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# A simple high performance liquid chromatography method for determination of rebamipide in rat urine

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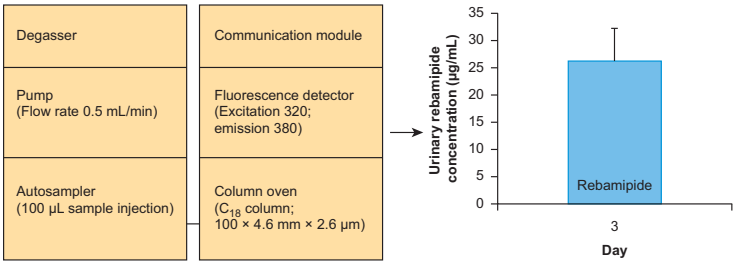
GRAPHICAL ABSTRACT




ABSTRACT

Rebamipide is a mucoprotective agent commonly used to prevent nonsteriodal anti-inflammatory drug-induced gastrointenstinal side effects [1]. Human plasma and urine analysis of rebamipide utilizing high performance liquid chromatography (HPLC) have been reported [2]. Recently, we reported on the plasma levels of rebamipide in presense or absence of celecoxib or diclofenac in rats [3] using a modified HPLC method of detection developed by Jeoung *et al.* [4]. To tailor the method towards use in urinary rebamipide extraction and analysis, the following modifications were made:

- To compensate for high concentrations of rebamipide found in urine, a new rebamipide stock solution was prepared with a final concentration of 50,000 ng/mL.
- Rat urine calibration standards were obtained within the range of 50–1000 ng/mL and 1000–50,000 ng/mL.
- Plasma samples were replaced with urine samples.



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## Method details

Rebamipide urine analysis was carried out using samples obtained from a previous study [5]. In the study, male Sprague-Dawley rats were dosed with rebamipide (30 mg/kg) twice daily for two days via gastric intubation. On day three, animals received one dose of rebamipide in the morning then were transferred to metabolic cages for a 12 h urine collection period.

Analysis of rebamipide concentrations in the urine involved preparation of rebamipide stock solution, development of low and high linear standard calibration curves to compensate for altered rebamipide concentrations noted in urine samples, use of acetonitrile and hydrochloric acid for urinary rebamipide extraction, and HPLC analysis of rebamipide using a Shimadzu HPLC system.

HPLC analysis was carried out using a $C_{18}$ analytical column with a flow rate of 0.5 mL/min and a fluorescent detector set at an excitation wavelength of 320 nm with an emission wavelength of 380 nm.

### *Materials*

Rebamipide powder (Tokyo Chemical Industry CO., Tokyo, Japan) (Cat. R0085). Ofloxacin powder (Sigma–Aldrich, St. Louis, MO, USA) (Cat. PHR1168-1G) HPLC grade methanol (Fisher Scientific, Pittsburg, PA, USA) (Cat. A9984). HPLC grade acetonitrile (Fisher Scientific, Pittsburg, PA, USA) (Cat. BP2405SK-4) 36% hydrochloric acid (Sigma–Aldrich, St. Louis, MO, USA) (Cat. 258148) HPLC grade water (Fisher Scientific, Pittsburg, PA, USA) (Cat. W5-4). HPLC grade glacial acetic acid (Fisher Scientific, Pittsburg, PA, USA) (Cat. A35-500). Drug free rat urine samples. Experimental urine samples from rats dosed with rebamipide.

### *Preparation of stock solutions*

Rebamipide stock solution was prepared by dissolving 10 mg rebamipide powder in 200 mL methanol for a final concentration of 50,000 ng/mL. Ofloxacin was used as an internal standard (IS). Ofloxacin stock solution was prepared by dissolving 100 μg ofloxacin powder in 200 mL acetonitrile. Standard solution was then vortex mixed until fully dissolved for a final concentration of 500 ng/mL.

### *Development of calibration curves*

To develop new standard calibration curves based on higher rebamipide stock concentrations, the following steps were followed:

1. Drug free rat urine (100 μL) was placed in a clean glass tube.
2. Samples were spiked with 100 μL IS.
3. Using methanol, rebamipide stock solution was serial diluted to 50,000, 25,000, 10,000, 5,000, 2,500, 1,000, 500, 250, 100, and 50 ng/mL.
   a. From the dilutions, two calibration curves with different concentration ranges were constructed.
      i. A low range calibration curve was prepared to include 50, 100, 250, 500, 1000, 2500, 5000, and 10,000 ng/mL rebamipide (Fig. 1A).
      ii. A high range calibration curve was prepared to include 10,000, 25,000, and 50,000 ng/mL (Fig. 1B).