

An Indirect Spectrophotometric Determination of Mesna in Pharmaceuticals and Environmental Samples

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Abstract

Two indirect spectrophotometric methods were proposed for the determination of mesna in pure and pharmaceutical preparations using sodium hypochlorite and two indicators, methyl orange and congo red. The methods involved addition of a known excess of sodium hypochlorite to mesna in acidic medium, followed by determination of residual sodium hypochlorite by reacting with a fixed amount of either methyl orange, measuring the absorbance at 505nm (method A) or congo red measuring the absorbance at 605nm (method B). In both methods the amount of sodium hypochlorite reacted correspond to the amount of mesna and the measured absorbance was found to increase linearly with the concentration of mesna, which is corroborated by the correlation coefficient of 0.9996 and 0.9998 for method A and B respectively. The two methods obey Beer's law for 0.08-1.6 $\mu\text{g mL}^{-1}$ and 0.2-2.0 $\mu\text{g mL}^{-1}$ for method A and B respectively. The apparent molar absorptivities were calculated to be 1.02×10^4 and $1.5 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ for method A and method B respectively. The limit of detection (LOD) and quantification (LOQ) were calculated to be 0.016 and 0.05 $\mu\text{g mL}^{-1}$ for method A and 0.048 and 0.15 $\mu\text{g mL}^{-1}$ for method B. The intra-day and inter-day precision and accuracy of the two methods were evaluated according to the current ICH guidelines. Both methods of comparable accuracy (relative error) better than $\pm 1.8\%$. and precision of $< 2\%$. The proposed method was proved to be selective and applied to the determination of mesna in pharmaceutical preparations and environmental water samples.

Keywords: Mesna, Spectrophotometry, Pharmaceutical preparations, Environmental samples.

() 605

0.9998 0.9996

0.2-2.0 0.08-1.6

1.5×10^3 1.2×10^4

0.05 0.016

1- 1-

/ 0.15 0.048

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. %2 % 1.6 ()

Introduction

Mesna, (sodium 2-sulfanylethane sulfonate) with a molecular formula of $C_2H_5NaO_3S_2$ and molecular weight of (164.18 $g \text{ mol}^{-1}$). Its structural formula is as follows:



A nucleophilic thiol doner often used for the prevention of urothelial toxicity in patients treated with the antineoplastics ifosfamide or cyclophosphamid. In the kidney, dimesna, the inactive metabolite of mesna is redused to free mesna which has thiol groups that react with the metabolited of ifosfamide and cyclophosphamide, including acrolein, considered to be responsible for the toxic effect on the bladder⁽¹⁻³⁾.

The aim of mesna therapy is to ensure adequate levels of mesna in the urine throughout the period during which these toxic metabolites, are present and the duration of mesna treatment should therefore equal that of the antineoplastic treatment. Plus the time taken for the concentration of antineoplastic metablites in the urine to fall to non-toxic levels. Urinary output should be maintained and the urine monitored for hoematuria and

proteinuria throughout the treatment period. However, frequent emptying of the bladder should be avoided. recently mesna used as antioxidant agent against acetaminophen toxicity⁽⁴⁾. Mesna is rapidly oxidized to dimesna, so in pharmaceutical formulation it must be stabilized using EDTA, sodium hydroxide and inert gas atmosphere. The reducing character of mesna should be taken into account in the design of any analytical methods⁽⁵⁾. The literatures revealed that mesna has been determined by means of a few analytical methods. These include: HPLC⁽⁵⁻⁸⁾. Chemiluminescence-flow injection analysis⁽⁹⁾. B.P method described a titrimetric assay for pure drug only⁽¹⁰⁾. The present work is to provide a simple, sensitive spectrophotometric method for the determination of mesna in dosage forms and water samples, utilize sodium hypochlorite and two dyes methyl orange and congo red.

Experimental

1- Apparatus

Optima SP 3000 plus UV-Visible spectrophotometer (Optima .Japan) with 1.0 cm quartz cells was used for the absorbance measurements.

2- Reagents

All chemicals used were of analytical purity grade and all solutions were prepared with distilled water. A standard sodium hypochlorite solution (0.1%) was prepared by dilution of 1.25mL of 8% sodium hypochlorite to 100 mL with distilled water, store in a dark bottle and standardized iodometrically every 4-5 days. This solution stored in a dark bottle⁽¹¹⁾.

Congo red: 0.1 % was prepared by dissolving 0.1 g accurately weighed dye in water, and diluting to 100 mL in volumetric flask.

Methyl orange 0.1% was prepared by dissolving 0.1 g accurately weighed dye in water and diluting it to 100 mL in volumetric flask.

