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Urinary mesna and total mesna measurement by high performance liquid chromatography with ultraviolet detection

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Abstract

We describe in this report a method for determination of mesna and total mesna in urine by high performance liquid chromatography with ultraviolet detection. The method involves a treatment of the urine sample with tri-*n*-butylphosphine in order to convert mesna disulfides to its reduced counterpart mesna, ultraviolet labelling with 2-chloro-1-methylquinoluinium tetrafluoroborate, reversed-phase HPLC separation, and detection and quantitation at 350 nm. The result corresponds to total mesna that is sum of mesna, dimesna and its mixed disulfides with endogenous thiols. For determination of mesna the reduction step is omitted. Content of disulfide forms of mesna can be calculated by subtracting the concentration of mesna from the total mesna concentration. The separation of 2-*S*-quinolinium derivatives of mesna from those of endogenous urinary thiols and internal standard was achieved on an analytical Waters Nova-Pak C18 (150 mm × 3.9 mm, 5 μm) column. A mixture of an aqueous solution of pH 2.3, 0.05 M trichloroacetic acid and acetonitrile (88:12, v/v) was used as a mobile phase at flow rate of 1.2 ml/min and ambient temperature.

The assay for mesna and total mesna in urine was proved to be linear over the studied ranges of 0.2–30 and 0.2–800 nmol/ml urine, respectively. The mean recoveries over the calibration ranges were 95.4% for mesna and 99.7% for total mesna. The lower limits of detection and quantitation were 0.1 and 0.2 nmol/ml for both the procedures, respectively. The imprecision did not exceed 8.5%. No interference from endogenous substances was observed.

Keywords: Mesna; Total mesna; Urine; HPLC; Derivatization

1. Introduction

Numerous studies have demonstrated that sulfhydryl-containing nucleophiles like *N*-acetylcysteine and mesna (2-mercaptoethanesulfonate) can antagonize the dose-limiting effects of alkylating anticancer agents on the genitourinary tract. The superiority of mesna as a chemoprotector to other available thiols has been confirmed clinically in an ifosfamide trial [1]. The urotoxic oxazaphosphorine metabolites are detoxified by their reactions with the sulfhydryl group of mesna [2]. Mesna does not block the antitumor action of oxazaphosphorines most likely due to its rapid formation of the inactive dimer dimesna in the blood-stream [3]. The active thiol monomer is reformed by reduction of dimesna in renal tubule cells, thereby inactivating toxins like acrolein to the bladder. In spite of routine use of mesna in patients receiving high doses of oxazaphosphorines, hemorrhagic cystitis occurs in some cases. This may be ascribed, among others, to insufficient urinary concentration of mesna [4]. In order to ensure constant protection of genitourinary tract sufficient levels of free thiol must be maintained during the period when the toxic metabolites are excreted. Monitoring the urinary levels of mesna, is therefore, clinically important.

Apart from non-specific and not very sensitive colorimetric, based on Ellman's reagent, methods [5,6] several HPLC procedures for determination of mesna in biological samples [4,7–12] have been elaborated. Ultraviolet detection is not

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enough sensitive for this purpose unless pre-column [8] or post-column [10] derivatization is applied. A majority of the HPLC procedures take advantage of electrochemical detection [9–12].

In a previous paper [8], we have reported a sensitive and accurate method for determination of mesna in plasma with the use of standard HPLC system equipped with by far the most popular UV–vis detector. The method measures mesna in the form of its 2-*S*-quinolinium derivative after pre-column derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate a thiol specific UV-tagging reagent [13]. Below we describe an extension of this method to cover mesna and its oxidized forms in urine. Total mesna is measured as mesna after pre-column treatment with tri-*n*-butylphosphine.

2. Experimental

2.1. Apparatus and instruments

HPLC analyses were performed with a Hewlett–Packard (Waldbronn, Germany) HP 1100 Series system equipped with quaternary pump, an autosampler, thermostated column compartment, vacuum degasser and diode-array detector and controlled by HP ChemStation software. UV spectra were recorded on a Hewlett–Packard HP 8453 (Waldbronn, Germany) diode-array UV–vis spectrophotometer. Water was purified using a Millipore Milli-QRG system (Vien, Austria). For pH measurement, a Hach One (Loveland, USA) pH meter was used.

