International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 7 Issue 5, May-2018, Impact Factor: 4.059

Determination of mesna in pharmaceutical preparations and environmental samples: Application to content uniformity testing

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ABSTRACT

A new, simple and sensitive indirect spectrophotometric method for the determination of mesna has been developed. The method is based on the oxidation of the drug by a known excess sodium hypochlorite in acidic medium, followed by the reaction of the unconsumed oxidants with indigo carmine and measurement of absorbance at 610 nm. The absorbance values increased linearly with increasing concentration of drug .The calibration graph is linear over the range 0.2-2 ppm. The apparent molar absorbance and Sandell, s sensitivity values are 4.2 x104 l.mol-1.cm-1 and 3.9 ng.cm-2,respectively.The relative standard deviation (RSD) is less than 1.9 (n=10) and the accuracy (average recovery) is 100±1.2 .The proposed method was applied successfully as a routine quality control and content uniformity tests for determination of mesna in some pharmaceutical formulations (tablets and injections).and in environmental wastewater sample

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

HOW TO CITE THIS ARTICLE

Nief Rahman Ahmed, Zena Sattam Hamed, Mohamad Yasin Kalaf, "Determination of mesna in pharmaceutical preparations and environmental samples: Application to content uniformity testing", International Journal of Enhanced Research in Science, Technology & Engineering, ISSN: 2319-7463, Vol. 7 Issue 5, May -2018.

INTRODUCTION

Mesna, (sodium 2-mercaptoethane sulfonate) has a molecular formula of

$C_2H_5NaO_3S_2$ =164.2

Chemical Structure of Mesna

Mesna is 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 reduced to free mesna which has thiol groups that react with the metabolitied of ifosfamide and cyclophophamide, including acrolein, considered to be responsible for the toxic effect on the bladder. [1-3] .Mesna used as antioxidant agent against acetaminophen toxicity [4].Mesna is rapidly oxidized to disulfide, so in pharmaceutical formulation it must be stabilized using ethylene di amine tetra acetic acid, 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 literature revealed that mesna has been determined by means of a few analytical methods. These include: HPLC [5-8].Chemiluminescence-flow injection [9]. British pharmacopoeia described a tedious titrimetric assay for pure drug only [10], kinetic fluorimetric method [11], pectrophotometric methods [12-16] The

proposed method was proved to be selective and applied to the determination of mesna in pharmaceutical preparations and environmental water samples using sodium hypochlorite and indigo carmine

EXPERIMENTAL

Apparatus
Optima SP 3000 plus UV-Visible spectrophotometer with 1.0 cm quartz cells was used for absorbance measurements.

Reagents
All chemicals used were of analytical grade and all solutions were prepared by distilled water.

Standard sodium hypochlorite solution :(0.1%) was prepared by dilution of 2.5 ml of 4% sodium hypochlorite to 100 ml by distilled water, store in a dark bottle and standardized every 4-5 days. This solution stored in a dark bottle [17, 18].

Indigo carmine: 0.01 % was prepared by dissolving 0.01 gm accurately weighed dye in distilled water, and diluting it to 100 ml in volumetric flask.

Standard solution of mesna: (100 ppm). This solution was prepared by dissolving 0.1 gm of pure drug in 1L distilled water. It was later diluted with water to get concentration of 10 ppm.

Recommended procedures
Different aliquots of standard mesna solution equivalent 5 -50 µg were transferred into a series of 25 ml volumetric flasks, 5 ml of 1N HCL , and 2.5 ml of sodium hypochlorite solution were added. The content was mixed and let stand for 5min with occasional shaking. Finally, 5ml of 0.01% Indigo carmine 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 610 nm against a reagent blank as shown in Figure 1

Procedures for pharmaceutical preparations
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 recommended procedure.

