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Pharmaceutical drugability of peptide drugs by analytical characterisation.

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SUMMARY

Peptide drugs gain increased interest as a new supra-group of therapeutics between the classic-organic, small-molecule drugs and the large biotechnology-derived bio-drugs. They are used in different therapeutic or diagnostic areas like allergy, anti-infection, oncology, obesity, etc… but also as functional excipients (*e.g.* CPPs). Due to their particular structure and biochemical origin, the pharmaceutical development of a peptide drug poses special challenges.

After the initial synthesis of the active pharmaceutical ingredient (API) or functional excipient, analytical characterisation is aimed at integrity and purity evaluation of the compound, which is also required for initial biomedical experiments. In this analytical characterisation, sample treatment issues like solubility, adsorption and degradation are important aspects to be looked after. The chemical and metabolic stability, critical parameters for peptides, is to be assessed to obtain kinetic and mechanistic information. Functionality is tested *in-vitro*, using cell- and organ-based protocols including ligand binding studies, as well as *in-vivo* encompassing ADME and target-organ confirmation like brain behaviour.

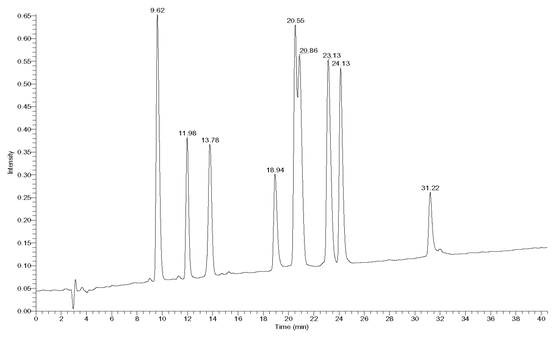
The pharmaceutical drugability information thus obtained allows further development decisions including required drug modifications, synthesis and purification modifications, quality specification settings as well as proof-of-principle drug delivery formulations.

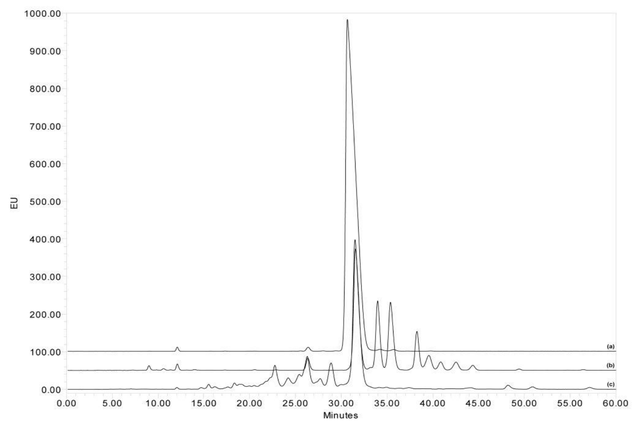
RESULTS

Data-mining **Bio-informatics** **Peptidomics** **Synthesis** **Morphology**

→ **Potential peptide drug**

✓ **HTS by LC-MS^n** [1]**:** **from** general pep-gradient :



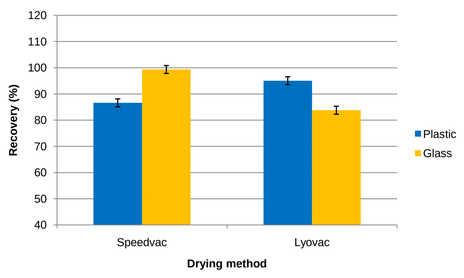


to peptide-specific optimised gradient :

identity: must: *e.g.* m/h-obestatin: 1 of 5 suppliers: was NOT ob [2]

LC-MS required (not LC-UV only)

purity: preferably ≥ 95%, but minimally ≥ 90%:



