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FACULTEIT FARMACEUTISCHE WETENSCHAPPEN

Qbd analytical development of calcitonin bioadhesive formulation.

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INTRODUCTION

Calcitonin is a 32-amino acid polypeptide hormone ubiquitous in humans and animals. It acts *i.a.* to reduce blood calcium (Ca^{2+}), opposing the effects of parathyroid hormone (PTH). Both human (hu) and salmon (sa) calcitonins are clinically effective and currently approved as active pharmaceutical ingredients (APIs).

A new bioadhesive nasal formulation is currently under development, which contains low dose sa-calcitonin in polymeric excipients (carbomer and starch).

The analytical development is confronted with several challenges: the low dose of the peptide in the formulation, its inherent instability, the polymeric matrix interacting with the peptide influencing sample preparation and its undefined degradation impurity profile in this formulation.

The aim of this investigation was to develop a suitable method to determine the concentration of sa-calcitonin in this formulation and to establish its degradation profile, using experimental designs which will also give us mechanistic information.

EXPERIMENTAL

➢ ***Sample preparation development (spiked plcaebo) :***

1. Placket Burman design (PBD):
   HPβCD (0-10 mg/ml), temperature (50-70°C), incubation time (1-2 h),
   number of steps (1-2), mixing velocity (300-600 rpm), concentration FA (1-5 % V/V).
   => FA and temperature have a significant influence ($p < 0.05$), BUT: too low recoveries
2. Onion design (with change FA to TFA):
   Temperature (20-70°C), incubation time (30-90 min), concentration TFA (0.1-0.75 % V/V)
   => Concentration TFA significant ($p < 0.05$), others regional ($p < 0.10$)
3. Verification robustness with PBD:
   Temperature (50-60°C), incubation time (40-50 min), concentration TFA (0.45-0.65 % V/V)
   => Incubation temperature significant influence hence: range ± 2°C

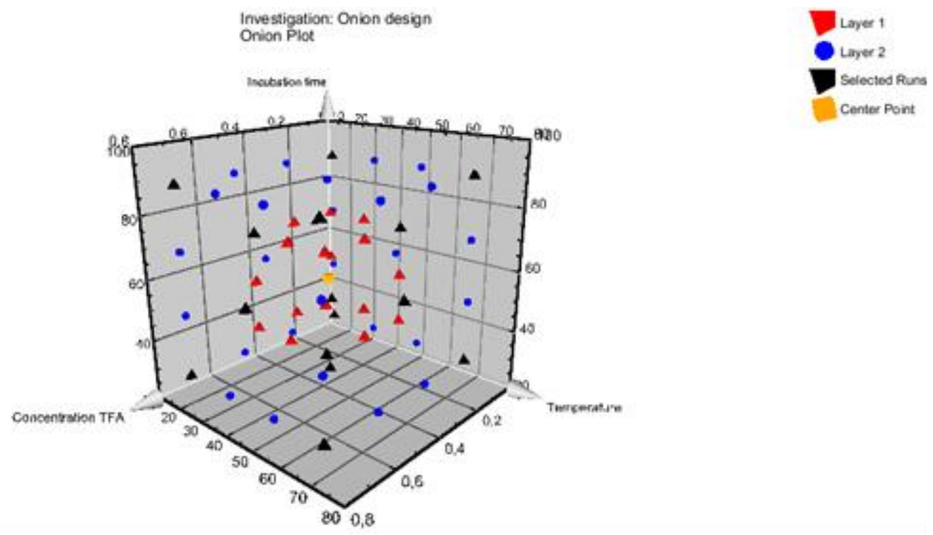
➢ ***HPLC-UV :***

Variables optimised with PBD: acid (TFA vs FA), column temperature, ACN gradient slope