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

Procedures for real wastewater sample.
To demonstrate the practical applicability of the proposed method, industrial waste water sample from the state company for drug industries and medical appliances Mosul-Iraq, were analyzed, by spiked with the concentrations ranging from 0.5-2 $\mu g.ml^{-1}$ of mesna and aliquot of this solution was treated as described above for recommended procedures.

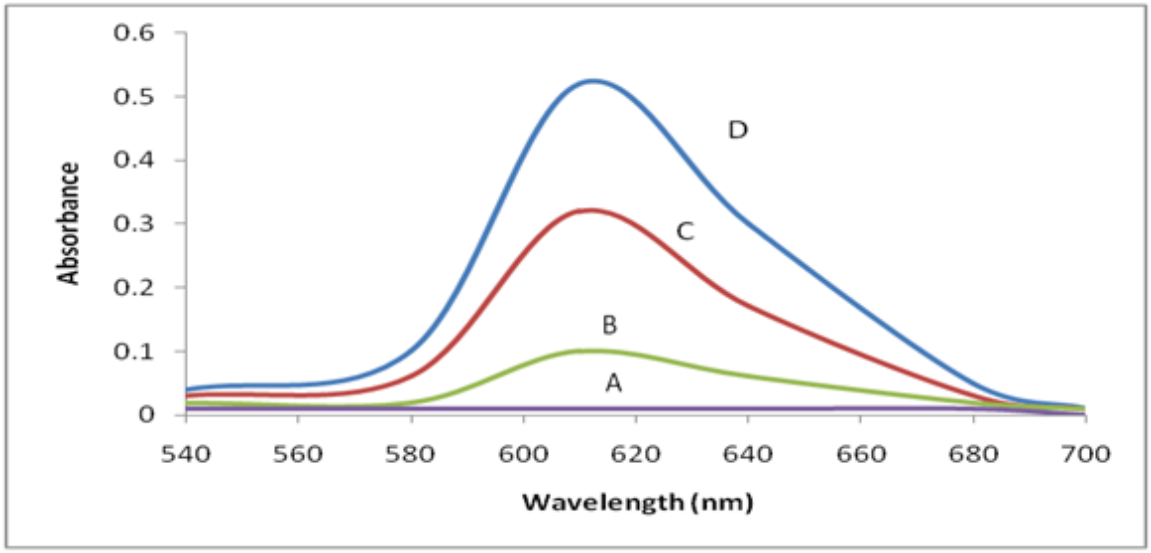
RESULT AND DISCUSSION

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. [19-22].The use depends mainly on its ability effect the oxidation of diverse functional groups. Taking advantage of the rapid oxidation reaction of sodium hypochlorite with mesna. The proposed method is based on the oxidation of mesna to dimesna by a known excess of sodium hypochlorite in hydrochloric acid medium, and subsequent determination of residual sodium hypochlorite by reacting it with a fixed amount of indigo carmine and measuring the change in absorbance of the latter at 610nm, Mesna ,when added in increasing amounts to a fixed amount of sodium hypochlorite , consume the latter and there will be a concomitant decrease in its concentration .When a fixed amount of indigo carmine is added to decreasing concentration of sodium hypochlorite ,a concomitant increase in the concentration of the dye is obtained .This is observed as a proportional increase in absorbance of the colored species on increasing the concentration of mesna. The first step in the assay procedure is the determination of the upper limit of indigo carmine that can be measured at 610 nm, this was found to be 5ml of 0.01%.This was completely destroyed to a colorless product by 2.5ml of 0.1% of sodium hypochlorite. Hence, different amounts of drug was reacted

with 2.5ml of 0.1% of sodium hypochlorite and the unreacted oxidant was determined as described under recommended procedure.

