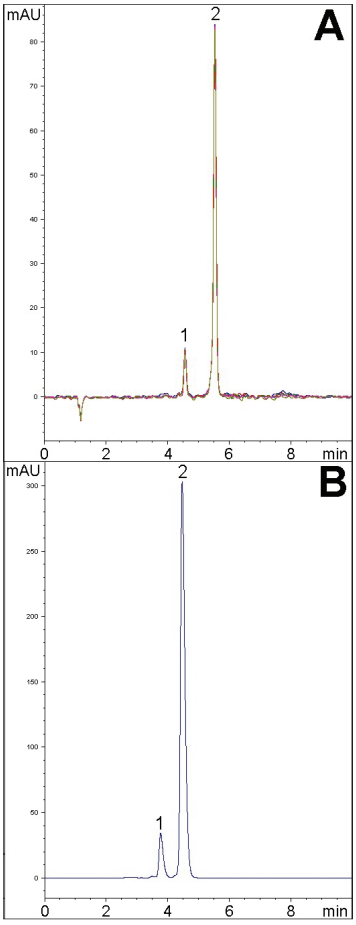
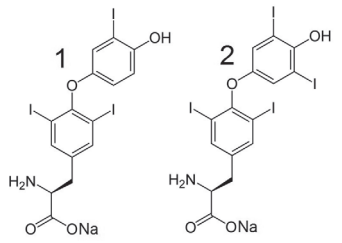
Assay Method for Levothyroxine

Superior resolution, reproducibility, and peak shapes compared to USP method





Note: 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: Cogent Phenyl Hydride™**, 4µm, 100Å
Fig. B: Type B silica-based cyano, 5µm, 100Å

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 H_2O / 0.1% formic acid (v/v)
B: 97% acetonitrile / 3% DI H_2O / 0.1% formic acid (v/v)
Fig. B: 60% DI H_2O / 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

Injection vol.: **Fig. A:** 2µL **Fig. B:** 100µL

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

Sample: Mix of levothyroxine and liothyronine standards.
Stock Solution: 0.4 mg levothyroxine or liothyronine dissolved with 1 mL 10 mM NaOH in 50:50 DI H_2O: 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 Cogent Phenyl Hydride method.

MANUFACTURED BY:
MICROSOLV TECHNOLOGY CORPORATION
9158 Industrial Blvd NE
Leland, NC 28451
p: 1.732.380.8900
f: 1.910.769.9435
customers@mtc-usa.com
www.Cogent-HPLC.com