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Note

# Determination of sodium 2-mercaptoethanesulphonate by high-performance liquid chromatography using post-column reaction colorimetry or electrochemical detection

BEATE SIDAU and IAN C. SHAW*

*Laboratory of Toxicology and Pharmacokinetics, Department of Clinical Pharmacology, University College London, 5 University Street, London WC1 (U.K.)*

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Sodium 2-mercaptoethanesulphonate (mesna) is a new drug which is used to ameliorate the urotoxicity associated with oxazaphosphorine (e.g. cyclophosphamide) chemotherapy [1]. The urotoxic oxazaphosphorine metabolites are detoxified by their reaction with the free sulphydryl group of mesna [2]. In order to ensure constant protection of the bladder and urinary tract, sufficient levels of free thiol must be maintained during the period when the toxic metabolites are excreted. Monitoring the urinary levels of free thiol is therefore important clinically.

Mesna in biological samples can be measured by the non-specific Ellman's free thiol assay [3, 4] which detects other thiols (endogenous thiols and thiol drugs) as well. A high-performance liquid chromatographic (HPLC) method with ultraviolet (UV) detection has also been reported [5]; however, its sensitivity is not sufficiently high to make it suitable for application to clinical samples.

The method described here allows the determination of mesna per se in pharmaceutical and biological samples. It is based on the separation of mesna from other thiols by ion-pair HPLC followed either by post-column derivatization and colorimetric detection (system A) or by electrochemical detection (system B).

## MATERIALS AND METHODS

### *Chemicals*

Mesna was provided by WB Pharmaceuticals (Bracknell, U.K.); other thiol

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compounds (L-cysteine, DL-homocysteine, glutathione, D-penicillamine) were purchased from Sigma (Poole, U.K.) and the ion-pairing reagents heptanesulphonic acid (HSA) and tetrabutylammonium phosphate (TBAP) from Magnus Scientific (Aylesbury, U.K.). All other chemicals were of analytical grade.

*HPLC equipment*

Two HPLC systems were used which consisted of the same basic set-up, but utilized different ion-pairing reagents and detection systems (Table I). They comprised a Constametric III solvent pump (Laboratory Data Control, Stone, U.K.) and a Rheodyne injection valve (Rheodyne, Berkeley, CA, U.S.A.) with a 50-$\mu$l injection loop. Separation was performed on a Hypersil ODS analytical column (25 cm × 4 mm I.D., particle size 5 $\mu$m).

TABLE I

DETAILS OF THE HPLC SYSTEMS USED FOR THE DETERMINATION OF MESNA

Both systems were used with conventional HPLC equipment (see Materials and methods).

| | System A | System B |
|---|---|---|
| Mobile phase | Phosphate buffer (0.25 *M*, pH 7.4)—methanol (95:5) | Phosphate buffer (0.25 *M*, pH 7.4) |
| Ion-pairing reagent | TBAP (0.005 *M*) | HSA (0.005 *M*) |
| Detection system | Post-column detivatization with Ellman's reagent followed by colorimetric detection at 412 nm | Electrochemical detection with glassy carbon electrode, potential + 800 mV |

*System A.* The mobile phase consisted of a mixture of aqueous phosphate buffer (0.25 *M*, pH 7.4) and methanol (95:5) containing 0.005 *M* TBAP as the ion-pairing reagent. The flow-rate was 1 ml/min. For the post-column reaction a stainless-steel column (20 cm × 4 mm I.D.) packed with glass beads (100—120 mesh, dichloromethylsilane-treated) was used [6]. The stock Ellman's reagent [7] for the post-column derivatization consisted of 5,5'-dithiobis(2-nitrobenzoic acid) (0.2%, w/v) and tripotassium citrate (10%, w/v) in phosphate buffer (0.25 *M*, pH 7.4); it was diluted 1:10 with water before use. The flowrate was 0.5 ml/min.

Absorbance was measured using a Varichrom variable-wavelength detector at 412 nm (Varian, Walton-on-Thames, U.K.).

*System B.* The mobile phase consisted of HSA (0.005 *M*) in aqueous phosphate buffer (0.25 *M*, pH 7.4) and was used at a flow-rate of 1 ml/min. The electrochemical detector (LCA 15, EDT Research, London, U.K.) with a glassy carbon electrode was used at a potential of + 800 mV [8].

*Preparation of standard solutions and calibration graph*

Standard solutions containing mesna, D-penicillamine, L-cysteine, DL-homocysteine and glutathione were made up in water or urine. EDTA (final concentration 0.1%, w/v) was added to prevent disulphide formation [9]. Calibration curves were prepared up to 3 $\mu$g of mesna (on column).

