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ORIGINAL ARTICLE

Spectrophotometric determination of the sulfhydryl containing drug mesna

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Abstract Four simple and sensitive spectrophotometric methods were developed for the determination of the sulfhydryl containing drug mesna (MSN). Methods I and II rely on nucleophilic aromatic substitution reactions using two UV tagging reagents namely: 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) for method I and 2,4-dinitrofluorobenzene (DNFB) for method II. Both reactions took place in alkaline buffered medium and the obtained yellowish products were measured at 414 and 332 nm for methods I and II, respectively. Methods III and IV are indirect spectrophotometric methods based on the suppressive effect of MSN on the absorption of two ternary complex systems which are composed of 1,10-phenanthroline, silver and eosin for method III and 1,10-phenanthroline, silver and bromopyrogallol red for method IV. The decrease in absorbance of the ternary complexes was measured at 547 and 635 nm for methods III and IV, respectively. All the experimental parameters affecting these reactions were carefully studied and optimized. The methods were validated as per the ICH guidelines. The methods were applicable in the linearity ranges 4–18 μg/mL for method I, 4–16 μg/mL for method II, 0.25–2.25 μg/mL for method III and 0.25–1.75 μg/mL for method IV. The proposed methods were successfully applied for the analysis of MSN in its commercial ampoules and no interference was encountered from the present excipients as indicated by the satisfactory percentage recoveries. The results obtained were in a good agreement with those obtained from a previously published method of the investigated drug.

1. Introduction

Mesna (MSN; Fig. 1) is chemically known as sodium 2-sulfanylethanesulfonate.[1] It is an antioxidant used for the prevention of urothelial toxicity in patients being treated with the antineoplastics ifosfamide or cyclophosphamide. In the kidney, dimesna, the inactive metabolite of mesna, is reduced to free mesna. This has thiol group that reacts with the metabolites of ifosfamide and cyclophosphamide, including acrolein, which are considered to be responsible for the toxic

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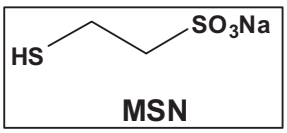

Figure 1 Chemical structure of mesna (MSN).

effects on the bladder. MSN is also used as a mucolytic in the management of some respiratory-tract disorders.[2] Besides these indications, MSN was found to be effective in the treatment of intestinal inflammation probably by scavenging reactive oxygen species.[3] Moreover, recent studies on MSN have shown that its antioxidant and antifibrotic properties can be of potential therapeutic value in protecting the liver against fibrosis and oxidative injury due to biliary obstruction.[4] Because of the increased interest in using MSN for treating various disorders, it was assumed that its quantitative determination would be of significant importance in clinical practice. MSN is official in both BP 2013[1] and USP 34,[5] both describe an indirect iodimetric titration method for the determination of the drug in its pure powder form. Screening the literature revealed various techniques that were used for MSN quantitation. Of these we can mention: a kinetic spectrofluorimetric method using cerium(IV) as an oxidizing agent,[6] and different spectrophotometric methods utilizing several reagents such as potassium permanganate,[7] methyl orange and congo red,[8] N,N-dimethyl-p-phenylenediamine[9] and ferric solution.[10] Other techniques include high performance liquid chromatography (HPLC),[11–13] capillary electrophoresis (CE)[12] and Raman spectroscopy.[14] In biological fluids, most of the methods published for MSN quantitation were based on HPLC.[15–17]

The aim of this work was to develop simple, sensitive, reliable and inexpensive spectrophotometric methods for MSN quantitation in bulk form and in its commercial ampoules. Methods I and II depend on the reaction of MSN with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and 2,4-dinitrofluorobenzene (DNFB) in borate buffer, to yield yellowish colored condensation products which were measured at their λ_{max}. Methods III and IV involve two indirect spectrophotometric methods based on the suppressive effect of MSN on the absorbance of two ternary complexes: 1,10-phenanthroline, silver and eosin $[(\text{phen-Ag-phen})^{+}]_2{\cdot}\text{TBF}^{2-}$ for method III and 1,10-phenanthroline, silver and bromopyrogallol red $[(\text{phen-Ag-phen})^{+}]_2{\cdot}\text{BPR}^{2-}$ for method IV. The decrease in absorbance of the ternary complexes was found to be proportional to the drug concentration. All the experimental conditions affecting these reactions were studied and optimized.

2. Experimental

2.1. Instrumentation

- Specord S600 spectrophotometer, associated with WinAspect software version 2.3, Analytik Jena AG, Germany. A 1-cm quartz cell (Analytik Jena) was used.
- A thermostatically controlled water bath, Köttermann, Germany.
- J.P. SELECTA, S.A. sonicator, Spain.
- Digital pH meter 3310 Jenway.

2.2. Materials and reagents

- All solvents, chemicals and reagents were of pure analytical grade.
- Pharmaceutical grade of MSN was purchased from Fluka (Sigma Aldrich, St. Louis, USA). It was certified to contain 99.04%.
- 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) 98%, (purchased from Sigma, St. Louis, USA) was prepared as 0.1% w/v solution in methanol. The reagent was stable for two weeks if kept under refrigeration.
- 2,4-Dinitrofluorobenzene (DNFB) (Hopkin and Williams Co., Essex-UK) was prepared as 0.2% w/v solution in methanol. The reagent was freshly prepared and protected from light.
- Silver nitrate, assay >99% (Fluka, packed in Switzerland) was used as 5×10^{-4} M solution in double distilled water and it was protected from light.
- 1,10-Phenanthroline (Prolabo) was prepared as 1×10^{-3} M solution in warm distilled water.
- Eosin (TBF) (2,4,5,7-tetrabromofluorescein) (Riedel-De Haën AG) was prepared as 1×10^{-4} M aqueous solution.
- Bromopyrogallol red (BPR) (Aldrich Chem. Co.). Its solution was prepared by dissolving 25 mg of BPR in 100 mL of 1% ammonium acetate. It should be freshly prepared.
- Ethylenediaminetetraacetic acid (EDTA) (Chemajet, Alexandria, Egypt) was prepared as 0.1 M aqueous solution.
- Gelatin (Veb Laborchemie Apolda, Germany) was prepared as 0.5 g% solution in warm distilled water.
- Borate buffer (disodium tetraborate) (Gateway Co., Egypt) was prepared as 0.05 M aqueous solution.
- Boric acid (Gomhouria Co., Alexandria, Egypt) was prepared as 0.1 M solution in warm distilled water.
- Sodium acetate (El. Nasr Pharmaceutical Chemicals Co. Egypt) was prepared as 1 M aqueous solution.
- NaOH (FEMICO Co., Alexandria, Egypt) was prepared as 0.1 and 1 M aqueous solutions.
- Acetic acid (El. Nasr Pharmaceutical Chemicals Co. Egypt) was prepared as 10% aqueous solution.
- Methanol (SDFCL, Mumbai, India).
- Fresh double distilled water was used.

2.3. Pharmaceutical preparation

Uromitexan® ampoules (Batch No. 3A109A) are manufactured by Baxter oncology GmbH, Germany. The ampoules are labeled to contain 400 mg mesna/4 mL ampoule.

2.4. Standard stock solutions

For methods I and II, a stock solution of MSN was prepared as 20 mg% in methanol. While for methods III and IV, it was prepared as 12.5 mg% solution in methanol then it was diluted to obtain a working solution of final concentration 1.25 mg%. These stock solutions were stored in the refrigerator at 4 °C.