Standard material and pharmaceutical preparations (mesnan tablet 400 mg, and mesnan 100mg injection) were provided from state company of drug industries and medical appliance (NDI) Ninavah- Iraq . Standard solution of mesna ($100\mu\text{g ml}^{-1}$).

This solution was prepared by dissolving 0.1 g of pure drug in 1L distilled water. It was later diluted with water to get concentration of $10\mu\text{g ml}^{-1}$.

3 -Analytical procedures

Method A:-

Different aliquots of standard mesna solution equivalent 2-40 μg were transferred into a series of 25mL volumetric flasks, 1mL of 1N HCL, and 5 ml of sodium hypochlorite solution were added. The content was mixed and let stand for 5 min with occasional shaking. Finally, 1mL of 0.1% methyl orange solution was

added and the volume was diluted to the mark with distilled water and mixed well. The absorbance of each solution was measured at 505 nm against a reagent blank .

Method B:-

Different aliquots of standard solution equivalent to 5-50 μg of mesna solution were transferred into a series of 25 mL volumetric flask, 1mL of HCL and 3 mL of sodium hypochlorite were added. The content was mixed well and the flasks were kept aside for 5 min with intermitten shaking. Finally, 3mL of 1% congo red solution was added to each flask, the volume was diluted to the mark with distilled water, mixed well and absorbance was measured against a reagent blank at 605nm.

4- Preparation of Mesna drugs

1 .Tablets

To minimize a possible variation in the composition of the tablets, the mixed content of 20 tablets, were weighed and grounded, then the powder equivalent to 100 mg of mesna was stirred well with water for 15min and the volume was made to 1L with distilled water ,filtered through whatman No. 42 filter paper and 10 mL of this solution was diluted to 100 ml by distilled water and aliquot of this solution was treated as described above for analytical procedure.

2 Injection

1ml vial containing 100 mg of mesna was transferred into 1L volumetric flask and diluted up to the mark with distilled water, 10mL of this solution was diluted to 100mL with distilled water and a aliquot of this solution was treated as described above for analytical procedure.

3 Preparation of spiked water samples

To demonstrate the practical applicability of the proposed method, real water samples were analysed by

this method. Drinking water from the mosul water-supply net work , industrial waste water from the state company for drug industries and medical appliances Mosul-Iraq, were spiked with the concentrations ranging from 0.1-1.5mg/ml of mesna and a liquot of this solution was treated as described above for analytical procedure.

Result and Discussion

The versatility of sodium hypochlorite as an analytical reagent can be gauged by its applications in the spectrophotometric determination of many organic compounds of

therapeutic importance⁽¹²⁻¹⁶⁾. The use depends mainly on its ability to effect the oxidation of diverse functional groups. Taking advantage of the rapid oxidation reaction of sodium hypochlorite with mesna. Mesna is a reducing agent owing to the presence of thiol group(-SH) in its structure. The proposed spectrophotometric methods are indirect and based on the determination of residual sodium hypochlorite after bringing the reaction between mesna and sodium hypochlorite to completion. The residual sodium hypochlorite was determined by methyl orange or congo red indicators.



Di mesna

+ Un reacted NaOCl

Method A

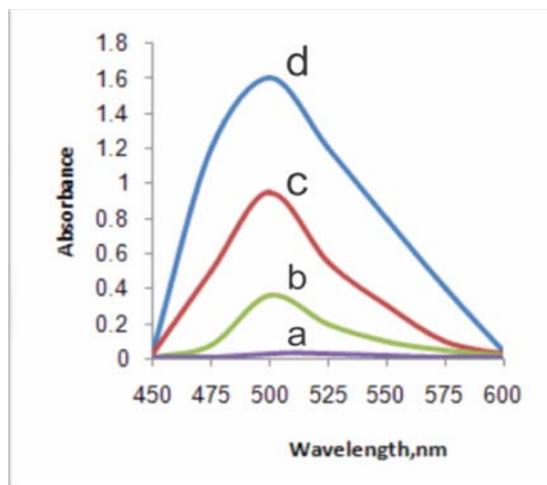
Un reacted NaOCl + Methyl orange → Bleached methyl orange (measured at 505nm)

Method B

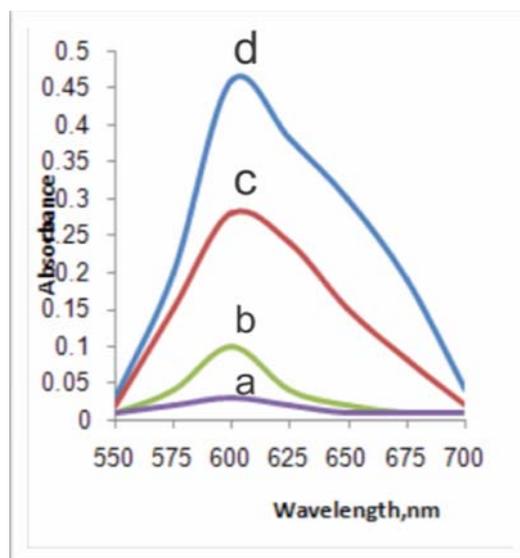
Un reacted NaOCl + Congo red → Bleached congo red (measured at 605nm)

