

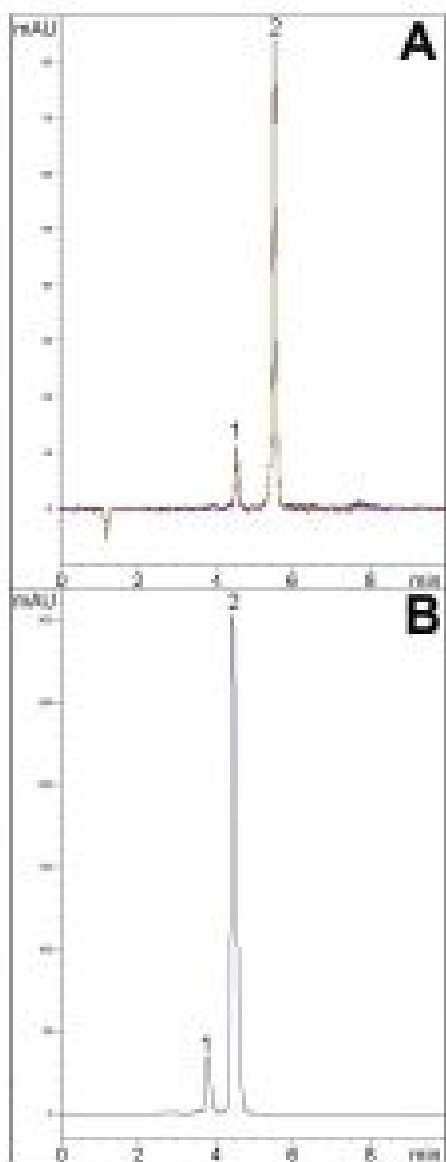
## Levothyroxine Analysis - AppNote

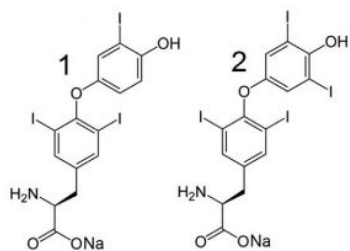
### Superior Resolution, Reproducibility, & Peak Shapes Compared to USP Method

Click [HERE](#) for Column Ordering Information.

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.





## Peaks:

1. Liothyronine Sodium
2. Levothyroxine Sodium

## Method Conditions

### Columns:

Fig. A: Cogent Phenyl Hydride™, 4µm, 100Å

Fig. B: Type B Silica Based Column, 5µm, 100Å

### Catalog Nos.:

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 (v/v)

B: 97% Acetonitrile / 3% DI Water / 0.1% Formic Acid (v/v)

Fig. B: 60% DI Water / 40% Acetonitrile / 0.05% Phosphoric Acid

### Gradient:

Time (minutes)	%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 / minutes

Fig. B: 1.5 mL / minutes

**Sample Preparation:** 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 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.

**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.



## Attachment

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