

Dosage of α_{S1} -casein (f91-100) in lactium™ by HPLC. Translation of Ingredia method # MLB C119B

1- ASSAY TITLE

Determination of α_{S1} -casein (f91-100) content in lactium™ powder by HPLC

2- SCOPE

This assay can be used to determine α_{S1} -casein (f91-100) natural decapeptide content in lactium™ powder by HPLC

3- PRINCIPLE

lactium™ powder is solubilised in a mix of water/acetonitrile/trifluoroacetic acid and analyzed by HPLC against external standard of synthetic α_{S1} -casein (f91-100). The HPLC column is C18 XTerra®, 5 μ m, 4.6 x 250 mm. The mobile phase is a gradient, consisting of 0.1% trifluoroacetic acid in water and acetonitrile with a flow rate of 0.8 mL/min and detection is UV at 280 nm.

4- SAFETY PRECAUTIONS

Consult the Material Safety Data Sheet (MSDS) and of any chemical used that is unfamiliar. All chemicals should be considered hazardous – avoid direct physical contact. MSDS of lactium™ is available upon request.

5- STANDARD

Synthetic α_{S1} -casein (f91-100), >85% by HPLC (Neosystem, France)
Each standard purity can precisely determined using HPLC and a spectrophotometer.

6- APPARATUS

Balance, analytical, accurate to minimum ± 0.1 mg
Filter 0.45 μ m, PFDF or GHP
High performance liquid chromatography apparatus
HPLC column, C18 XTerra®, 5 μ m, 100Å, 4.6 x 250 mm (Waters)
Magnetic stirring bars
Multipoint magnetic stirrer
Pipettes, class A, assorted sizes
Polypropylene syringes (without needle)
Semi micro cuvettes
Spatula
Spectrophotometer UV/visible
Vials, chromatography with caps
UV/visible detector (preferably a photodiode array detector)
Volumetric flasks and cylinders, class A, assorted sizes

7- REAGENTS

Trifluoroacetic acid, HPLC grade

Acetonitrile, HPLC grade

Water, HPLC grade

8- STANDARD PREPARATION

Accurately weigh about 10 mg (± 0.1 mg) of synthetic α_{S1} -casein (f91-100) into a 50-mL polypropylene or glass flask. Add accurately a mix of eluents A and B (74 volumes of eluent A with 26 volumes eluent B) to obtain a concentration of 1.0 mg/ml α_{S1} -casein (f91-100) after a first slow moistening with the same solution. Keep the solution under stirring during 30 min. Dilute this stock solution if needed. Suggested dilutions are around 1:20. Filter a portion through a 0.45 μ m filter into an HPLC vial.

9- SAMPLE PREPARATION

Accurately weigh about 20 mg (± 0.1 mg) of lactiumTM into a 50-mL polypropylene or glass flask. Add accurately a mix of eluents A and B (74 volumes of eluent A with 26 volumes eluent B) to obtain a concentration of 2.0 mg/ml of lactiumTM after a first slow moistening with the same solution. Keep the solution under stirring during 30 min. Filter a portion through a 0.45 μ m filter into an HPLC vial.

10- CHROMATOGRAPHIC CONDITIONS

Column: C18 XTerra®, 5 μ m, 100Å, 4.6 x 250 mm (Waters)

Column temperature: 37 °C

Flow rate: 0.8 mL/min

Mobile phase: linear gradient

Eluent A

Mix 0.1% (v/v) of trifluoroacetic acid with water

Eluent B

Mix 0.1% (v/v) of trifluoroacetic acid with acetonitrile

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0.0	0.8	74	26
20.0	0.8	66	34
20.1	0.8	1	99
25.0	0.8	1	99
25.1	0.8	74	26
34.0	0.8	74	26

Detector: UV between 200 and 310 nm

Injection volume: 100 μ L

Run Time: 34 min

Retention time of α_{S1} -casein (f91-100) : 16 min

11- PROCEDURE

Prepare reference standard solutions and sample preparations as directed.
Make injections of the standard preparations.
Create a linearity plot of standard peak areas versus standard concentrations.
Make injections of the sample preparations.
Calculate α_{S1} -casein (f91-100) concentration in the samples.

12- SYSTEM SUITABILITY:

The correlation coefficient of the linear regression must be ≥ 0.999 for α_{S1} -casein (f91-100) linearity curve.

13- IDENTIFICATION

The peak of α_{S1} -casein (f91-100) in the chromatogram obtained with the sample solution (Figure 1) is similar in retention time (17min) to the principal peak in the chromatogram obtained with standard solution (Figure 2). The peptide absorption is characteristic between 215 and 290 nm due to 2 tyrosine residues. Its spectrum between 210 and 290nm is particularly relevant with 2 significant maxima of absorption at 223 and 275nm (Figure 3). The ratios A_{215}/A_{280} and A_{223}/A_{275} are about 7.0 and 6.8 respectively and allow to checking purity of the peak.