When added in increasing concentration to a fixed concentration of sodium hypochlorite, mesna consumes the latter proportionally and there is a concomitant drop in the remaining concentration of sodium hypochlorite. When a fixed indicator

concentration is added to decreasing concentration of sodium hypochlorite, a concomitant increase in the indicator concentration result a proportional increase in absorbance at the respective λ max is observed with increasing concentration of mesna. Fig (1 and 2).



Fig[1]:Absorption spectra of method A; a ,reagent blank against water.,b,c and d after addition of 10,30 and 50µg of mesna.



Fig[2]:Absorption spectra of methodB ; a ,reagent blank against water.,b,c and d after addition of 10,30 and 50µg of mesna.

Preliminary experiments were performed to fix the upper concentration of the indicator that could be determined spectrophotometrically and this was found to be 1 and 3mL of 0.1% of methyl orange and congo red respectively. A sodium hypochlorite concentration 500 µg was found to bleach the red color due to 100 µg of methyl orange where as 300µg was required to destroy the blue color due to 300µg congo red. Hence, different

concentration of mesna were reacted with hypochlorite solution 5 ml of 0.1% method A and 3mL of 0.1% sodium hypochlorite in method B, followed by determination of residual sodium hypochlorite under the recommended procedure for both methods. The reaction between mesna and sodium hypochlorite and the determination of the latter by reacting with the indicators. HCL medium (1mL of 1N) was found to be ideally suited. Reaction time of 5mint is not

critical and any delay up to 6h did not affect the absorbance reading.

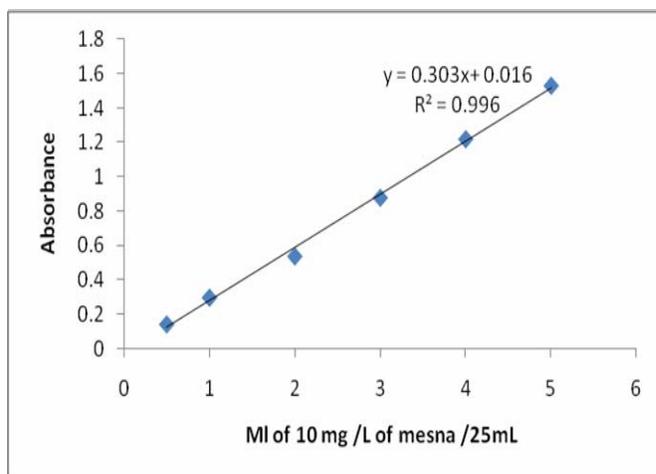
3-1 Method Validation

Under the optimized conditions, A liner correlation was found between absorbance at λ max and mesna concentration. Fig (3 and 4).

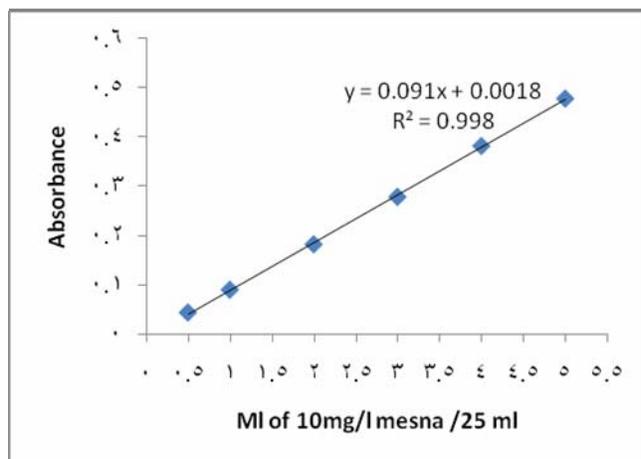
$$A = 0.016 + 0.303 C \quad R = 0.996 \quad n = 6 \text{ (method A)}$$

$$A = 0.0018 + 0.091C \quad R = 0.998 \quad n = 6 \text{ (method B)}$$

Where A is the absorbance and C is concentration in $\mu\text{g.ml}^{-1}$, R is the regression coefficient and n is the number of concentration levels.



Fig[3]: Calibration curve of mesna with methyl orange.



Fig[4]: Calibration curve of mesna with congo red

Table 1: Method validation of the spectrophotometric determination of mesna.

