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## Simultaneous Determination of 2-Mercaptoethanesulfonate and Its Disulfide in Human Urine by Isotachophoresis

ISAO IKEUCHI\* and TAMEYUKI AMANO

*Shionogi Research Laboratories, Shionogi & Co., Ltd.,  
Sagisu 5-12-4, Fukushima-ku, Osaka 553, Japan*

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A simple, specific and accurate method for simultaneous measurement of 2-mercaptoethanesulfonate (mesna) and its metabolite 2,2'-dithiobis(ethanesulfonate) (dimesna) in human urine was developed by using capillary isotachophoresis with 0.01 M hydrochloric acid and  $\beta$ -alanine (leading electrolyte, pH 3.20) and 0.01 M *n*-caproic acid (terminating electrolyte). Mesna and dimesna were sufficiently separated from each other and were recovered quantitatively without interference from urine constituents. The detection limit was 1.3  $\mu\text{mol/ml}$  of mesna and 0.8  $\mu\text{mol/ml}$  for dimesna.

The present method was used to determine mesna and dimesna in urine samples of healthy adult volunteers after intravenous administration of mesna.

**Keywords**—sodium 2-mercaptoethanesulfonate; 2,2'-dithiobis(sodium ethanesulfonate); isotachophoresis; human urine; simultaneous determination; intravenous administration

Sodium 2-mercaptoethanesulfonate (mesna) is a therapeutic drug used to alleviate the urotoxic side-effect of oxazaphosphorine antitumor agents such as cyclophosphamide and ifosfamide. The mechanism of mesna action is likely to be based on the formation of a nontoxic additive compound with acrolein as a causative substance derived from the oxazaphosphorines.<sup>1)</sup> Currently, the determination of mesna and its metabolite 2,2'-dithiobis(ethanesulfonate) (dimesna) in urine can be performed by the colorimetric method using 5,5'-dithiobis(2-nitrobenzoic acid).<sup>2)</sup> However, determination of dimesna requires the tedious procedure of reduction of the disulfide bond for color development.

This report describes a simple, specific and accurate method for the simultaneous determination of mesna and dimesna in human urine using capillary isotachophoresis.

### Experimental

**Apparatus and Conditions**—Isotachophoretic analysis was performed with a Shimadzu IP-1B isotachophoretic analyzer equipped with a PG-1 potential gradient detector and two tubes consisting of a pre-separation tube (1.0 mm i.d.  $\times$  15 cm) and a separation tube (0.5 mm i.d.  $\times$  20 cm). The leading electrolyte was 0.01 M HCl adjusted to pH 3.20 by adding  $\beta$ -alanine; 0.2% Triton X-100 was also added to prevent diffusion of the electrophoretic zone. The terminating electrolyte was 0.01 M *n*-caproic acid.

**Materials**—Sodium 2-mercaptoethanesulfonate (mesna) and 2,2'-dithiobis(sodium ethanesulfonate) trihydrate were used as received from Asta-Werke AG, West Germany. Other chemicals were of special grade.

Urine samples were obtained from healthy adult volunteers who had received multiple intravenous administration of mesna. The samples were kept at  $-20^\circ\text{C}$  in a freezer until just before use.

**Standard Solution**—A standard solution of mesna was prepared by dissolving 16 mg in 10 ml of 0.5% dithiothreitol. Further dilutions were made with 0.5% dithiothreitol to obtain the desired concentrations.

Standard solutions of dimesna were prepared by dissolving 30 mg in 10 ml of distilled water, followed by dilution to the desired concentrations. These standard solutions of mesna and dimesna were freshly prepared each time.

**Procedure**—One to 4  $\mu\text{l}$  of a urine sample was introduced directly without pretreatment into the apparatus by means of a 10- $\mu\text{l}$  syringe. The analysis was run at 250  $\mu\text{A}$  for 30 min and the current was then reduced to 100  $\mu\text{A}$ . The isotachopherograms were recorded at a chart speed of 20 mm/min.

