 743-751.
[7]. Ahmed, N. R. *Iraqi Nat. J. Chem.* **2011**, *44*, 492-500.
[8]. Rizk, M.; Taha, E. A.; Mowaka, S.; Abdallah, Y. M. *Chem. Sci. Rev. Lett.* **2012**, *1*, 140-147.
[9]. El-Didamony, A. M. *J. Chin. Chem. Soc.* **2009**, *56*, 755-762.
[10]. Taha, E. A.; Hasan, N. Y.; Abdel Aal, F.; Abdel Fattah, L. E. *B. Fac. Pharm. Cairo Univ.* **2005**, *43*, 9-22.
[11]. Darwish, I. A.; Khedra, A. S.; Askala, H. F.; Mahmoud, R. M. *Farmaco* **2005**, *60*, 555-562.
[12]. Mohamed, F. A.; Mohamed, H. A.; Hussein, S. A.; Ahmed, S. A. *J. Pharmceut. Biomed. Anal.* **2005**, *39*, 139-146.
[13]. Mahgoub, H. *J. Pharmceut. Biomed. Anal.* **2003**, *31*, 767-774.
[14]. Alarafaj, N. A. A. *J. Pharmaceut. Biomed. Anal.* **2002**, *28*, 331-335.
[15]. Walash, M. I.; Belal, F.; El Enany, N.; Abdelal, A. A. *Int. J. Biomed. Sci.* **2009**, *5*, 146-157.
[16]. The United States Pharmacopeia, USP 34, National Formulary, The United States Pharmacopeial Convention, Inc., Rockville, 3433-3434.
[17]. Trost, B. M.; Ley, S. V.; Fleming, I. Comprehensive Organic Synthesis, 1st ed., Pergamon Press, London, 1991.
[18]. Tabrizi, A. B. *B. Kor. Chem. Soc.* **2006**, *27*, 1199-1202.
[19]. Ramadan, N. K. *J. Appl. Pharm. Sci.* **2012**, *2*, 112-119.
[20]. El-Didamony, A. M. *J. Chil. Chem. Soc.* **2012**, *57*, 1404-1408.
[21]. Suganthi, A. *Int. J. Pharm. Chem. Sci.* **2013**, *2*, 938-941.
[22]. Jeffery, G. H.; Bassett, J.; Mendham, J.; Dennery, R. C. Vogel's Textbook of Quantitative Chemical Analysis, 5th ed., Bath Press: England, 1989.
[23]. Yatsimirskii, K. B. Kinetic Methods of Analysis, Pergamon Press, London, 1966.
[24]. Martin, A. Physical Pharmacy, 3rd ed. , Lea & Febiger, USA, 1983.
[25]. The United States Pharmacopeia, USP 34, The National Formulary, The United States Pharmacopeial Convention, Inc., Rockville, 779-782.
[26]. Manufacturer (EMIC) Method of analysis of mesna in Uromes ampoule by personal communication.
[27]. Calcut, R.; Boddy, R. Statistics for Analytical Chemists, Chapmann & Hall, London, 1983.
[28]. Rose, J. Advanced Physicochemical Experiments, Pitman, London, 1964.
[29]. Tang, B.; Wang, X.; Jia, B.; Niu, J.; Ying, Y. W.; Chen, Z.; Wang, Y. *Anal. Lett.* **2003**, *36*, 2985-2997.