Parameter	Method A	Method B
λ_{\max} (nm)	505	605
Beer's law limit ($\mu\text{g mL}^{-1}$)	0.08-1.6	0.2-2
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.02×10^4	1.5×10^3
Limit of detection ($\mu\text{g mL}^{-1}$)	0.016	0.05
Limit of quantification	0.048	0.15
Intercept	0.016	0.0018
Slope	0.303	0.091
Correlation coefficients, (r)	0.996	0.998

The optical characteristics such as Beer's Law, limits and molar absorptivity values is given in Table (1). The limit of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines⁽¹⁷⁾ as the ratio of 3.3 and 10 standard deviations of the blank (n = 6) and the slope of the calibration line.

3-2 Accuracy and Ruggedness of the proposed methods

To evaluate the accuracy and intra-day precision of the methods a pure drug solution was analysed at two different concentrations, each determination being repeated six times. The relative error (%) and relative standard

deviation (%) values are summarized in table (2). From Table (II), it is clear that method A with a relative error of 1.3% is as accurate as method B with 1.6%. Moreover, both methods are found to be precise with RSD values < 2%. For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed, in which the standard drug solution was determined at three different levels each day for six days, with all solutions being prepared a fresh each day. The day-to-day relative standard deviation values were in the range of 1.1-1.8 % and represent the best appraisal of repeatability of the proposed methods.

Table (2) Accuracy and precision of the proposed methods.

Method	Mesna conc. taken μg	Conc. found (μg)	Er (%) ^a	RSD(%)
A	20	20.22	1.1	1.3
	40	40.52	1.3	1.8
B	10	10.12	1.2	1.5
	30	30.48	1.6	1.1

a: Mean of six determinations

3-3 Interference studies

In order to assess the possible applications of the two proposed methods, the effect of substances that often accompany with mesna in various pharmaceutical products (Tablets and injections) were studied by adding different amount of

interferents to 20 μg of mesna. An attractive feature of the method is its relative freedom from interference by the usual diluents and excipients in amounts for in excess of their normal occurrence in pharmaceutical preparations. The results are given in table (3)

Table (3) Determination of 20µg of mesna in the presence of excipients and other substances

Interfering substances	Amount of interferences taken/mg	Amount found/µg		RSD %	
		Method A	Method B	Method A	method B
Na ₂ - EDTA	1	19.8	18.9	0.78	0.68
Benzyl alcohol	1	19.5	19.6	0.88	0.64
Chlorobutanol	10	19.4	19.3	0.91	0.93
Lactose	40	19.5	19.6	0.71	0.77
Microcrystalline cellulose	20	18.9	19.5	0.64	0.79
Calcium phosphate	10	19.4	19.5	0.81	0.77
Corn starch	30	19.8	19.6	0.78	0.68
Povidone	30	18.9	19.5	0.79	0.64
Magnesium stearate	40	19.4	19.4	0.91	0.93
Hydroxypropyl methyl cellulose	40	19.5	19.3	0.93	0.91
Poly ethylene glycol	20	19.8	19.5	0.91	0.64
Titanium dioxide	10	19.4	19.5	0.88	0.68

*Average of six determinations.

3-4 Analytical applications

Table(4),(5) gives the results of the assay and reveals that there is close agreement between the results obtained by the proposed methods and the table

claim for pharmaceutical preparations and the recovery results of real water samples was higher than 98%,indicating that successfully applicability of the proposed methods.

Table (4): Determination of mesna in pharmaceutical formulations.

Pharmaceutical formulation	Label amount (mg)	Found* (mg)	
		Method A	Method B
Mesnan tablet(NDI)	400	402	401.3
Mesnan ampoule(NDI)	100	99.7	99.9

*Mean value of six determinations.

Table(5): Determination of mesna in spikedwater samples

Water samples	Mesna(mg/ml)			% Recovery(n=6)	
	Taken	Found		Method A	Method B
		Method A	Method B		
Drinking water	0.1	0.101	0.101	101	101
	1.0	0.98	0.99	98	99
	1.5	1.48	1.47	98.66	98
Industrial waste water	0.1	0.1	0.1	100	100
	1.0	1.02	1.01	102	101
	1.5	1.5	1.51	100	100.6

*Mean of ten determinations

4- Conclusions

An indirect spectrophotometric methods were established for the analysis of mesna in pure form ,pharmaceuticals and water samples

The proposed methods are simple ,rapid ,sensitive ,accurate ,selective, and no need of heating or solvent extraction. To the best of our knowledge, this is the first report of the reaction between mesna and excess of

sodium hypochlorite (NaOH) and then indirectly determined the drug after the residual hypochlorite bleaches the dyes

which absorb at different wavelength maxima.

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