UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Peptibolomics of the cow's udder: peptide profiling of the teat canal

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Introduction

Peptibolomics is a new interdisciplinary area in the *–omics* family, encompassing the specific study of functional peptides available *in vivo*. Functional means not only a biologically relevant effect or property, but also potentially serving directly or indirectly a diagnostic or therapeutic pharmaceutically relevant purpose. In the innate immunity system of the bovine mammary gland, the epithelial cells may play an important role, *i.a.* through the secretion of antimicrobial peptides. To investigate the role of peptides in udder-diseases, the proposed strategy is to obtain and compare peptide profiles of anatomically specified samples taken from physio-pathologically defined cows. The methodology currently under investigation is a multi-dimensional LC with ESI-ion trap MS^n detection. Model-peptides are used for its development and evaluation. This system will be used for the comparative peptide-profiling of the teat canal fluid, the teat canal epithelium and the cistern mucosa of the cow udder.

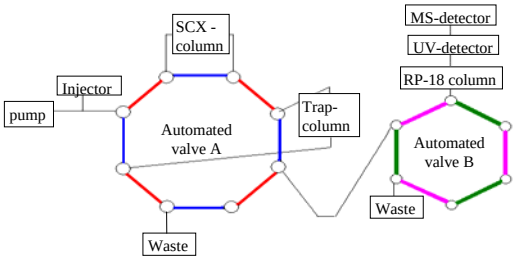
Experimental

1D Evaluation of two SCX columns

Column A	ZORBAX 300-SCX (2.1 x 50 mm, 5 µm) (Agilent Technologies, USA)
Column B	PolySULFOETHYL A (2.1 x 100 mm, 5 µm, 300 Å) (Phenomenex, USA)
Column temp.	25 ± 5 °C
Injection vol.	20 µL
Run time	33 min (excl. 17 min equilibration)
Flow	0.2 mL/min
Sample temp	20 °C
Mobile phase (pH = 3 , HCOOH)	A: Water/ACN (98/2, V/V) B: 1M $HCOONH_4$/ACN (98/2, V/V) Gradient: see table below
UV (DAD)	190 nm to 400 nm
Fluorescence detector	Excitation: 230 nm Emission: 240 nm to 800 nm

Time (min)	Flow (mL/min)	A	B
0	0.2	100	0
3		100	0
33		0	100

2D-LC UV/ESI-ion trap MS



Red line: Represents position 1 of valve A

Blue line: Represents position 2 of valve A

Green line: Represents position 1 of valve B

Purple line: represents position 2 of valve B

Pump: P1000RX (Thermo) quaternary pump

(low pressure mixing)

1D Evaluation of two trap columns and one RP-18 column

Trap Column A	Security Guard Cartridges (C18, 3.0 x 4.0 mm) (Phenomenex, USA)	
Trap Column B	MassPREP On-line Desalting Cartridges (2.1 x 10 mm) (Waters, USA)	
RP-18 Column	Vydac Monomeric C18 238EV52 (2.1 x 250 mm, 5 µm, 300 Å) (Grace, USA)	
Column temp.	23 ± 2°C	
Injection volume	20 µl	
Flow	0.2 mL/min	
Sample temp.	Room temperature	
UV Detection	215 and 275 nm	
ESI-Ion trap MS detector parameters: methods A and B		
Ion spray voltage	4.5 kV	4.5 kV
Capillary temp	225 ° C	225 ° C
Capillary voltage	39 V	45 V
# micro scans	3	1
Max inj. time	50 msec	2000 msec
m/z range	100-2000 Da/e	1000-2000 Da/e
Zoom scan	Data dependent	Data dependent
MS/MS	Data dependent	Data dependent
Mobile Phase		
Trap columns	10 min isocratic (2% ACN in H_2O + 0.1% HCOOH), followed by ACN gradient.	
RP-18 gradient	5 min ACN/H_2O 5/95, V/V (0.1% HCOOH m/V) 45 min ACN/H_2O 60/40, V/V (0.1% HCOOH m/V) 60 min ACN/H_2O 90/10, V/V (0.1% HCOOH m/V)	

