UNIVERSITEIT GENT
FACULTY OF PHARMACEUTICAL SCIENCES

ADSORPTION OF CYCLIC DEPSIPEPTIDE MYCOTOXINS TO GLASS

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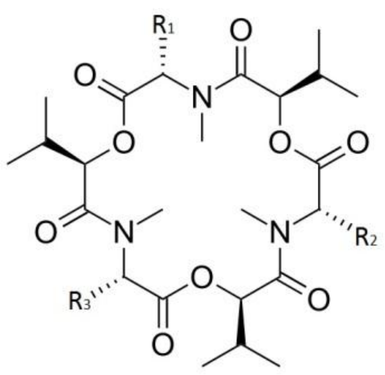
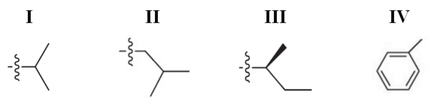
INTRODUCTION



Compound	R_1	R_2	R_3
Beauvericin	IV	IV	IV
Enniatin A	III	III	III
Enniatin A1	III	I	III
Enniatin B	I	I	I
Enniatin B1	I	III	I
Enniatin C	II	II	II
Enniatin D	I	I	II
Enniatin E_1	I	II	III
Enniatin E_2	I	III	II
Enniatin F	II	III	III

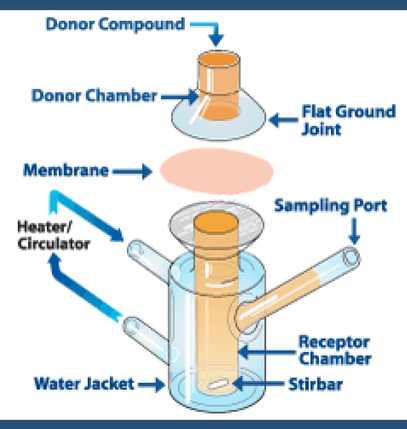


During analytical processes, adsorption of peptides, which is believed to be mostly due to non-covalent interactions and depending upon the experimental conditions, cannot only lead to significant loss of the analyte, but also to increased analytical variability. This undesirable aspect, however, has been given scant attention [1-2].
Some cyclic depsipeptides are considered as mycotoxins, *i.e.* beauvericin (BEA) and enniatins (ENNs). To quantitatively evaluate their transdermal behaviour, *ex-vivo in-vitro* Franz Diffusion Cell (FDC) experiments are performed. The adsorption of the cyclic depsipeptide analytes to the FDC glass wall, of which the quality differs from analytical volumetric glassware, was not yet investigated.

EXPERIMENTAL

UHPLC-MS² settings
Acquity UHPLC with Xevo TQ-S MS detector
Acquity UHPLC CSH C_{18} column (2.1 mm x 150 mm x 1.7 µm)
Injection volume: 10 µL
Column temperature: 45°C
Mobile phase: 70/30 ACN/H_2O (V:V) + 0.1% FA + 0.1% 2-propanol
Isocratic flow: 0.6 mL/min
ESI+ with capillary voltage 3.50 kV and cone voltage 50 V
Multiple reaction monitoring (MRM) modus

FDC adsorption conditions
Six solvent mixtures tested in duplicate:
EtOH/H_2O and ACN/H_2O: 10/90, 50/50, 95/5 (V/V)
Reference: 95/5 (V/V) = no adsorption assumed
Static (Logan model FDC-6)
With stirrer bar (600 rpm)
Receptor compartment volume: ± 5.0 mL
Equilibration 24h at 25 ± 0.5°C
Aliquot analysed in triplicate

