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Application of Chloramine-T, Methylene Blue in the Assay of Mesna in Tablets, Injections and Wastewater Samples

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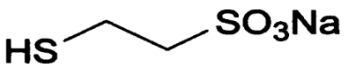
ABSTRACT

A new, accurate, sensitive and simple indirect spectrophotometric method for the estimation of sodium 2-mercaptoethanesulfonate (mesna) has been developed. This procedure base on the drug oxidation with excess Chloramine-T in acidic solution, followed by the reaction of the excess oxidants with methylene blue, and read the absorbance at 664 nm .The absorbance results increased with increasing concentration of mesna .The calibration curve was linear at the range 0.1-2.0 ppm. Apparent molar absorbance, Sandell's sensitivity values were 7.832×104 $Lmol^{-1}cm^{-1}$. And 47 $ngcm^{-2}$, respectively. The (RSD) of this method was less than 1.8 and average recovery (accuracy) is 100 ± 0.95. The method applied successfully for estimation of mesna in waste water sample and in (tablets and injections pharmaceutical formulations).

INTRODUCTION

Mesna is an important thiol compound. The chemical name is sodium 2-mercapto ethane sulfonate **Figure 1.** Mesna used as antioxidant for prevention of urothelial toxicity in patients treated by antineoplastic, cyclophosphamide or ifosfamide. In the kidney, by neutralizing the highly reactive urotoxic metabolites of oxazaphosphorines locally in the urine [1-3], mesna recently used as antioxidant against acetaminophen toxicity [4]. Mesna oxidized to disulfide, and stabilized using EDTA, sodium hydroxide and inert gas atmosphere in pharmaceutical formulation [5]. The reducing character of mesna should be considered in the design of any analytical methods [5].



$C_2H_5NaO_3S_2$=164.2

Figure 1. Chemical Structure of Mesna.

The literature revealed that mesna has been determined by means of a few analytical methods. These include these include: HPLC [5-8]. Chemiluminescence-flow injection [9], spectrophotometric methods [10-12], Spectrofluorimetric [13]. BP and USP described a tedious titrimetric estimations [14,15]. The present method work is accurate, simple, sensitive spectrophotometric method for estimation of mesna in pure, pharmaceutical dosage forms and waste water sample (Industrial waste water sample provided from the state company for drug industries and medical appliances Mosul-Iraq (NDI). Utilize Chloramine-T and methylene blue as a new reagents.

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EXPERIMENTAL

Apparatus

Optima SP 3000 plus UV-Visible spectrophotometer with 1.0 cm quartz cells was used (OPTIMA USA, INC).

Reagents

The chemical reagents used were of pharmaceutical and analytical purity grade, distilled water, ethanol were used throughout.

- **A standard Chloramine T (CAT) solution (Merck) (0.1%):** Prepared by dissolving 0.1 g in 100 mL of distilled water. This solution stored in a dark bottle [15].
- **Methylene blue (Merck) 0.01 %:** Prepared by dissolving 0.01 g by distilled water and diluting it to 100 mL in calibrated flask.
- **Sulfuric acid solution (2M)**
- **Standard solution of mesna (100 ppm):** Prepared by dissolving 0.1 g of mesna in 1 L distilled water. It was later diluted to get concentration of 10 ppm by distilled water.

Recommended Procedure

Different amount of standard solution of mesna equivalent 2.5-50 µg were transferred into a series of 25 mL calibrated flasks, 2 mL of 2M H_2SO_4, and 3 mL of CAT solution were added. Mixed well, let stand for 5 min with occasional shaking, and then added 5 mL of 0.01% methylene blue solution, the volume was diluted to the mark with distilled water. The absorbance was measured at 664 nm against a reagent blank.

Procedures for Pharmaceutical Dosage Forms

Tablets: To minimize a possible variation in the composition of the tablets (were provided from the company for pharmaceutical industries (NDI) Mosul-Iraq). The mixed content of 10 tablets (Mesnan tablets 400 mg of Mesna/tablet), supplied from (NDI) Mosul- Iraq were weighed and grounded, then powder equivalent to 100 mg of mesna was dissolved well in 1 L distilled water, filtered by filter paper and 10 mL of this solution was diluted to 100 mL by distilled water and 3 mL of this solution was treated as under recommended procedure.

Injection: Ampoule of 100 mg of mesna (provided from (NDI) Mosul- Iraq). The content of 5 ampoules was mixed well in 500 mL beaker. An aliquots equivalent to 100 mg of mesna was transferred into 1 L volumetric flask and diluted up to the mark with distilled water, 10 mL of this solution was diluted to 100 mL with distilled water and 3 mL of this solution was treated as recommended procedure.

Procedure for Real Waste Water Sample

Industrial waste water sample provided from the state company for drug industries and medical appliances Mosul-Iraq (NDI) were analyzed, by added the amount 5-50 µg/25 mL of mesna and this solution was treated as recommended procedures.