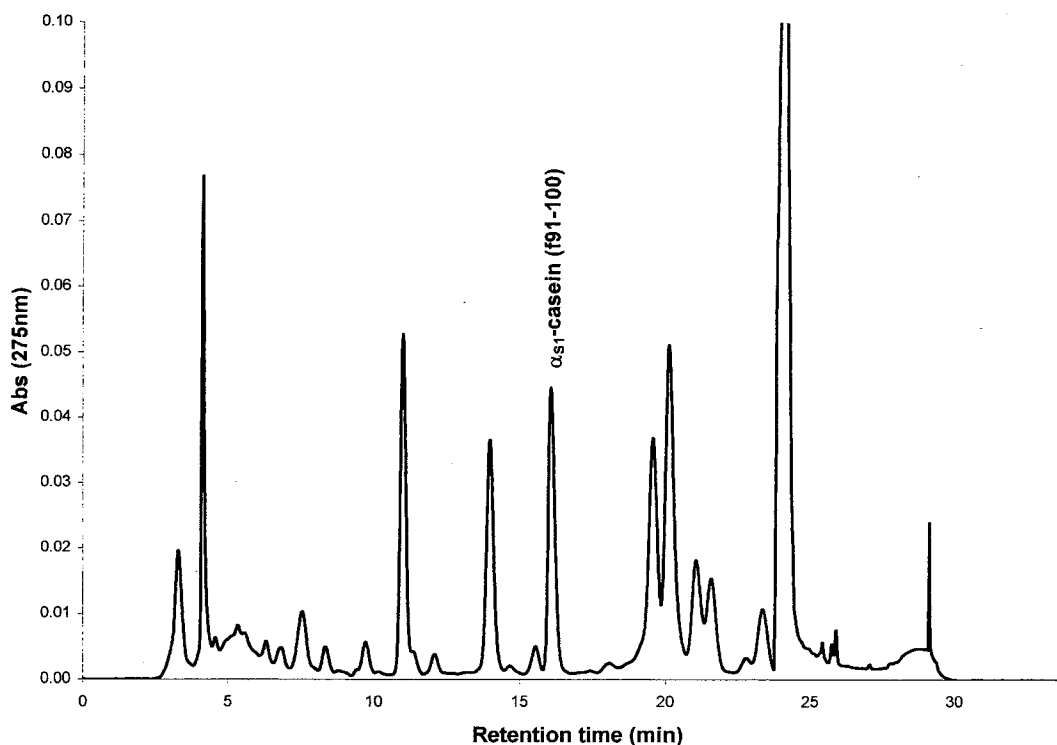


Figure 1. High performance liquid chromatography of lactium™ at 275nm

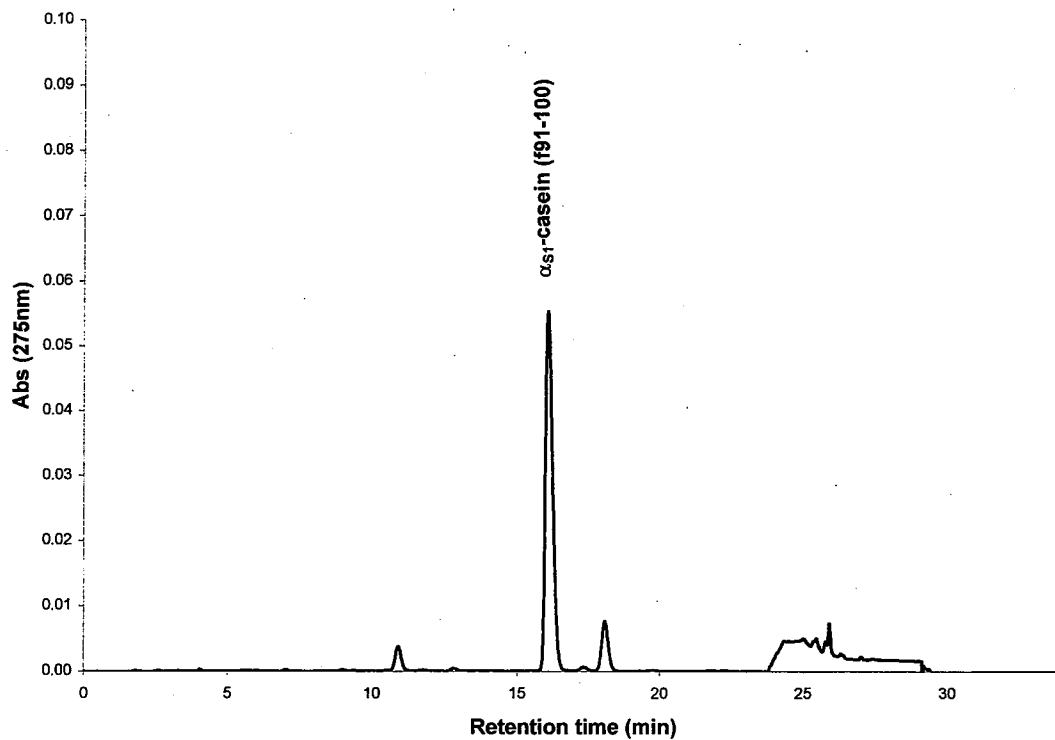


Figure 2. High performance liquid chromatography of the synthetic peptide α_{S1} -CN (f91-100) at 275nm

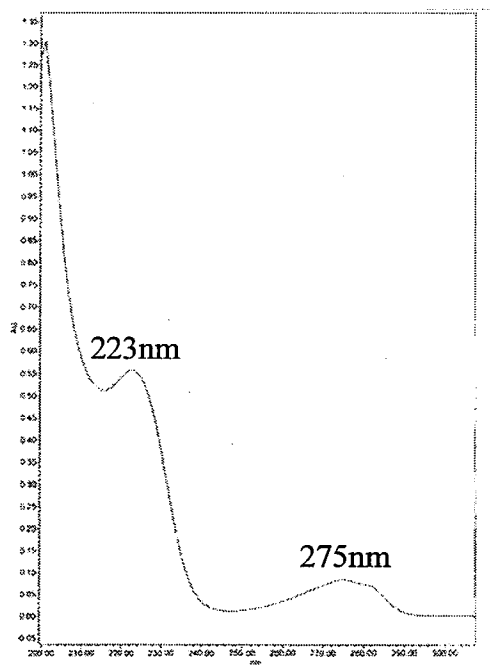


Figure 3. UV spectrum of peptide α_{S1} -CN (f91-100)