Tryptophan quantification using UV fluorescence measurements on the CLARIOstar® multi-mode microplate reader

Mark Gröne and Franka Maurer, BMG LABTECH, Ortenberg, Germany

BMG LABTECH
The Microplate Reader Company

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- Tryptophan dilution curve shows linearity over a broad concentration range
- Sensitivity was determined to be < 2 nM tryptophan
- Combination of filters for excitation and LVF monochromator™ for emission provides excellent UV fluorescence results

Introduction

Tryptophan is one of the 22 amino acids of which proteins are built. It is an essential amino acid in the human diet as it cannot be synthesized by the human organism and has to be externally taken from food. Furthermore, tryptophan is used as a precursor substance for neurohormones, neurotransmitters as well as for vitamins.

Due to its essential nature, tryptophan is one of the most investigated amino acids. Originally, researchers aimed at identifying nutrients with high tryptophan content for dietary recommendations. Initially, tryptophan was determined by spectrophotometry[1] (Fig. 1) as its aromatic residue enables detection by absorbance (Fig. 2).

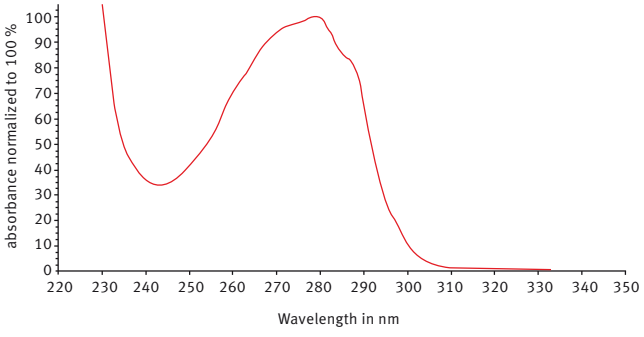

Fig. 1: Tryptophan absorbance spectrum recorded on the CLARIOstar® using 1 nm resolution. In the MARS Data Analysis software the spectral curve was normalized to 100 % for the OD value at 280 nm.

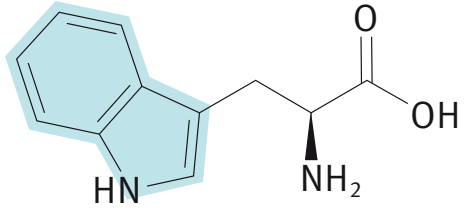

Fig. 2: Structural formula of L-tryptophan. The blue marked part shows the aromatic residue.

With the further development of analytical instrumentation, researchers realized that fluorescence could be exploited for tryptophan determination. Moreover, it was observed that the position of tryptophan within the protein affected its fluorescence.[2] In particular, if the aromatic tryptophan residue is present on the surface of the protein, the fluorescence is much higher compared to the fluorescence that can be captured when tryptophan is located inside the protein. Thanks to this observation, fluorescence could be used to determine the conformational state of a protein and its folding process.

In this application note, we use the CLARIOstar® multi-mode microplate reader from BMG LABTECH for the measurement of tryptophan with fluorescence intensity detection. The CLARIOstar can use either filters or combine filters and LVF monochromators™ for an accurate and sensitive detection of tryptophan.

Materials and Methods

- ☐ L-tryptophan from SigmaAldrich, # T-0254
- ☐ PBS from Biochrom, # L1825
- ☐ 96-well UV-star microplates from Greiner, #655801
- ☐ CLARIOstar® microplate reader from BMG LABTECH (Fig. 3)

Fig. 3: CLARIOstar multimode microplate reader

Standard Curve Preparation

A 10 µM tryptophan stock solution was prepared in PBS buffer. From the stock a 10 point dilution was prepared containing the concentrations shown in table 1. Pure PBS buffer served as blank. In every well of the 96-well plate 300 µl of standard or blank was pipetted. Triplicates for every standard and 18 blanks were pipetted.