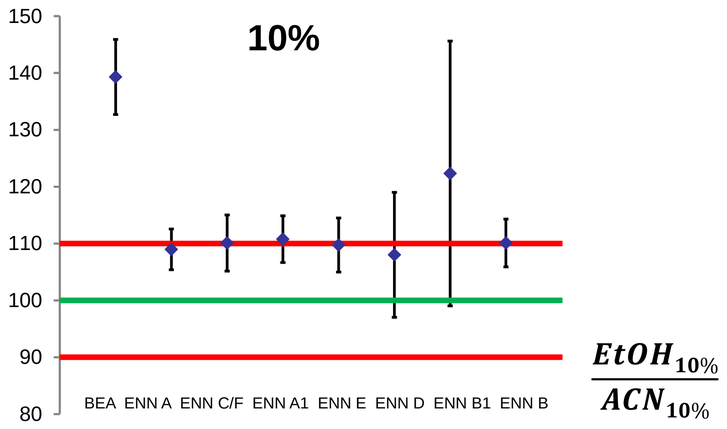


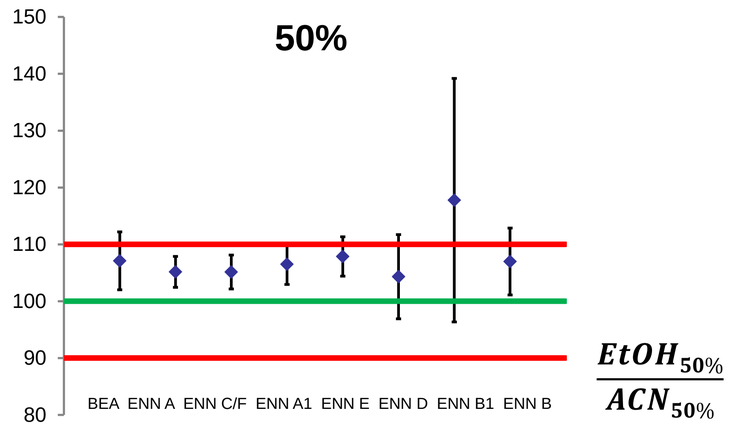
RESULTS and DISCUSSION

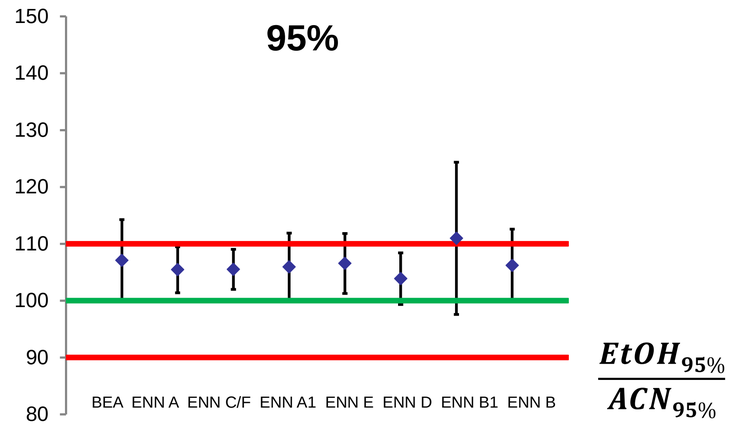
Statistics
Responses were analysed using ln-lin models to obtain mean response ratios (in %). These were fitted using generalised estimating equations with unstructured covariance to account for correlation within the duplicates [3]. QQ-plots confirmed the normality of the raw residuals in these models. Bonferroni corrected p-values and 95% confidence intervals were determined to account for multiplicity in the analysis of each compound separately.

Results
For each compound separately, mean response ratios (in %) were evaluated:

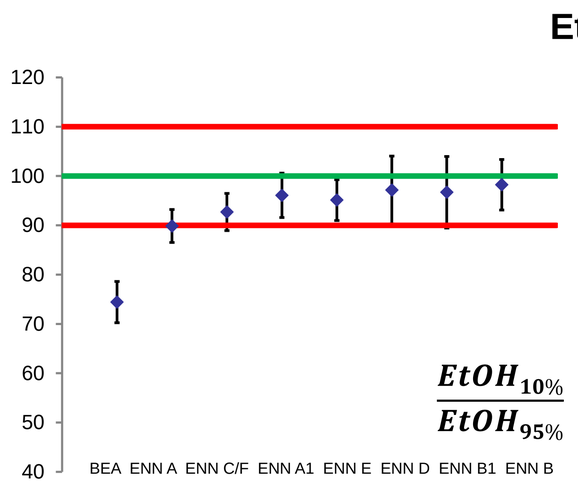
1) Per concentration level (10, 50 or 95% organic solvent) ACN and EtOH were compared