RESULTS AND DISCUSSION

The proposed method was based on the oxidation of mesna to dimesna by a constant excess of CAT in sulfuric acid medium, and then estimation of residual CAT by reacting with a constant amount of methylene blue and measuring at 664 nm. The first step in the assay procedure is the estimation of the upper limit of methylene blue that can be measured at 664 nm, this found 5 mL of 0.01%.This was completely destroyed to a colorless product by 3 mL of 0.1% of CAT. Hence, different amounts of drug was reacted with 3 mL of 0.1% of CAT and the un-reacted oxidant was determined as described under recommended procedure. The formation of the results might be written as [16].

$2HS\text{-}CH_2\text{-}CH_2\text{-}SO_3Na$+CAT (Known excess) → $NaO_3\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH_2\text{-}SO_3Na$ + Un-reacted CAT

Di mesna

Excess CAT + Methylene blue→ Bleached methylene blue (measured at 664 nm).

Mesna reacted with CAT and the determination of CAT by reacting with methylene blue. H_2SO_4 medium (2 mL of 2 M) was found to be good result. The reaction time of 5 minute up to 6 hours was good. A linear graph was found between absorbance and concentration **Figure 2.**

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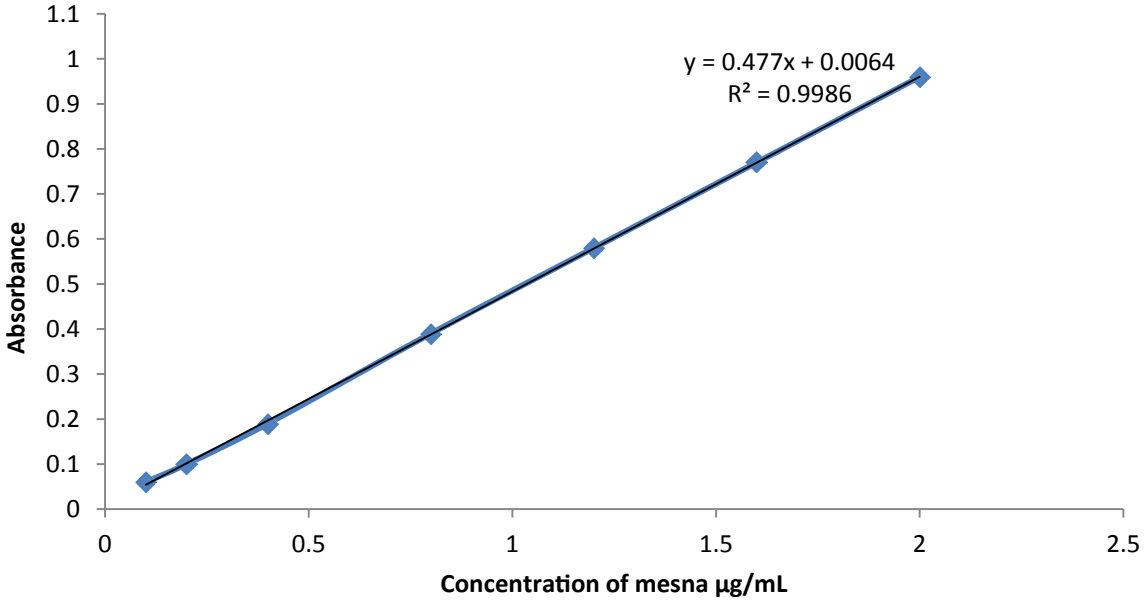

Figure 2. The calibration graph of mesna.

The optical parameters values were given in **Table 1.**

Table 1. Analytical parameters of the present method.

Parameter	Proposed method
ƛ max (nm)	664
Beer's law limit (µg/mL)	0.1-2.0
Molar absortivity ($Lmol^{-1}cm^{-1}$)	7.832x104
Sandell,s Sensitivity ($ngcm^{-2}$)	47
Intercept	0.0064
Slope	0.477
Correlation coefficient, R	0.998
Recovery %	100 ± 0.95
Relative standard deviation (%)	Less than 1.8

Precision, Accuracy of the Present Method

The precision and accuracy of the present method was analyzed at four different concentrations, each estimation repeated six times. The accuracy and (RSD) values were shown in **Table 2.** From **Table 2**, it was clear that relative error of ±0.95% was as accurate Moreover ,the method was found to be sensitive and accurate with (RSD) values <1.8%. The standard deviation at concentration 0 (blank) was calculated and this value was used for the calculation of the limit of detection and limit of quantification. The Limits of Detection (LOD) and Quantification (LOQ) were calculated using the following equation: LOD=(3.3 σ/s) and LOQ=(10 σ/s) where σ is the standard deviation of the response and s is the slope of the regression line [17]. Limit of Detection (LOD) and Limit of Quantification (LOQ) were found 0.01 µg/mL and 0.03 µg/mL respectively. The results indicate that the method was sensitive enough to detect a concentration of 0.01 µg/mL and able to quantify concentrations above 0.03 µg/mL.