Table 1: Tryptophan dilution series

content	Concentration (M)	content	Concentration (M)
S1	5.0E-6	***S6***	5.0E-8
S2	2.5E-6	***S7***	2.5E-8
S3	1.0E-6	***S8***	1.3E-8
S4	5.0E-7	***S9***	6.3E-9
S5	1.0E-7	***S10***	1.3E-9
		Blank	0

Instrument settings

Measurement method: Fluorescence Intensity, Endpoint mode
Number of flashes: 100
Settling time: 0.2

Optical Settings:

Using filter – LVF monochromator combination		
excitation	***dichroic***	***emission***
F: 280-12	automatic monochromator	monochromator 362-25
Using filters		
excitation	***dichroic***	***emission***
F: 280-12	F: Trp-LP	F: 360-20

Automated focus and gain adjustment were performed to obtain the highest dynamic range. The optimal focus for a fill volume of 300 µl resulted in 11.0 mm. Fixed gain and focus values were used for plate-to-plate comparisons with the same filling volume.

Sensitivity (LOD) calculation

The LOD was calculated according to IUPAC standards:

$$LOD = 3^* SD_{blank} / slope_{standard\ curve}$$

Results and Discussion

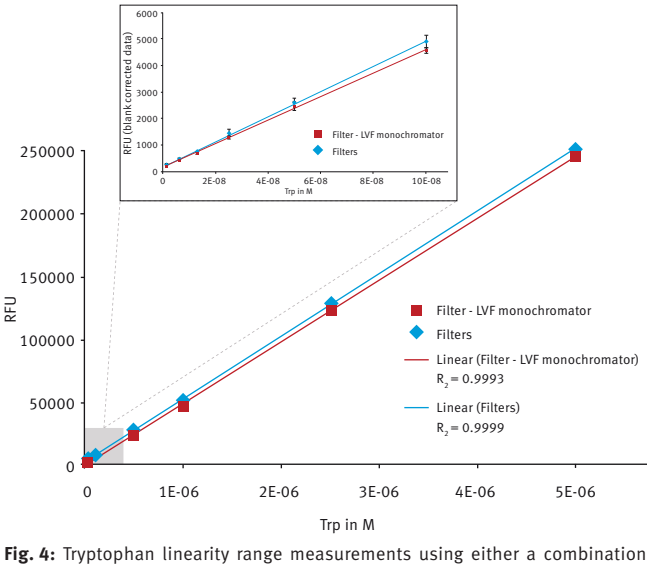

Fig. 4: Tryptophan linearity range measurements using either a combination of filter and LVF monochromator or just filters. The insert zooms into the low nM concentration range.

The standard curve measurement showed a high linearity relation between tryptophan concentration and fluorescence output over a broad concentration range (Fig. 4).

The use either of an emission filter or of the emission LVF monochromator did not affect the sensitivity and accuracy of the measurement, demonstrating that the LVF monochromator has filter-like sensitivity.

Conclusion

The data shown in this application note demonstrate that the CLARIOstar performs accurate and highly sensitive tryptophan fluorescence measurements. The limit of detection was determined to be ‹ 2 nM. This corresponds to a tryptophan concentration of 0.4 ng/ml. This sensitivity can be achieved using either filters for both excitation and emission, or with a filter for excitation and the LVF monochromator for emission.

References

1. Goodwin TW and Morton RA. (1946) The spectrophotometric determination of tyrosine and tryptophan in proteins. Biochem. J. **40**(5-6): 628-632.

2. Imoto T, Forster LS, Rupley JA and Tanaka F. (1972) Fluorescence of lysozyme: emissions from tryptophan residues 62 and 108 and energy migration. Proc. Natl. Acad. Sci. USA **69**(5): 1151-1155.

Germany:	**BMG LABTECH GmbH**	**Tel: +49 781 96968-0**
Australia:	BMG LABTECH Pty. Ltd.	Tel: +61 3 59734744
France:	BMG LABTECH SARL	Tel: +33 1 48 86 20 20
Japan:	BMG LABTECH JAPAN Ltd.	Tel: +81 48 647 7217
UK:	BMG LABTECH Ltd.	Tel: +44 1296 336650
USA:	BMG LABTECH Inc.	Tel: +1 877 264 5227
Internet:	**www.bmglabtech.com**	**applications@bmglabtech.com**