

## ASSAY FOR CITRIC ACID/CITRATE AND PHOSPHATE

The following ion chromatographic general procedure is provided for the determination of citric acid/citrate and phosphate in compendial articles, when specified in the individual monographs.

The procedure for preparation of the Standard Preparations used for the assay depends on whether or not citrate and phosphate are being assayed concomitantly, as indicated below.

**Mobile Phase**— Transfer an appropriate volume of water (resistivity not less than 18 megohm-cm) to a suitable container, and degas with helium for not less than 20 minutes. Add an appropriate volume of 50% (w/w) carbonate-free sodium hydroxide or potassium hydroxide to obtain a 20 mM sodium hydroxide or potassium hydroxide solution. Alternatively, a 20 mM sodium hydroxide or potassium hydroxide eluant can be generated electrolytically using an automatic eluant generator. [note—Protect the Mobile Phase from atmospheric carbon dioxide.]

**Standard Preparations**— Use Standard Preparation 1 for an assay for citric acid/citrate only. Use Standard Preparation 2 when a concomitant assay for citrate and phosphate is intended.

**Standard Preparation 1** —Dissolve [USP Citric Acid RS](#) in freshly prepared 1 mM sodium hydroxide to obtain a solution having a known concentration of about 20 µg per mL of citrate (C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>).

**Standard Preparation 2** —Dissolve [USP Citric Acid RS](#) and monobasic sodium phosphate in freshly prepared 1 mM sodium hydroxide to obtain a solution having known concentrations of about 20 µg per mL and 12 µg per mL of citrate and phosphate (PO<sub>4</sub>), respectively.

**Assay Preparation for Citric Acid/Citrate Assay**— Unless stated otherwise in the monograph, dissolve an appropriate quantity of a solid dosage form in freshly prepared 1 mM sodium hydroxide to obtain a solution containing about 20 µg per mL of citrate. If the dosage form is a liquid formulation, dilute with water, and add a freshly prepared sodium hydroxide solution to obtain a solution containing about 20 µg per mL of citrate in 1 mM sodium hydroxide.

**Assay Preparation for Phosphate Assay**— Unless stated otherwise in the monograph, dissolve an appropriate quantity of a solid dosage form in freshly prepared 1 mM sodium hydroxide to obtain a solution containing about 12 µg per mL of phosphate. If the dosage form is a liquid formulation, dilute with water, and add a freshly prepared sodium hydroxide solution to obtain a solution containing about 12 µg per mL of phosphate in 1 mM sodium hydroxide.

**Chromatographic System**—The liquid chromatograph is equipped with a suitable anion trap

column; a 4-mm × 50-mm guard column and a 4-mm × 250-mm analytical column, both packed with L61 packing; and an electrochemical detector with suppressed conductivity detection using either a micromembrane anion autosuppressor or a suitable chemical suppression system. All columns are maintained at a temperature of 30°C and eluted at a flow rate of 2 mL per minute.

**[note]**—An anion trap column designed to remove trace anion contaminants in the Mobile Phase should be added to the column assembly before the injector.] Chromatograph Standard Preparation 1 or Standard Preparation 2, as appropriate, and record the peak area responses as directed for Procedure: the tailing factor is not more than 2.0; and the relative standard deviation of the peak areas for citrate (and phosphate where appropriate), for six replicate injections of Standard Preparation 1 or Standard Preparation 2, is not more than 1.5%.

**Procedure**— Separately inject 10 µL each of the appropriate Standard Preparation and the Assay Preparation into the chromatograph, record the chromatograms, and measure the peak areas for citrate and phosphate, as appropriate. Determine the concentrations of citrate or phosphate in the portion of Assay Preparation taken by the formula:

$$CS (rU / rS)$$

in which CS is the concentration of citrate or phosphate, in µg per mL, in the appropriate Standard Preparation; and rU and rS are the peak areas of citrate or phosphate obtained from the Assay Preparation and the Standard Preparation, respectively.