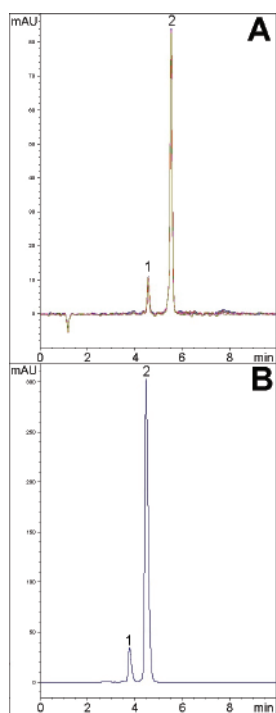
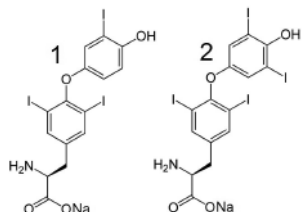


Assay Method for Levothyroxine

Superior Resolution, Reproducibility, & Peak Shapes Compared to USP Method



Notes:

Levothyroxine is the L-isomer of the main thyroid hormone thyroxine (T4). It is used as a replacement for the thyroxine that is deficient in patients with hypothyroidism. Liothyronine is the L-isomer of another thyroid hormone, triiodothyronine (T3). T3 is produced from T4 and is the metabolically active form of the hormone.

Method Conditions

Column: Fig. A: MTC Phenyl Hydride™, 4µm, 100A
 Fig. B: Type B silica-based Cyano, 5µm, 100A

Catalog No.: Fig. A: 69020-7.5P Fig. B: N/A

Dimensions: Fig. A: 4.6 x 75 mm Fig. B: 4.6 x 250 mm

Mobile Phase: Fig. A: A: DI water/ 0.1% formic acid
 B: 97% Acetonitrile/ 3% DI water/
 0.1% formic acid
 Fig. B: 60% DI water/ 40% acetonitrile/
 0.05% phosphoric acid

Gradient: Fig. A

time (min.)	%B
0	20
6	50
7	20

Temperature: Fig. A: 35 °C Fig. B: ambient

Flow rate: Fig. A: 1.0 mL/min Fig. B: 1.5 mL/min

Injection volume: Fig. A: 2 µL Fig. B: 100 µL

Sample: Mix of levothyroxine and liothyronine standards

Stock Solutions: 0.4 mg levothyroxine or liothyronine dissolved with 1 mL 10 mM NaOH in 50:50 DI water: methanol. Working Solution (Fig. A): Aliquots of stock solutions were mixed and diluted with 50:50 A:B to obtain concentrations of 40 mg/L and 4 mg/L for levothyroxine and liothyronine respectively. Working Solution (Fig. B): Aliquots of stock solutions were mixed and diluted with the mobile phase to obtain concentrations of 10 mg/L and 0.2 mg/L for levothyroxine and liothyronine respectively.

Peaks:
 1. liothyronine sodium
 2. levothyroxine sodium

Discussion

The USP assay method for levothyroxine requires that a resolution of not less than 5.0 must be demonstrated between levothyroxine and related compound liothyronine. A chromatogram obtained from following the USP method using a Type-B silica based L10 column is shown in Figure B. The average resolution between the two compounds over five runs is 2.8, which does not satisfy the system suitability for resolution for this assay. Figure A shows the five-run overlay obtained from a method developed with the Cogent Phenyl Hydride™ column. The average resolution in this case was 5.3. In addition, the peak shapes and reproducibility were far superior for the Phenyl Hydride™ method.

For more information visit www.MTC-USA.com

Cat. No. Description

69020-7.5P Phenyl Hydride™ HPLC Column, 100A, 4µm, 4.6mm x 75mm