2 HS-CH_2-CH_2-SO_3Na + NaOCl (Known excess) → NaO_3-CH_2-CH_2-S-CH_2-CH_2-SO_3Na

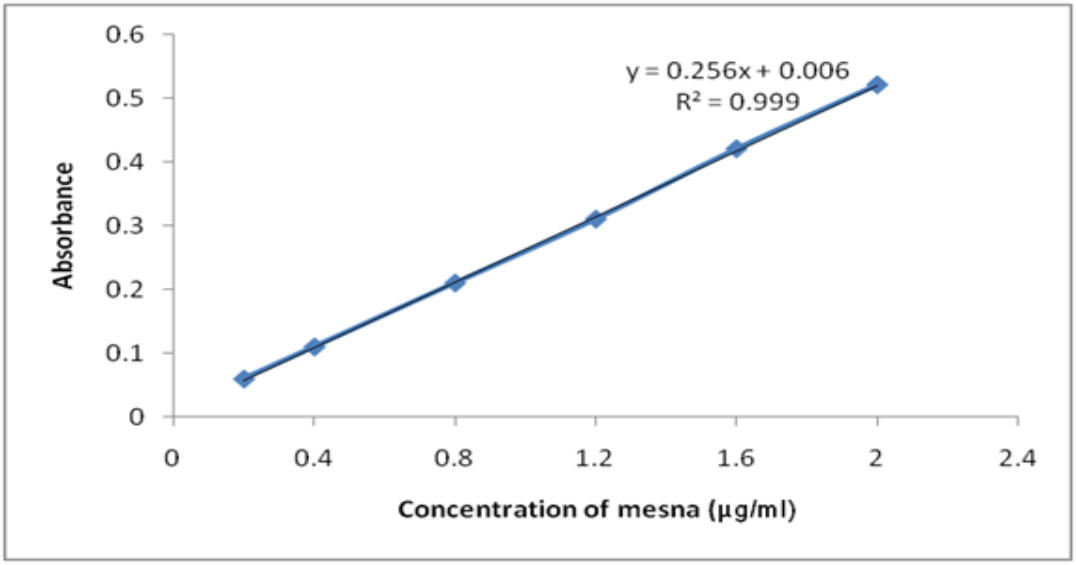
Di mesna
+ Unreacted NaOCl
Unreacted NaOCl +Indigo carmine → Bleached Indigo carmine (measured at 610nm).[22]



Figure(1):Absorption spectra of; A-reagent blank against water., B,C and D-after addition of 10,30 and 50µg/25 ml of mesna.

The reaction between sodium hypochlorite and mesna and the determination of the latter by reacting with the indigo carmine HCL medium (5 ml 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. A liner correlation was found between absorbance at ג max and mesna concentration. Figure (2).A = 0.256x + 0.006, R^2 = 0.999

Where A is the absorbance and x is concentration in µg.ml^{-1}, and R is the regression coefficient.



Figure(2); Calibration curve of mesna.

The optical characteristics such as Beer's Law, limits and molar absorptivity values is given in(Table 1).

Table (1): Analytical and regression parameters of the proposed methods.

Parameter	Proposed method
ג max (nm)	610
Beer's law limit (µg/ml)	0.2-2
Molar absorbtivity ($L.mol^{-1}.cm^{-1}$)	4.2×10^4
Sandell's Sensitivity ($ng.cm^{-2}$)	3.9
Intercept	0.006
Slope	0.256
Correlation coefficient, R^2	0.999

Accuracy and precision

To evaluate the accuracy and precision of the method a pure drug solution was analyzed at four different concentrations, each determination being repeated six times. The relative error (%) and relative standard deviation (RSD) values are summarized in (Table 2). From (Table 2), it is clear that relative error of 1.2 % is as accurate Moreover, the method was found to be precise with RSD values <1.9 %..

Table (2): Accuracy and precision of the proposed method.

Mesna taken µg/ml	Mesna found µg/ml	Er (%)[a]	RSD(%)
0.5	0.505	1.0	1.3
1.0	1.01	1.0	1.8
2.0	2.04	1.2	1.5

a: Mean of six determinations

Effect of interferences

In order to assess the possible applications of the proposed method, the effect of substance that often accompany with mesna in various pharmaceutical products (Tablets and injections) were studied by adding different amount of substances to 50 µg of mesna. An attractive feather 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 50 µg/25 ml of mesna in the presence of interferences.

