

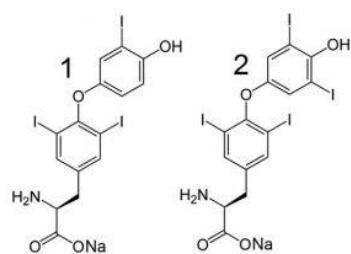
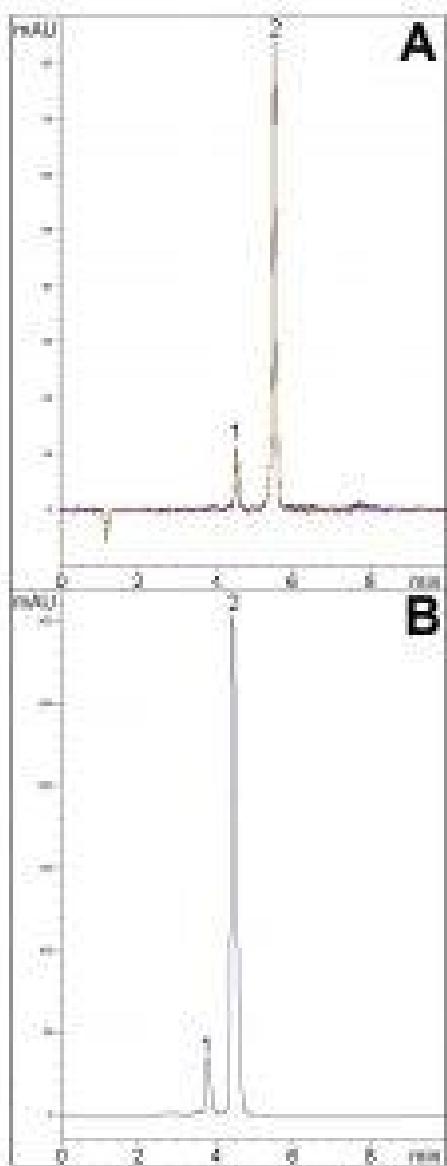
## Levothyroxine and Liothyronine Sodium Analysis by HPLC - AppNote

### 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 <sup>TM</sup>, 4 $\mu$ m, 100 $\text{\AA}$   
 Fig. B: Type B Silica Based Column, 5 $\mu$ m, 100 $\text{\AA}$

### 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.

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**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.

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**Attachment No 128 Levothyroxine Assay pdf 0.3 Mb [Download File](#)**

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**MicroSolv Technology Corporation**

9158 Industrial Blvd. NE, Leland, NC 28451

Tel: (732) 380-8900

Fax: (910) 769-9435

Email: [customers@mtc-usa.com](mailto:customers@mtc-usa.com)

Website: [www.mtc-usa.com](http://www.mtc-usa.com)