2.2. Reagents and solutions

2-Chloro-1-methylquinolinium tetrafluoroborate (CMQT) was prepared in this laboratory as described in our previous report [13]. 2-Mercaptoethanesulfonic acid, sodium salt (mesna, MES) and 3-mercaptopropane sulfonic acid, sodium salt (MPS) was purchased from Aldrich Europe (Beerse, Belgium). Dimesna $(MES)_2$ and 3,3′-dithiodipropanesulfonic acid, disodium salt $(MPS)_2$ were prepared in this laboratory. Thiomalic acid (TMA), tri-*n*-butylphosphine (TNBP), 2-mercaptopropionic acid (2MPA), 3-mercaptopropionic acid (3 MPA) and thioglicolic acid (TGA) were from Fluka (Buchs Switzerland), ethylenediaminetetraacetic acid, disodium salt (EDTA), perchloric acid (PCA), HPLC-grade acetonitrile (ACN) and methanol (MeOH) were provided by J.T. Baker (Deventer, The Netherlands). Tris(hydroxymethyl)aminomethane (Tris) and trichloroacetic acid (TCA) were from Merck (Darmstadt, Germany). All other chemicals and solvents were of the highest purity available from commercial sources and used without further purification.

TCA and Tris buffer solutions of appropriate concentration were adjusted to the desired pH by potentiometric titration with lithium hydroxide and hydrogen chloride solution, respectively. The titration systems were calibrated with standard pH solutions. Standard thiol solutions (10 μmol/ml) were prepared in water or dilute hydrochloric acid, standardized with *o*-hydroxymercurybenzoate [14] and kept at 4 °C. The working solutions were prepared daily as needed.

2.3. Chromatography

An autosampler handed 20-μl volume of the final analytical solution onto a Nova-Pack C18 column (150 mm × 3.9 mm, Waters, USA) packed with 5 μm particles. Mobile phase consisted of 0.05 M TCA buffer, adjusted to pH 2.3 with lithium hydroxide solution of the same concentration, and acetonitrile in ratio of 88:12 (v/v). The flow rate was 1.2 ml/min, the temperature 25 °C and the detector wavelength 350 nm. Identification of peaks was based on comparison of retention times and diode-array spectra, taken at real time of analysis, with corresponding set of data obtained with authentic compounds.

2.4. Calibration standards

Stock solutions of 10 μmol/ml mesna, dimesna and internal standards sodium 3-mercaptopropanesulfonate and disodium 3,3′-dithiodipropanesulfonate were prepared by dissolving an appropriate amounts of each compound in 2 ml of 0.1 M hydrochloric acid and diluting to the volume of 10 ml. These solutions were kept at 4 °C for several days without noticeable change in the solutes concentrations. The working solutions were prepared by appropriate dilutions with water as needed. For preparation of calibration standards for total mesna (dimesna) in urine, portions of 0.5 ml of urine from apparently healthy donors were each placed in a sample tube and spiked with increasing amounts of working standard solution of dimesna. Assuming 100% of the future reduction of the disulfide bonds, concentration of mesna in the consecutive tubes was 0.2, 0.5, 2.0, 8.0, 15.0, 30.0, 100.0, 400.0, 600.0 and 800.0 nmol/ml urine. For mesna the same amount of urine was spiked with standard mesna solution giving concentration of 0.2, 0.5, 2.0, 6.0, 8.0, 15.0 and 30 nmol/ml urine.

2.5. Search for internal standard

Several thiols such as 2- and 3-mercaptopropionic acid, thioglycolic acid, thiomalic acid and 3-mercaptopropanesulfonic acid and their disulfides (in concentration 10 nmol/ml) were added first to mesna water standard and next to urine spiked with mesna or dimesna and the resulted mixtures were subjected to all steps of the appropriate analytical procedure. *N*-Acetylcysteine, *N*-(2-mercaptopropionyl)glycine and penicillamine were not taken into consideration because they can be present as drugs.

2.6. Analytical procedures

2.6.1. Determination of total mesna, procedure 1

To 500 μl of urine 0.25 ml of 0.1 M EDTA, 0.5 ml of 1 M, pH 7.3 Tris buffer, 75 μl of 0.1 μmol/ml $(MPS)_2$ (internal