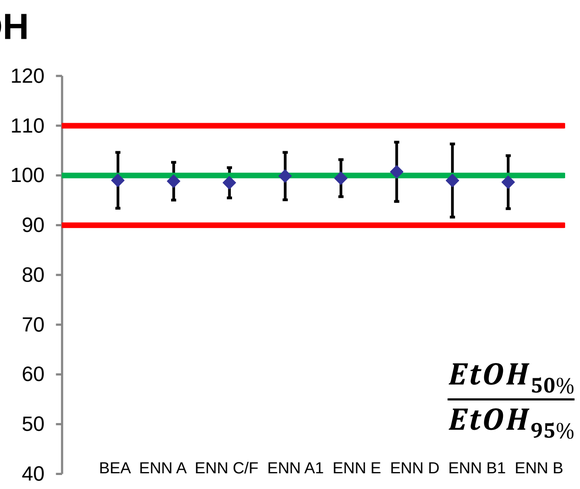


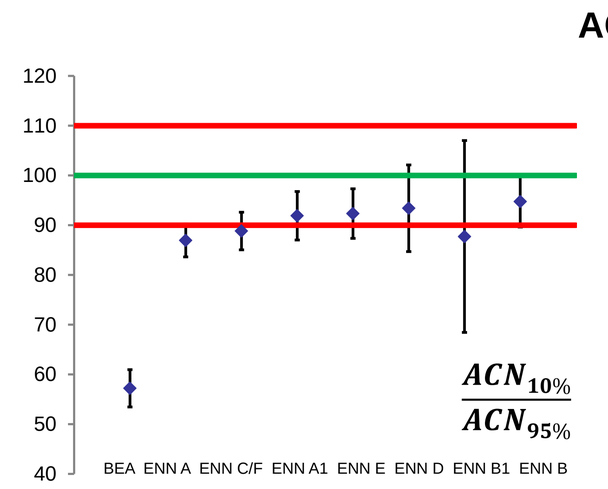


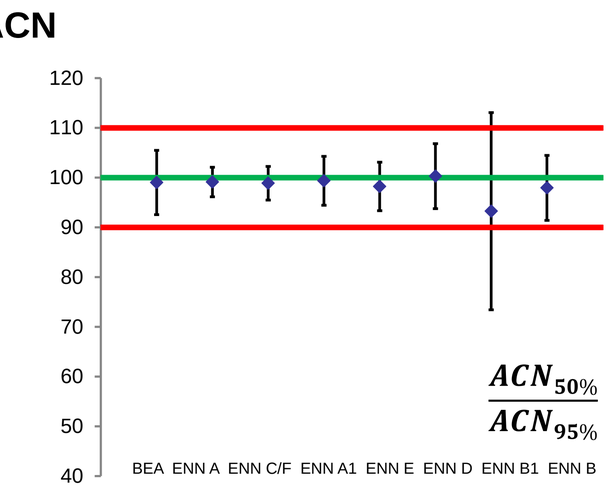


2) Per organic solvent (ACN or EtOH) the concentration levels (10 and 50%) were compared to the reference (95% = no adsorption assumed)









CONCLUSIONS

EtOH formulations: no significant adsorption effect at a concentration of ≥ 50% EtOH.
ACN formulations: also no significant adsorption effect at a concentration ≥ 50%, except for ENN B1: a possible adsorption effect cannot be excluded.
Lower levels of organic solvents: a significant adsorption effect cannot be excluded, *i.e.* up to approximately 45% adsorption at 10% ACN.
Comparing EtOH with ACN formulations: for BEA (the most lipophilic compound) a significant difference is observed: ± 40% less adsorption with EtOH.
Hydrophobicity of the compounds most likely plays a major role in the observed adsorption effects: higher log P values ~ more adsorption to glass.

REFERENCES

[1] Herath, H.M.D.R.; Kim, R.-R.; Cabot, P.J.; Shaw, P.N.; Hewavitharana, A.K. Inaccuracies in the quantification of peptides – A case study using β-endorphin assay. LCGC North America, 2013, 31(1): 58-61.
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[3] Liang, K-Y.; Zeger, S.L. Longitudinal data analysis using generalized linear models. Biometrics, 1986, 78(1): 13-22.