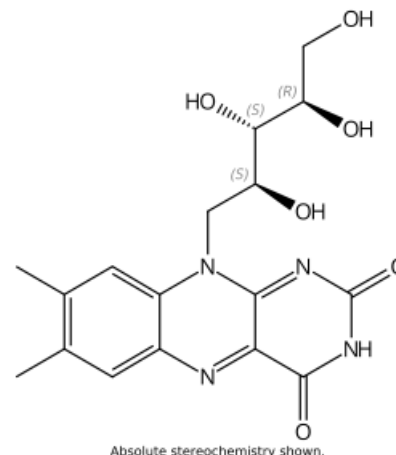


# Certificate of Analysis - Analytical Standard

## Riboflavin (B2)

**Product no.:** 47861  
**Lot no.:** LRAD4427  
**Description of CRM:** Orange powder  
**Expiry date:** December 2025  
**Storage:** REFRIGERATE  
**Certificate version:** LRAD4427.01 (Note: Certificates may be updated due to the availability of new data. Check our website at: [www.sigma-aldrich.com](http://www.sigma-aldrich.com) for the most current version.)  
**Chemical formula:** C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>  
**Molecular mass:** 376.4  
**CAS No.:** 83-88-5



Analyte	Purity (mass balance / as is basis)
Riboflavin (B2)	98.7 % (mass balance / as is basis)

**Metrological traceability:** Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. When applicable, additional traceability to Primary Standards is established through comparative assay determinations. See "Details on metrological traceability" on page 2.

**Measurement method:** Where applicable, the certified value is based on a purity determination by mass balance. See section "Certification process details".

**Intended use:** Intended for Laboratory Use only. Not for drug, household or other uses

**Minimum sample size:** 20 mg

**Health and safety information:** All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.

**Certificate issue date:** 19 DEC 2022

[Andy Ommen - Quality Control]

[Shawn Stetler - Quality Assurance]



**Instructions for handling and correct use:**

Do not dry, use on the as is basis. Protect from light. The internal pressure of the container may be slightly different from the atmospheric pressure at the user`s location. Open slowly and carefully to avoid dispersion of the material. All values reported on the CoA are for the contents of the unopened standard and apply to the initial use of the standard. Any unused portions remaining after the container has been opened should be carefully stored in accordance with prudent laboratory procedures. Many variables are outside of the control of MilliporeSigma. Therefore, MilliporeSigma makes no warranties concerning the continued suitability of previously opened CRMs. Decisions concerning the proper use of previously opened CRMs are the responsibility of the user. Expiration is at end of month given on certificate and label.