Results and discussion

Unidimensional LC characteristics of the model peptides

Peptides	Characteristics		SCX A			SCX B			RP-C18			Trap A	Trap B
	pI	AA (n)	RT (min)	k'	$HCOONH_4$ (M)	RT (min)	k'	$HCOONH_4$ (M)	RT (min)	k'	AcCN (%)	RT (min)	RT (min)
Leucine-enkephalin	5.9	5	12.42	11.42	0.27	12.87	11.86	0.26	25.45	7.37	28.9	-2	0.8
Bradykinin	12.4	9	-1	-	-	23.02	22.01	0.60	15.33	4.04	15	>10	-1
VYV	5.9	3	10.25	9.24	0.34	12.45	11.44	0.25	5.58	0.84	5	2.7	0.6
LLY	5.9	3	10.85	9.85	0.22	12.38	11.38	0.25	12.37	3.07	11	-2	1.5
GGYR	9.8	4	14.55	13.55	0.34	18.33	17.32	0.45	3.06	0.01	5.0	0.8	0.6
Gonadorelin	9.6	10	32.4	31.40	0.94	18.87	17.86	0.46	21.28	6.00	23.2	-2	0.8
b Insulin	5.3	51	2.44	1.44	0	33.77	32.76	0.96	30.83	9.14	36.3	-2	>10
Goserelin	n.a	9	38.03	37.03	>1	18.3	17.30	0.45	28.65	8.42	33.3	-2	-2
LY	5.9	2	9.63	8.63	0.18	12.39	11.39	0.25	3.78	0.24	5	1.2	0.7
Protirelin	~7	3	-1	-	-	-1	-	-	28.84	8.49	33.6	-2	-2
Buserelin	n.a	9	27.67	26.67	0.78	-2	-	-	29.58	8.73	34.6	-2	-2
Vancomycin	8.3	7	14.20	13.20	0.33	20.80	19.80	0.53	3.53	0.16	5	0.9	0.80
h Defensin HNP-1	8.3	30	-2	-	-	-2	-	-	26.87	7.84	30.9	-2	-1
h Defensin HNP-2	8.3	29	-2	-	-	-2	-	-	26.85	7.83	30.9	-2	-2
h β-defensin 1	8.6	36	-2	-	-	-2	-	-	31.86	9.48	37.8	-2	-2
h β-defensin 2	9.2	41	-2	-	-	-2	-	-	36.63	11.05	44.3	-2	-2
Bacitracin	8.8	11	-1	-	-	20.51	19.50	0.52	30.27	8.96	35.6	-2	-2
Polymixin B	n.a	9	13.77	12.77	0.32	33.43	32.43	0.95	20.77	5.83	22.5	-2	0.7
Aspartam	5.2	2	10.56	9.56	0.21	12.05	11.05	0.24	8.75	1.88	6	-2	0.70
m Obestatin	9.7	23	-2	-	-	22.20	21.20	0.58	27.72	8.12	32.1	-2	1.3

[1] Data were not obtained (not detected under the specified operating system1 or not injected2).

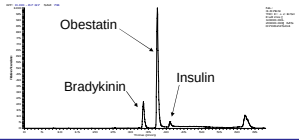
T0,A = 0.8 min (n=1)
T0,B = 0.27 ± 0.05 min (n= 18)

Combined 2D system

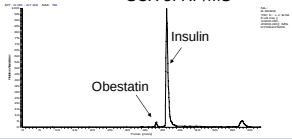
Five gradient fractions on SCX column B (0-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1 M $HCOONH_4$) retained on trap A and gradient-eluted on RP-18 column (5-60% ACN V/V + 0.1%HCOOH m/V).

Mixtures of model-peptides are injected and some typical 2-dimensional SCX-fractionated RP-chromatograms with MS detection are shown here.

SCX 4: RP/MS



SCX 5: RP/MS



Conclusions: current status & future investigations

- ✓ Uni-dimensional SCX and RP18: good results ==> others to evaluate and operational optimization (*e.g.* temperature)
- ✓ Evaluation optimal combination of dimensionality ==> criteria and final decision for maximal separation power
- ✓ Trap: loss of some peptides ==> needs further investigations and improvement
- ✓ 2-Dimensional system: operates well ==> robustness, qualitative and quantitative evaluation
- ✓ Sampling of udder tissues performed ==> sample treatment investigations