## Results and Discussion

Since mesna having the sulfhydryl group was oxidized especially in alkaline solution to its disulfide, the analytical conditions for the simultaneous determination of mesna and dimesna were examined using acidic and neutral electrolytes. To detect both anions of mesna and dimesna, 0.01 M hydrochloric acid- $\beta$ -alanine (pH 3.20) was used as the leading electrolyte and 0.01 M *n*-caproic acid as the terminating one. Under these analytical conditions, mesna and dimesna were distinctly separated from each other and were well resolved from urine constituents, as shown in Fig. 1. The potential unit (PU) value<sup>3)</sup> was determined to be 0.11 for mesna and 0.06 for dimesna.

Dithiothreitol was used as a nonionic protective reagent for oxidation of the sulfhydryl

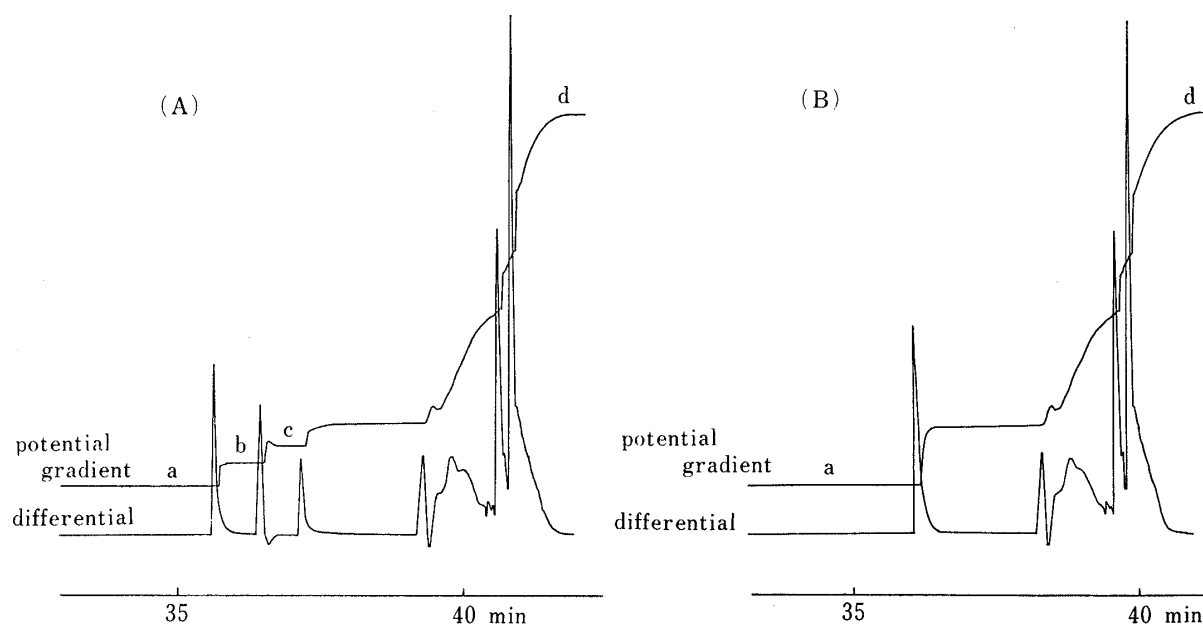


Fig. 1. Isotachopherogram of Mesna and Dimesna in Human Urine

(A) urine sample, (B) urine blank.

a, chloride; b, dimesna (PU value, 0.06); c, mesna (PU value, 0.11); d, *n*-caproic acid.

Leading electrolyte, 0.01 M HCl- $\beta$ -alanine (pH 3.20) containing 0.2% Triton X-100; terminating electrolyte, 0.01 M *n*-caproic acid; applied current, 250  $\mu$ A for 30 min and then 100  $\mu$ A.

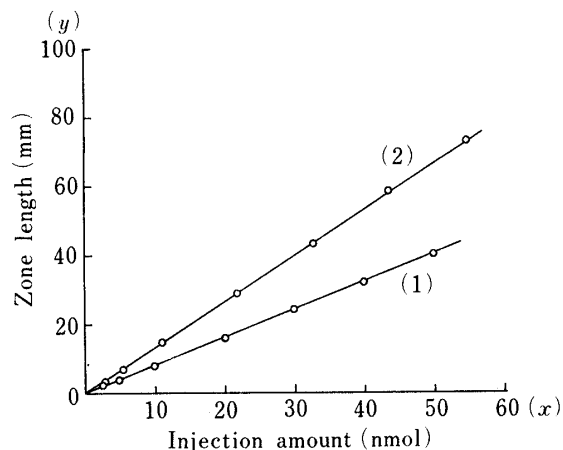


Fig. 2. Standard Curves for Mesna and Dimesna

(1) mesna: regression equation,  $y = 0.809x - 0.07$ ,  $s = 0.08$ ,  $n = 7$ .

