Fraunhofer
IGB

Analytical Report
27_21_1_D

HPLC analysis of Peptide Complex IPH AVN

to:
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1 Sample description and problem definition

A sample and a standard were supplied by the customer. The following HPLC analysis should be made: Comparison/identification of the respective peptide with the corresponding standard (IPH AVN with the standard peptide AVN) and determination of the purity by means of ultra high performance liquid chromatography with mass spectrometry (UHPLC/MS/MS) and ultra high performance liquid chromatography with diode array detection (UHPLC/DAD).

2 Testing period

The sample was measured and analysed from 18.-26.02.2021.

3 Sample description

Sample receipt	Client's sample name	Code
15.02.2021	Peptide AVN (Batch No: P180316-4)	27/21/1
15.02.2021	Peptide Complex IPH AVN	27/21/2

4 Description of the test procedure and results

The standard and the sample were weighted-in, diluted with ultrapure water and measured by high performance liquid chromatography (HPLC). For the detection of the signal a diode array detector (DAD) and also a mass spectrometer (MS) was used.

Used equipment:
UHPLC Agilent 1290 Infinitiy with diode array detector (DAD) and mass spectrometer Thermo Scientific LTQ XL.

Column: Hypercarb, 2,1 x 100 mm, 3 µm
Eluent: A: 0,1% formic acid in water
B: 0,1% formic acid in methanol

Time [min]	Eluent A	Eluent B
0 min	98%	2%
22 min	78 %	22 %
45 min	0 %	100%
50 min	0 %	100%

Flow: 0,2 ml/min
Wavelenght: 220 nm

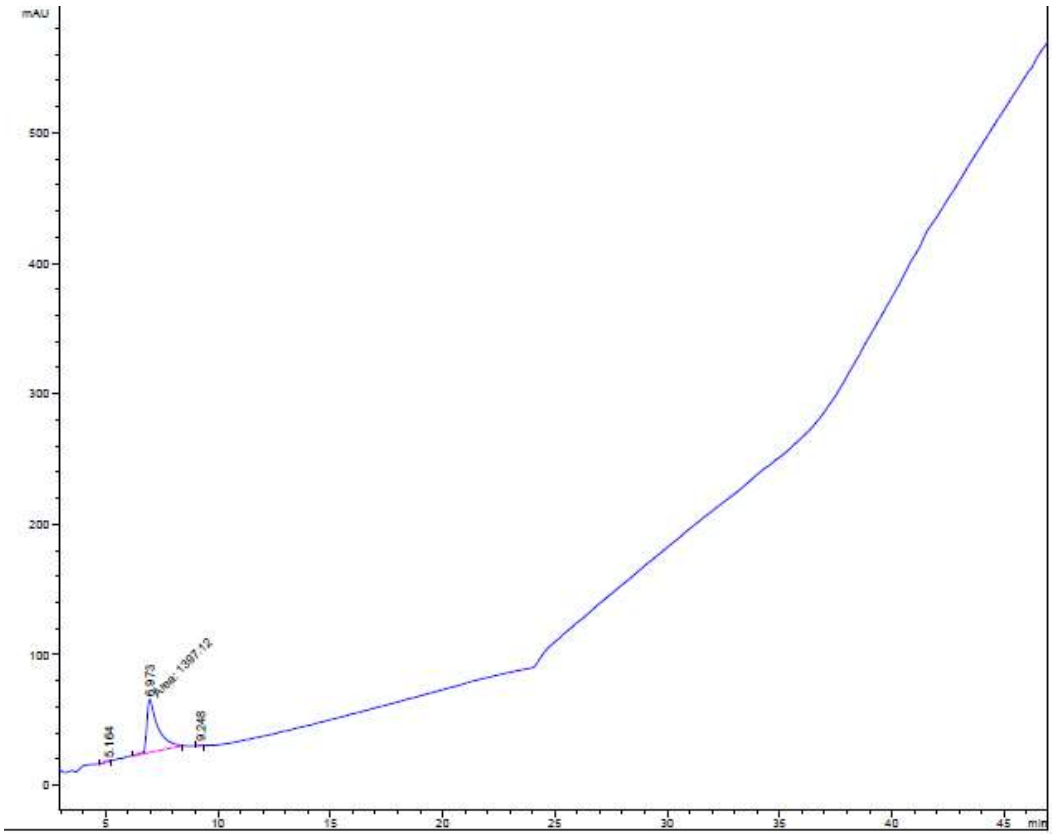

Figure 1: HPLC/DAD-chromatogram (220 nm) of the standard 27/21/1 (200 mg/L)