*Preparation and analysis of urine samples*

Urine samples were collected from patients receiving mesna (30 mg/kg body weight/dose intravenously; four doses were given each day at times 0, 3, 6 and 9 h after the cyclophosphamide dose) during cyclophosphamide therapy [10]. Control urine samples were obtained before the start of chemotherapy. Urine samples were preserved with EDTA (final concentration 0.1%, w/v) and analysed immediately.

A 50-$\mu$l volume of diluted urine (dilution range 1:3 to 1:39) was injected onto the column. The mesna concentrations were calculated using the calibration graphs.

## RESULTS AND DISCUSSION

Mesna was readily detectable by both of the HPLC and detection systems described and gave a linear calibration graph up to 3.5 $\mu$g (on column) in system A and 7.4 $\mu$g (on column) in system B. Quantitative variation in system A was 15 ng at the 1-$\mu$g level and 0.8 ng at the 25-ng level in system B ($n$=4).

In system B with HSA as the ion-pairing reagent the retention time for mesna is relatively short since it probably does not form an ion-pair with HSA at this pH; however, a sufficient separation from other thiols (Table II) and endogenous compounds (see Fig. 2A) is achieved because a number of these compounds do form ion-pairs with HSA at pH 7.4. HSA possibly modifies the reversed-phase HPLC column giving it ion-exchange characteristics and this could be an important mechanism of separation of mesna from endogenous compounds. Since a number of chemical groups give an electrochemical response at + 800 mV (e.g. phenolic hydroxyl groups), this is important when clinical samples are analysed, where interference from drugs and their metabolites during multiple drug treatment could be a problem.

TABLE II

CAPACITY FACTORS ($k'$) FOR SELECTED THIOL COMPOUNDS USING REVERSED-PHASE ION-PAIR HPLC AS DESCRIBED IN TABLE I

| Thiol compound | System A | System B |
|---|---|---|
| L-Cysteine | 0.1 | 0 |
| DL-Homocysteine | 0.1 | 0.3 |
| D-Penicillamine | 0.72 | 1.5 |
| Glutathione | 1.2 | 0.1 |
| Mesna | 5.0 | 0.2 |

Considerable interest in the application of electrochemical detection has resulted in numerous techniques for detection of phenolic hydroxyl groups (e.g. catecholamines); this study underlines the importance of this technique in the detection of thiol groups.

In system A the resolution is much greater (Table II) because mesna forms an ion-pair with TBAP and this results in discrete peaks for all thiol compounds tested apart from cysteine and homocysteine which are not separated under these conditions. The specific Ellman's assay only detects compounds containing free sulphydryl groups; therefore the number of possible inter-

fering substances is limited and no interfering peaks are present in the control urine samples analysed (Fig. 1A).

The elctrochemical detection has three-fold greater sensitivity when applied to urine samples (limit of detection 25 ng compared to 75 ng in system A); however, with minimum mesna levels in the urine in the order of 50 μg/ml during the first 24 h after mesna administration the sensitivity of the Ellman's assay is sufficient.