Interfering substances	Amount added(mg)	Amount found(µg) *	Recovery %
Na_2- EDTA	1	50.05	100.1
Benzyl alcohol	1	50.06	100.12
Chlorobutanol	10	50.07	100.14
Lactose	40	50.04	100.08
Microcrystalline cellulose	20	49.93	99.86
Calcium phosphate	10	49.95	99.9
Corn starch	30	50.05	100.1
Povidone	30	50.05	100.1
Magnesium stearate	40	49.97	99.94
Hydroxyl propyl methyl cellulose	40	49.97	99.94
Poly ethylene glycol	20	49.8	99.6
Titanrium dioxide	10	50.04	100.08

*Average of six determinations.

Analytical application

(Table 4,5) gives the results of the assay and reveals that there is close agreement between the results obtained by the proposed method and the label claim for pharmaceutical preparations and the recovery results of real wastewater sample was higher than 98%,indicating that successfully applicability of the proposed method.

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

Pharmaceutical formulations	Label amount (mg)	Found* (mg)	% Recovery(n=6)
Mesna tablet	400	402	100.5
Mesna ampoule	100	99.7	99.7

*Mean value of six determinations.

Table (5): Determination of mesna in real waste water sample.

Industrial wastewater samples	Mesna(µg/ml) Taken	Found	% Recovery(n=10)
1	0.5	0.501	100.2
2	1.0	0.98	98.0
3	2.0	1.98	99.0

*Mean of ten determinations.

Application the method to uniformity of dosage units (content uniformity)

The proposed method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Data presented in (Table6) indicate that the proposed method can accurately and precisely quantities mesna (400mg) in its commercially available tablets. The mean percentage (with RSD) of the labeled claim found in ten tablets was (1.4%) which falls within the content uniformity limits specified by the United State Pharmacopeia 33-NF28USP 33[23].

Table (6): Content uniformity testing of mesna tablets using the proposed method

Parameter	% of the label claim
Sample. 1	101. 24
Sample. 2	101. 56
Sample. 3	98. 83
Sample. 4	99.27
Sample. 5	101.85
Sample. 6	101. 65
Sample. 7	98.52
Sample. 8	98. 58
Sample. 9	101.61
Sample. 10	100.49
Mean ($\overline{x}$)	100.36
% RSD	1.4
Max. allowed unit [23]	±15%

COMPARISON OF METHODS

The proposed method was compared with other reported spectrophotometric methods and found to be more applications than other reported methods (Table 7).

Table (7): Comparison of the existing spectrophotometric methods with the proposed method for mesna.

Parameters	Method 1	Method 2	Method 3	Method 4
Ref	12	14	15	Proposed
λ Max(nm)	590	610	613	610
Linear range (µg/ml)	0.2-2	1-10	0.4-4	0.2-2
Molar absorptivity (l/mol.cm)	6.98×10^4	———	3.64×10^4	4.2×10^4
Application	Pharmaceuticals	Pharmaceuticals	Pharmaceuticals	Pharmaceuticals, industrial waste water, content uniformity test

CONCLUSION

The proposed method developed is 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 not effected by slight variations in the experimental conditions such as acidity and other reagents. The proposed method do not take more than 10 mints and successfully applied to determination of mesna in pharmaceutical preparations(tablets and vials), environmental industrial wastewater sample and application the method to uniformity of dosage units(content uniformity).

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ACKNOWLEDGMENTS

The first author (Dr. Nief Raman.) wishes to express gratitude to his former company [the state company for drug industries and medical appliance (NDI) Nineveh – Iraq for providing gift samples of mesna standard materials and pharmaceutical preparations (tablets and vials).

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