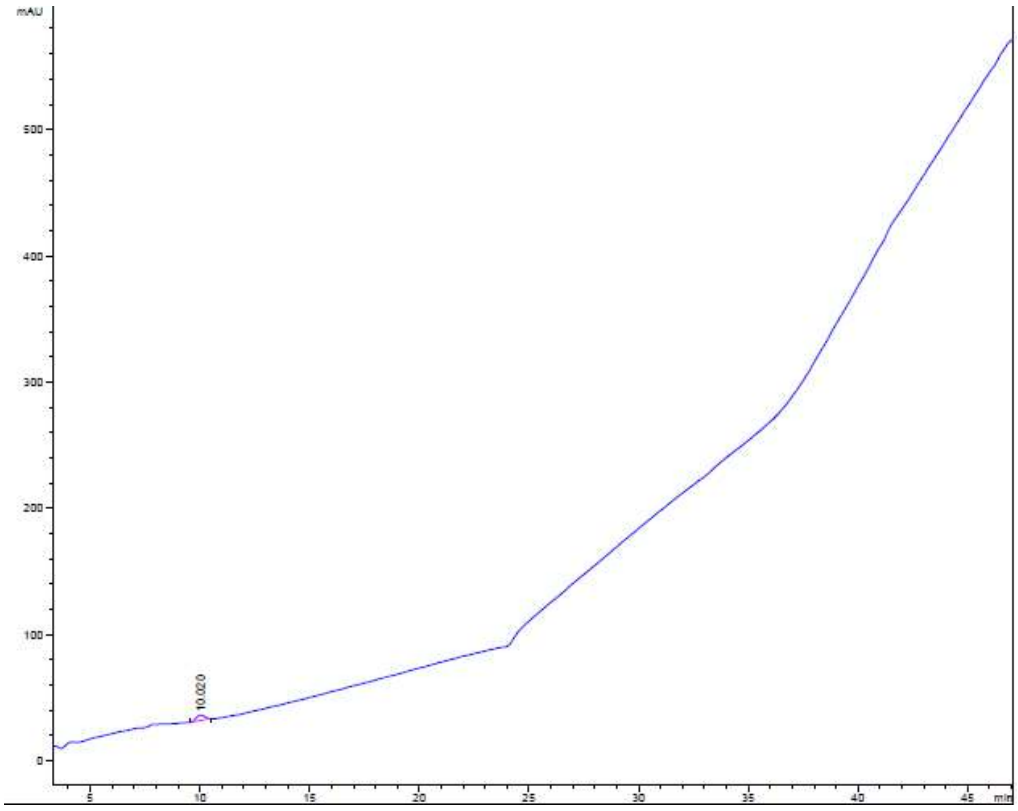

Figure 2: HPLC/DAD-chromatogram (220 nm) of the sample 27/21/2 (2000 mg/L)