**Packaging:**

1G IN AMBER VIAL

**CHROMATOGRAPHIC IMPURITY ANALYSIS**

**METHOD: HPLC (ref.: Riboflavin, Current Compendial Monographs )**

Column: Supelcosil LC-18-DB, 250 x 4.6mm, 5µm particle size

Mobile Phase A: 0.1% Phosphoric Acid in Water

Mobile Phase B: Acetonitrile

Flow Rate: 1.0 mL/min

Column Temperature: 30 °C

Injection Volume: 5 µL

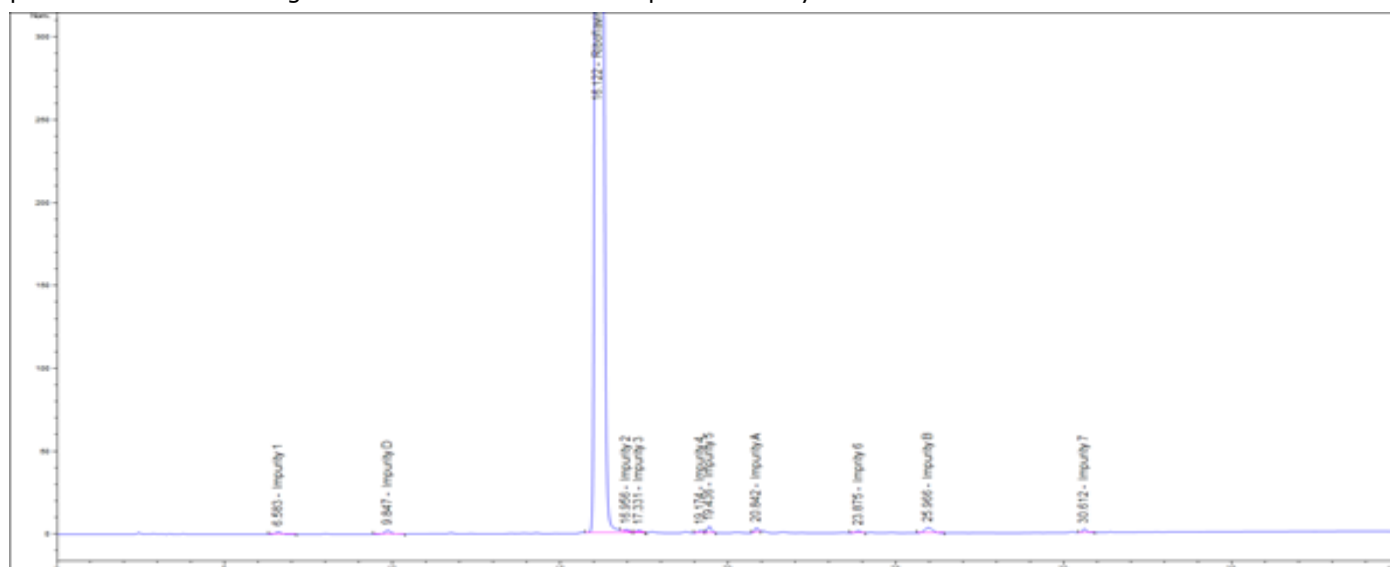
Detector: DAD

Wavelength: 267 nm

**GRADIENT:**

TIME (mins)	MPA (%)	MPB (%)
0	90	10
5	90	10
20	80	20
25	80	20
35	50	50
45	50	50
48	90	10
58	90	10

Representative Chromatogram from Lot: LRAD4427 Impurities Analysis



#### Impurities Detected:

Impurity 1:	0.067 %
Impurity D:	0.120 %
Impurity 2:	0.051 %
Impurity 3:	0.056 %
Impurity 4:	0.064 %
Impurity 5:	0.137 %
Impurity A:	0.135 %
Impurity 6:	0.057 %
Impurity B:	0.219 %
Impurity 7:	0.076 %
Total Impurities:	<b>0.982 %</b>

#### RESIDUE ANALYSIS

Method: Sulfated ash

Sample Size: ~300 mg

Mean of three measurements, Residue = **0.038 %**

#### LOSS ON DRYING/VOLATILES

Method:

Sample Size:

Mean of three measurements, Loss = **0.294 %**

#### PURITY BY MASS BALANCE

**98.7 % mass balance / as is basis**

#### **Homogeneity assessment:**

Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical method: HPLC

Sample size: 20 mg

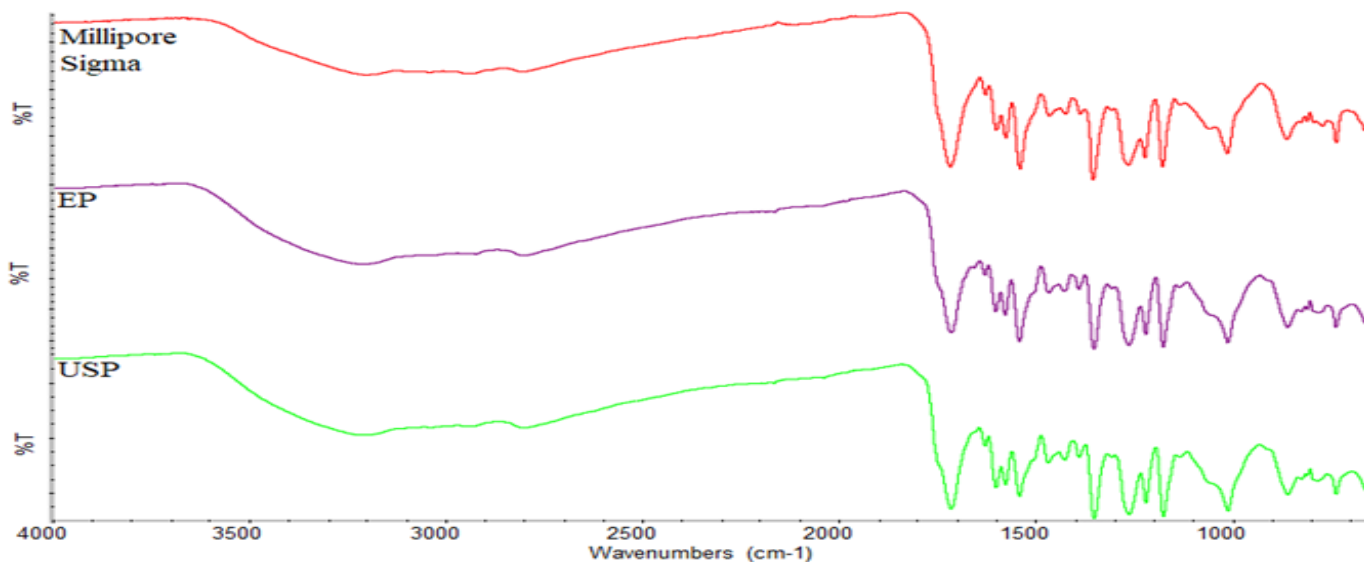
#### **Stability assessment:**

Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.

Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

### Identification Test:

**INFRARED SPECTROPHOTOMETRY** (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)



MilliporeSigma Lot: LRAD4427

### Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAD4427.01	19 DEC 2022	Original release date

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The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