➢ ***HPLC-MS analysis for determination of related substances :***

Evaluation using ESI-iontrap MS (SIM), with selectivity optimised gradient FA

Figure 1: 3D visualisation of the Onion design



RESULTS AND DISCUSSION

➢ The final HPLC conditions for the assay are listed in Table 1, while a typical chromatogram of the selecivity study for related compounds is given in Figure 2

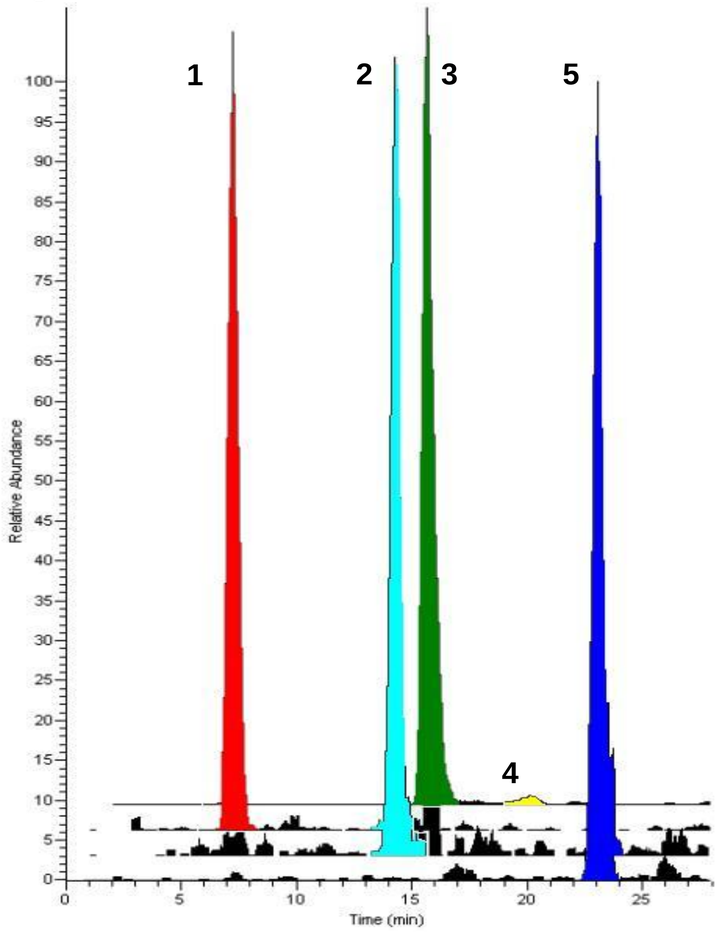
Table 1: HPLC method for sa-calcitonin assay

Parameter	Specification		
Column	Everest C_{18} (300 Å), 250 × 4.6 mm, 5 µm (+ guard column)		
Column temperature	40°C		
Mobile phase	- Solvent A: 0.1% V/V TFA in water. - Solvent B: 0.085% V/V TFA in acetonitrille.		
Gradiënt programme	Time (min)	Solvent constitution with TFA	
		%A	%B
	0	73	27
	20	63	37
	21	73	27
	40	73	27
Flow	1 ml/min		
Injection volume	100 µl		
Detection	UV at 195 nm		

Table 2: Peak identification corresponding to Figure 2

Peak identification	RRT	m/Z
1: cys-ser hydrolysis product	0.5	862.81-863.81
2: calcitonin trisulfide	0.9	866.30-876-30
3: sa-calcitonin	**1.00**	859.37-860-49
4: epimer	1.25	859.37-860-49
5: acetylated calcitonin	1.50	868.81-869.81

Figure 2: HPLC-MS for related substances



CONCLUSIONS

✓ Optimised sample preparation obtained with Plackett-Burman and Onion designs: 0.45% V/V TFA at 60°C during 40 minutes
⇒ accuracy (recovery) = 97.37%, precision = 3.34%

✓ HPLC-UV assay characterisation .

✓ A selective method for specified related substances profiling for nasal powder was established, using HPLC-ESI/MS (SIM).