NESTLE P T C KONOLFINGEN	HPLC Method N°1050-1(14-1) / Remis: 0089(79) **Peptide profiling by size exclusion SE-HPLC**	KR-0007 17-Sep-01 FEM, BC,BR

Method principle & application

Separation of soluble and redissolved peptides/proteins by size exclusion. Application for hydrolysate based formulas, such as hypoallergenic formulas, extensively hydrolysed formulas, clinical and dietetic products.

HPLC

Size Exclusion SE-HPLC

Instruments

HPLC HP 1050 (Agilent Technologies); Quaternary pump; Autosampler; Online degasser; DAD 1050

Detector 1 Diode Array Detector HP 1050

Wavelength A: **220 nm** Wavelength B: **214 nm** Ref. 360/100

Computer HP ChemStation Dell Optiplex Gxi 110 Pentium

Software LC 3D Dos Software G1307; Cirrus GPC-Software

HPLC Method File

TFAGFB.m

Column	TSK 2000 SWXL 25SWXN3101, Agilent Technologies
Stationary Phase	Spherical cross-linked polystyrene/divinylbenzene particles 8-10 µm.
Mobile Phase A)	HPLC grade water - TFA 0.1%

Conditions

Column Temperature	**off**	°C
Flow	**0.500**	ml/min
Injection Volume	**25**	µl

Solvent Gradient

Time (min)	Solvent A)	Solvent B)	Solvent C)	Solvent D)	Flow (ml/min)
0.01	100.0	0.0	0.0	0.0	0.500
45.00	100.0	0.0	0.0	0.0	0.500

Stop Time 45.00

CALIBRATION

Standard Solutions:

	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6
	alpha-lactalburnin	Cytochrom C	Trypsin Inhibitor	Insulin	Insulin chain B	Angiotesin I
Weight [mg] +/- 0.01mg	0.5	0.5	0.5	0.5	0.5	0.5

Standard Solutions:

	Comp. 7	Comp. 8
	Bradykinin	L-Tyrosine
Weight [mg] +/- 0.01mg	0.5	0.5

Each Compound is weighted separatly into an 1.5 ml Eppendorf tube and dissolved in 1000 µl of water - TFA 0.1% (Mobile Phase A). Store the solutions at -20°C.
The standard solutions are injected as samples at the beginning of the sequence (injection volume: 25 µl).

Remarks:

Determination of the peptide profile, the molecular weight distribution and median molecular weight.
(no complete validation)

SAMPLE PREPARATION (Prepare single determinations, with blank and standard in the HPLC sequence.)

	milk (3.3%p.)	MSK (35%p.)	IMP(25%p.)	ref. P.J.Powder (%p.)
Sample Weight [g] +/- 0.1mg*	0.9667	0.0911	0.1276	

Protein concentration: approx.31.9 mg/10ml sample solution (5mgTN/10mL sample solution)

* for both sample preparations the same sample-amount is weighted

Instructions

Soluble and redissolved peptides / proteins - urea 6M/DTE 50mM/Tris 50mM pH 7.8:

Weight the sample into a 10 ml graduated test tube, for powder samples dissolve with ~4 ml dist. water. Add 3.6 g urea, 77 mg dithioerythritol (DTE), 1.0 ml Tris-HCl buffer 0.5 M / pH 7.8*.
fill up to 9 ml with dist. water, close and mix the tubes and let stand for 1h at 20-25°C (to complete dissolution of urea). Fill to 10 ml with dist. water, mix well and wait for another 1h.
The clear sample solutions** filter through Anatop 25 plus 0.1 µm membrane filter into auto sampler vials by using 2 ml disposal syringes. Analyse by HPLC within 24 h (25°C).

Soluble peptides / proteins - TFA 0.1%:

Weight the sample into a 10 ml graduated test tube, dissolve and fill to10 ml with water - TFA 0.1%. Filter the sample-solutions throgh acrodisc 0.2 µm mebrane. Analyse by HPLC.

*6.06 g tris(hydroxymethyl)-aminomethan +80ml water, adj. pH with HCl 2M, fill up to 100 ml; stable for about 1 year at +4°C.
**For turbid solutions fill about 1.5 ml of the sample solution into a 2 ml Eppendorf tube, centrifuge for 5 min at 15800 x g.

Ref. : Seidelmeier W, Wieser H, Belitz H-D, Z Lebensm. Unters Forsch (1987)185:487-489

REAGENTS & MATERIAL LIST (equal quality can be used as well)

Remarks	Name	Order No.	Supplier
Std.No. 1	alpha-lactalbumin	L-5385	Sigma, St.Louis MO (USA)
Std.No. 2	Cytochrom C	18020	Serva
Std.No. 3	Trypsin Inhibitor	13718	Serva
Std.No. 4	Insulin	I-5500	Sigma, St.Louis MO (USA)
Std.No. 5	Insulin chain B	I-6383	Sigma, St.Louis MO (USA)
Std.No. 6	Angiotesin I Angiotensin	A-9650	Sigma, St.Louis MO (USA)
Std.No. 7	Bradykinin	B-3259	Sigma, St.Louis MO (USA)
Std.No. 8	L-Tyrosine	93829	Fluka
	HPLC grade water (ultra pure water equipment - Maxima)		USF Elga
Column	TSK 2000 SW 25XN3101	475533	Agilent Technologies
TFA	Trifluoracetic acid (puriss. p.a. for HPLC)	91707	Fluka, Buchs (CH)
Tris	Tris(hydroxymethyl)-aminomethan e	8382	Merck, Darmstadt (D)
	Urea (Harnstoff) GR	1.08487	Merck, Darmstadt (D)
DTE	1,4-Dithioerythritol >99% MicroSelect	43794	Fluka, Buchs (CH)
	Anotop 25 plus 0.2µm membrane filter (Whatman)	35 930 15	Merck, Darmstadt (D)