(2) dimesna: regression equation,  $y = 1.326x + 0.09$ ,  $s = 0.48$ ,  $n = 7$ .

TABLE I. Mean Urinary Excretions of Mesna and Dimesna from Human Volunteers after Multiple *i.v.* Administrations of Mesna

	1st day (mg)	2nd day (mg)	3rd day (mg)	Total	
				(mg)	(% of dose)
400 mg × 3/d					
Mesna	384 ± 61	375 ± 52	374 ± 64	1133 ± 138	31.5 ± 3.8
Dimesna	735 ± 61	766 ± 69	707 ± 56	2208 ± 169	61.7 ± 4.7
Mesna + dimesna	1123 ± 53	1145 ± 80	3353 ± 100	3353 ± 208	93.1 ± 5.8
800 mg × 3/d					
Mesna	773 ± 76	864 ± 77	948 ± 105	2585 ± 122	35.9 ± 1.7
Dimesna	1411 ± 113	1360 ± 173	1355 ± 89	4125 ± 198	57.6 ± 2.8
Mesna + dimesna	2193 ± 50	2233 ± 239	2310 ± 114	6735 ± 252	93.5 ± 3.5

The values are means ± S.D. of four human subjects.

group in standard solutions to maintain the actual mesna concentrations. In the presence of dithiothreitol, no dimesna was detected in the standard solutions of mesna. Therefore, we concluded that no oxidation of mesna occurred during the analysis. Standard curves for mesna and dimesna (Fig. 2) showed a linear relationship between the zone length and the amount of sample injected over the range of 2.5–50 nmol for mesna and 2.7–55 nmol for dimesna.

Regression analysis for the simultaneous determination of mesna and dimesna was conducted. First, the recovery of dimesna added to human urine was examined in the concentration range of 2–20  $\mu\text{mol/ml}$  (0.65–6.5 mg/ml). The relationship between the added ( $x$ ) and found ( $y$ ) values of dimesna was linear;  $y = 0.976x + 0.28$ , standard deviation ( $s$ ) = 0.71, coefficient of variation ( $c.v.$ ) = 3.31%,  $n = 7$ . The regression equation indicated that dimesna was quantitatively recovered from the urine. Second, the recovery of mesna added to human urine was examined in the concentration range of 1.5–25  $\mu\text{mol/ml}$  (0.25–6.2 mg/ml). Because of the instability of mesna, dimesna produced from mesna added in the urine was detected. Therefore, the amount of added mesna was evaluated as the total amount of the residual mesna and the produced dimesna. The relationship between the added ( $x$ ) and found ( $y$ ) values was again linear;  $y = 0.991x + 0.30$ ,  $s = 0.75$ ,  $c.v. = 3.21\%$ ,  $n = 7$ .

From these results, we concluded that the present method could exactly determine mesna and dimesna levels in urine within 3.5% coefficient of variation. The detection limit was 1.3  $\mu\text{mol/ml}$  for mesna and 0.8  $\mu\text{mol/ml}$  for dimesna in human urine.

The present method was applied to determinations of mesna and dimesna in urine samples of healthy adult volunteers. Mesna was intravenously administered to 8 volunteers at a dose of 400 or 800 mg three times a day at 4-h intervals for three days and 24-h urine samples were collected after administration. The results are summarized in Table I.

With multiple doses of 400 mg of mesna, the mean cumulative urinary excretions of mesna and dimesna for each 24-h period amounted to about 377 and 736 mg respectively; the urinary recoveries were estimated to be about 31.5 and 61.7%, respectively. These values did not change significantly from day to day during the administration. Total urinary recovery was calculated to be about 93.1%.

With doses of 800 mg of mesna, the mean cumulative urinary excretions of mesna and dimesna increased in comparison with the case of 400 mg administration, but the urinary recoveries (percent) were almost the same as those obtained when 400 mg of mesna was given.

These results confirmed that most of the injected materials were excreted in the urine.

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**References**

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