

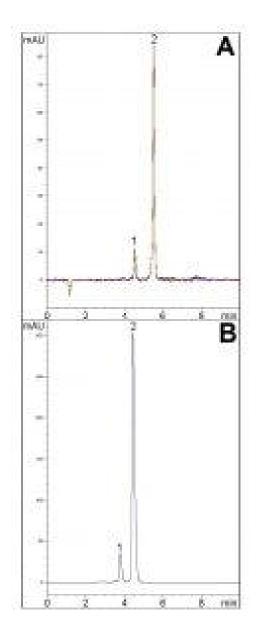
# Levothyroxine Analysis - AppNote

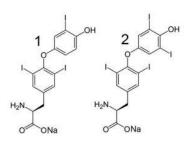
# Superior Resolution, Reproducibility, & Peak Shapes Compared to USP Method

Click <u>HERE</u> 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>™</sup>, 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)%B020650720Temperature:

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

No 128 Levothyroxine Assay pdf 0.3 Mb Download File

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