

## INTRODUCTION

**Riboflavin** (vitamin B2) is a vitamin that plays a **fundamental role in the synthesis of cofactors required for the respiratory chain**. It is used in the **management of mitochondrial cytopathies** that can lead to unexplained **comas** in **pediatric intensive care**.

**Beflavine®** (riboflavin) is **unavailable due to a marketing stop**. The availability of hospital preparations in the form of **riboflavin capsules** is essential to respond to **vital pediatric emergencies**. The European Pharmacopoeia requires **control of the active ingredient content** of hospital preparations as part of the **release control**.

**Objectif : to develop an analytical method for the assay of 50 mg riboflavin capsules by high performance liquid chromatography (HPLC).**

## METHOD

### Formulation :

50 mg riboflavin capsule

- Riboflavin
- Mannitol

### Analytical validation (ICH Guidelines) :

- Linearity : 5-point range of 80 – 120 µg/mL (target concentration : 100 µg/mL) - 3 days ≠
- 3 quality controls (QC) - 3 days ≠ :
  - QC1 : 85 µg/mL - QC2 : 100 µg/mL - QC3 : 115 µg/mL

### Development and validation of dosing method :

- High performance liquid chromatography (HPLC)
- Software : Chromeleon®
- Column : Polaris® C18 250 × 4,6 mm 5 µm
- Mobile phase (pH = 3,1), isocratic 1 ml/min :
  - 85 % citrate buffer 97 mM
  - 15 % acétonitrile
- Diode array UV detector : λ = 268 nm
- Dilution of samples in Dimethyl sulfoxide

## RESULTS

### Linearity

#### Linear regression

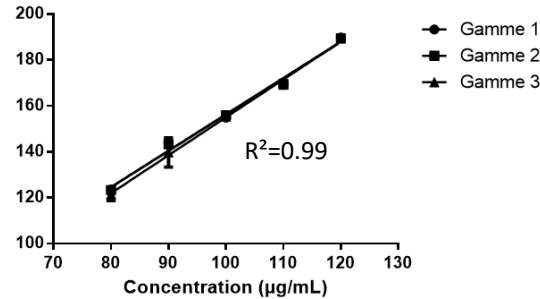


Fig. 1. Calibration range (concentration range 80 to 120 µg/mL)

### Repeatability

Table 1. Repeatability data

Concentration N = 6 (µg/mL)	Coefficient of variation (%)
QC1 = 85	1.7
QC2 = 100	1.1
QC3 = 115	1.2

limit of detection = 10 µg/mL

Limit of quantification = 29 µg/mL

### Accuracy

Table 2. Accuracy data

Concentration (µg/mL)	Recovery rate (%)	Limits of recovery rate
QC1 = 85	101.26	[101.20 ; 101.30]
QC2 = 100	99.75	[99.70 ; 99.80]
QC3 = 115	99.79	[99.70 ; 99.90]

## DISCUSSION-CONCLUSION

The **HPLC method** developed and validated according to **ICH Guidelines** allows the **control of the uniformity of content** required by the European Pharmacopoeia. It enables the **preparation of riboflavin capsules** in advance for immediate availability to **health care services**.