Table 2. Precision, accuracy and RSD of the method.

Mesna taken (µg)	Mesna exists (µg)	Recovery (%)a	RSD (%)
5	5.04	0.8	1.3
10	10.093	0.93	1.7
30	30.285	0.95	1.6
50	50.44	0.88	1.2
a: Mean of six estimations			

Analytical Application

Tables 3 and 4 gives the results of the estimation of mesna which reveals there was almost close agreement between the label claim for pharmaceutical preparations and present method and the accuracy results of real waste water sample was more than 98%, indicating that very good applicability of this method.

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Table 3. Estimation of mesna in pharmaceutical dosage forms.

Pharmaceutical formulations	Label claim (mg)	Exists* (mg)	% Recovery (n=6)	%RSD
Mesna tablet (NDI)	400	403	100.75	0.89
Mesna ampoule (NDI)	100	99.8	99.8	1.2
*Mean of six determinations.				

Table 4. Estimation of mesna in real waste water sample.

Industrial waste water samples	Mesna (µg/25mL)★		% Recovery (n=10)	%RSD
	Taken	Found		
1	5	5.05	101	1.3
2	10	9.95	99.5	0.75
3	20	19.8	99.0	0.83
4	30	30.3	101	1.2
★Mean of ten determinations				

CONCLUSION

The proposed method developed was simple, selective and offers the advantages of high sensitivity and a wide range of determinations without the need for heating or solvent extraction .The method un affected by slight variations in acidity and other reagents .This method do not take more than 10 minutes and can be used for routine quality control analysis of mesna in pure form and pharmaceutical formulations .and used to the estimation of mesna in pharmaceutical formulations (tablets and ampoules) .and environmental industrial waste water sample.

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REFERENCES

1. British National Formulary (BNF). Royal Pharmaceutical Society Pubication, Great Britain, UK; 2016.
2. Shaw IC and Graham MI. Mesna a short review. Cancer Treat Rev. 1987;14:67-86.
3. Martindale W. The Extra Pharmacopoeia. 35th ed. Pharmaceutical Press, London, UK, 2007.
4. Sener G, et al. Protective effect of Mesna (2-morcapto ethane sulphonate) against acetaminophen-induced hepatorenal oxidative damage in mice. J Appl Toxicol. 2005;25:20-29.
5. Verschragen M, et al. Quantification of mesna and total mesna in kidney tissue by high performance liquid chromatography with electrochemical etetion. J Chromatogr B. 2003;783:33-42.
6. Miranda V, et al. Simultaneous determination of BNP7787 and its metabolite mesna in plasma and tissue by micro-HPLC with a dual electrochemical detector. J Pharm Sci. 2003;92:1040-1050.
7. Suneetha M, et al. High performance liquid chromatography analysis of MESNA (2-mercaptoethane sulfonate) in biological samples using fluorescence detection. Biomed Chromatogr. 2005;19:80–86
8. Glowacki RX, et al. Urinary mesna and total mesna measurement by high performance liquid chromatography with ultraviolet detection. Talanta. 2005;66:534-539.
9. Capitan L, et al. Chemiluminescence determination of sodium.2-mercaptoethan sulphonate by flow injection analysis, using cerium (IV) sensitized by quinine. Talanta. 2000;51:1155-1161.
10. Haggag RS, et al. Elbardisy; Spectrophotometric and spectrofluorimetric determination of mesna, acetylcysteine and timonacic acid through the reaction with acetoxymercuri fluorescein. Anal Methods. 2016;8:2479-2493.
11. Ahmad NR. An indirect spectrophotometric determination of mesna in pharmaceuticals and environmental samples. Irq Nat J Chem. 2011;44:492- 500.
12. Mohamed R, et al. Kinetic Spectrophotometric Determination of Mesna in Drug Substance and Drug Product Using Alkaline Potassium Permanganate. Chem Sci Rev Lett. 2012;1:140-147.
13. Haggag RS, et al. Spectrofluorimetric Estimation of Some Sulfhydryl–Containing Drugs by Demasking Reaction of the Palladium chelate of 8-Hydroxyquinoline-5- Sulfonic Acid. Pharm Anal Acta. 2017;8:1-10.
14. https://www.pharmacopoeia.com/
15. https://www.who.int/medicines/areas/quality_safety/quality_assurance/resources/US_Pharmacopoeia.pdf

e-ISSN:2320-0812

16. Ahmed NR, et al. Determination of mesna in pharmaceutical preparations and environmental samples: Application to content uniformity testing. Int J Enhanc Res Sci Technol Eng. 2018;7:14-19.

17. Basavaiah K and Somashekar BC. Quantitation of rantidine in pharmaceuticals by titrimetry and spectrophotometry using dichromate as the oxidimetric reagent. J Iran Chem Soc. 2007;4:78-88.