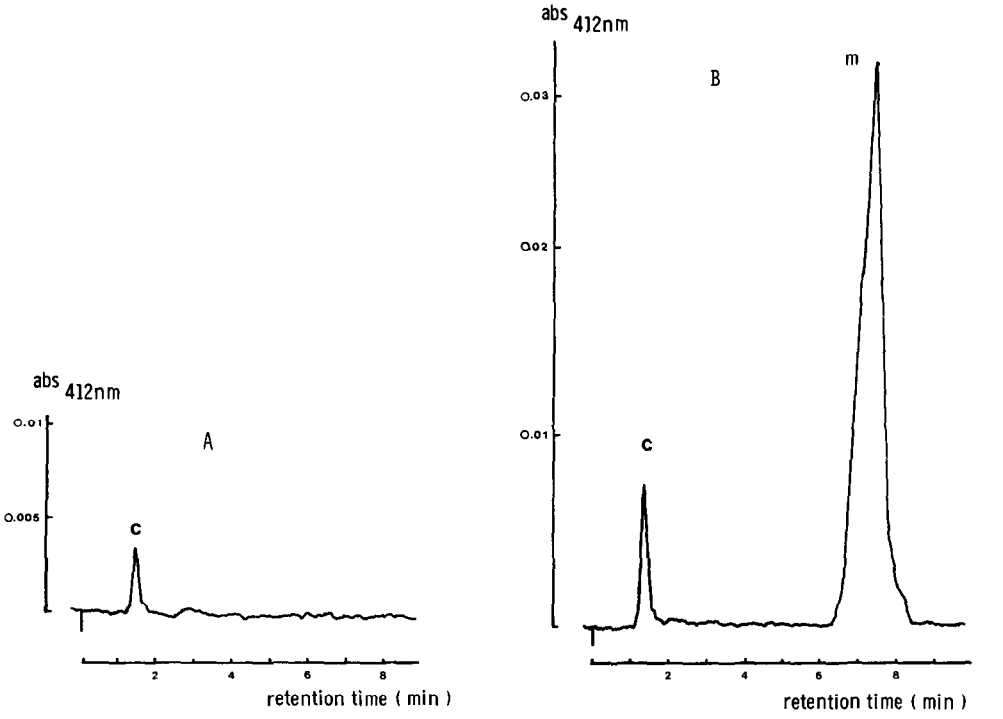


Fig. 1. Reversed-phase HPLC traces (system A) (A) of undiluted control urine showing a small peak for excreted cysteine (c), and (B) of diluted urine (1:5) from a patient receiving mesna during cyclophosphamide cancer chemotherapy. The trace shows enhanced excretion of cysteine (c) and excretion of mesna (m).

HPLC analysis of urine from patients receiving mesna (Figs. 1B and 2B) demonstrates enhanced cysteine excretion and underlines the necessity to use chromatographic separation prior to the colorimetric determination rather than the Ellman's free thiol assay alone (i.e. where total SH levels are measured) [4]. This results in artificially high "mesna" levels in urine from patients receiving mesna. The enhanced cysteine excretion during mesna administration is analogous to the treatment of cystinuria with the thiol drug penicillamine [11] and is presently being studied in our laboratory.

## CONCLUSION

Both HPLC systems followed by either the post-column reaction or electrochemical detection techniques described are suitable for application to rapid

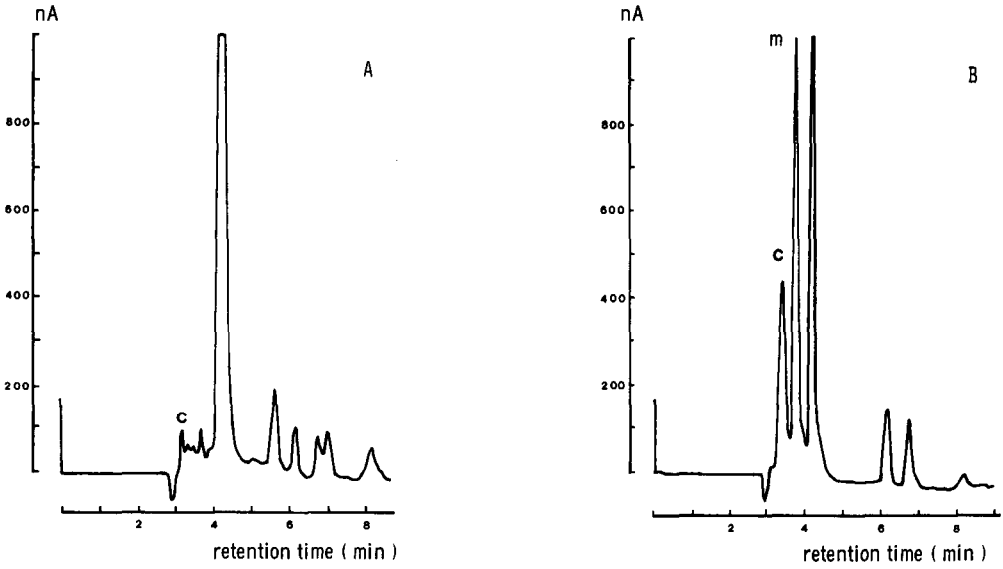


Fig. 2. Reversed-phase HPLC traces (system B) (A) of control urine showing a small peak for excreted cysteine (c), and (B) of urine from a patient receiving mesna during cyclophosphamide cancer chemotherapy. The trace shows enhanced excretion of cysteine (c) and excretion of mesna (m).

clinical assays, and may be used to monitor urinary mesna levels during oxazaphosphorine cancer chemotherapy using mesna uroprotection.

There is no reason why the ion-pairing reagents (TBAP and HSA) and the detection systems cannot be exchanged to produce the combination most suitable for a specific analytical problem.

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