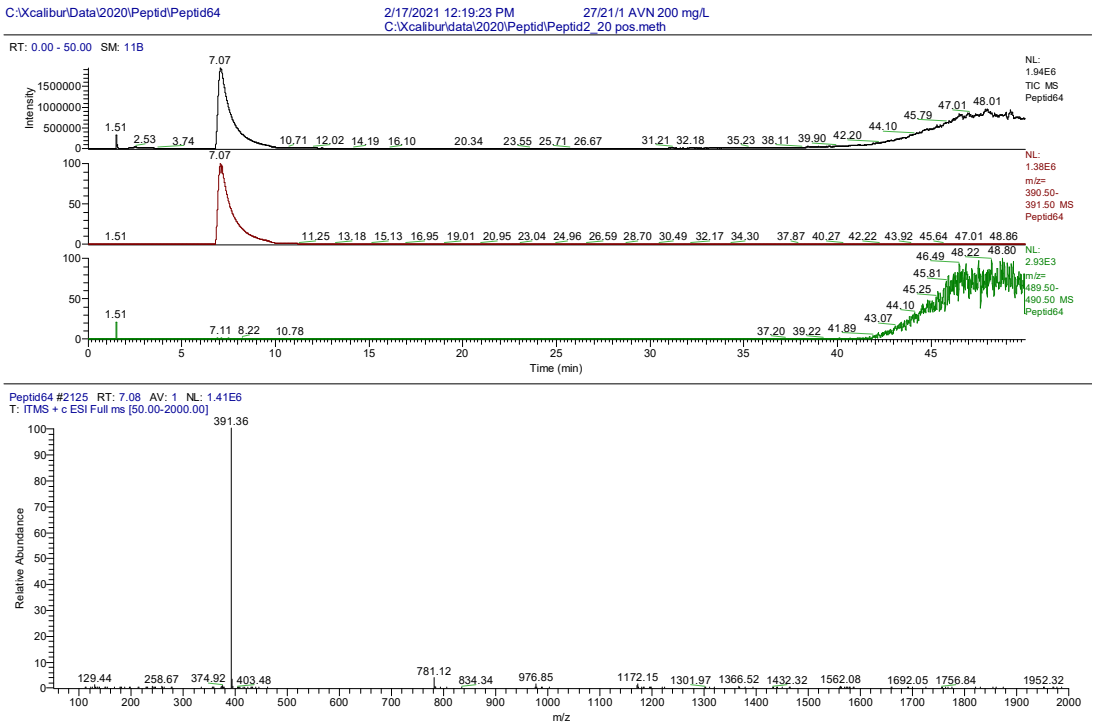

Figure 3: HPLC/MS-Chromatogram of the standard 27/21/1 with the spectrum of the peak at a retention time of 7,07 min

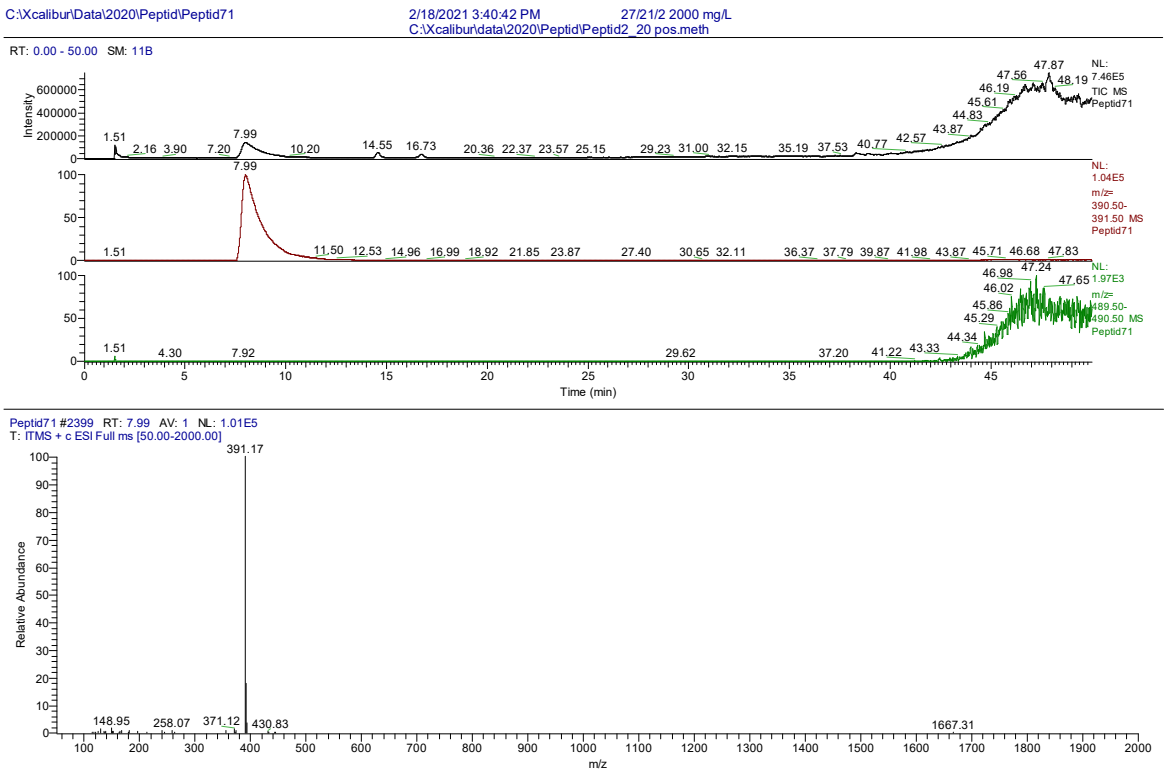

Figure 4: HPLC/MS-Chromatogram of the sample 27/21/2 with the spectrum of the peak at a retention time of 7,99 min

In the HPLC/UV chromatogram of sample 27/21/2, the peptide of the standard cannot be detected even though the sample weight is 10 times higher.

In the HPLC/MS chromatogram, the peptide of the standard can be identified in the sample.

The chromatograms of the sample look relatively clean. But by calculating the amount of peptide in the sample by comparison of peak areas between sample and standard, the sample roughly consists of only 1% of the peptide of the standard.

Katharina Wasmer

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