Sample	Supplier	Sum of impurities (%)	Largest impurity (%)	Number of impurities	
				Total	≥ 1.00%
Mouse obestatin	GLB	9.07	5.37	8	2
	CPR	0.38	0.12	5	0
	GPS	3.90	0.71	14	0
Human obestatin	GLB	7.26	2.65	6	3
	CPR	0.34	0.19	3	0
	GPS	6.91	1.66	12	3
	PB	0.67	0.17	7	0
	PP	6.82	1.99	7	4

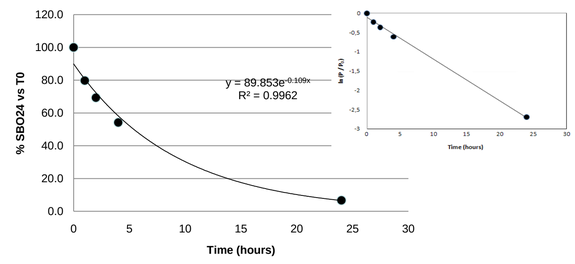
→ solubilization and adsorption (more for iodinated radio-peptides)

✓ **Stability:** **chemical** under various conditions [1],
incl. ligand binding conditions :

Stability of FPKPEGSQDKSLHN in radioligand binding study incubation buffer

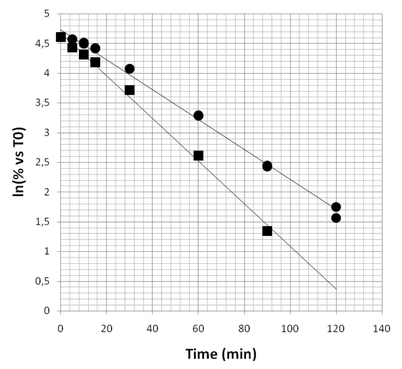
37°C
HEPES 20 mM pH 7.4 +
1 mg/ml BSA &
5 µl/ml PIC (in DMSO)

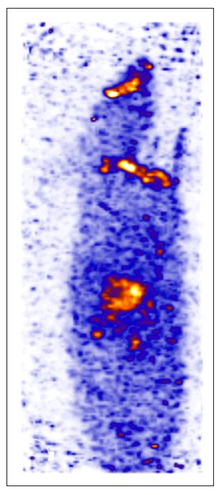
1st order kinetics
k_{obs} = 0.10 hours^{-1}
$t_{1/2}$ = ca 7 hours



metabolic in blood and different tissues [4], *e.g.* obestatin in brain:

Protease inhibitor (PI)	Half-life (min) [LC-UV mouse obestatin]	
	Crude brain homogenate	Brain membranes homogenate
No PI present	19	27
P8340 Protease Inhibitor Cocktail	52	74
EDTA	38	57
Soybean Trypsin Inhibitor	20	37
APMSF	20	15
1,10-Phenanthrolin	24	27
Captopril	20	27
Chymostatin	17	28





✓ **Functionality:** cellular response, receptor-binding and *in-vivo* distribution, *e.g.* mouse-SPECT:

Drugability information → **development decisions**

References: **[1]** V. Vergote, B. Baert and B. De Spiegeleer. HPLC analysis and stress stability of peptide drugs. Presented at Barcelona 2008: 6th World Meeting on Pharmaceutics, Biopharmaceutics & Pharmaceutical Technology. **[2]** B. De Spiegeleer, V. Vergote, A. Pezeshki et al. Impurity profiling quality control testing of synthetic peptides using LC-PDA-Fl and LC-ESI-MS: the obestatin case. Anal. Biochem. 376 (2008) 229-234. **[3]** B. De Spiegeleer, V. Vergote and C. Burvenich. Development of quality specifications of peptide drugs. Presented at Naples 2008: 11th Workshop on Bioactive Peptides. **[4]** V. Vergote, S. Van Dorpe, B. De Spiegeleer et al. In vitro metabolic stability of obestatin: kinetics and identification of cleavage products. Peptides 2008, in press.