UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

The blood-brain barrier (BBB) permeability properties of plant *N*-alkylamides

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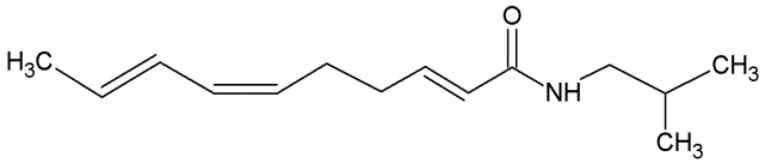
N-alkylamides

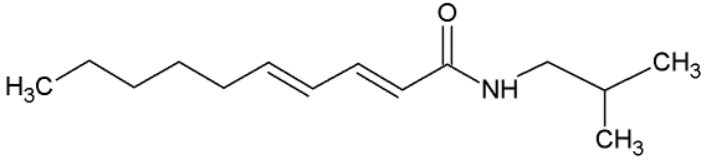
- Plant secondary metabolites
- Occurrence > 25 plant families
- Wide structural diversity

- Various functionalities *i.e.* antimicrobial, insecticidal, sensory, anti-inflammatory, immune-modulating, *central nervous system effects (CNS)*:
  * analgesic, anticonvulsant, antidepressant, anti-oxidant, anti-inflammatory activity
  * protection against neurodegeneration
  * cognitive enhancing effects

- 2 model *N*-alkylamides (NAAs): spilanthol (logP 3.39) and pellitorine (logP 3.65)



Spilanthol
deca-2*E*,6*Z*,8*E*-trienoic acid isobutylamide
(present in *Spilanthes acmella*)



Pellitorine
deca-2*E*,4*E*-dienoic acid isobutylamide
(present in *Anacyclus pyrethrum*)

N-alkylamides (spilanthol and pellitorine) enter the systemic blood circulation after different routes of administration:

- Oral:

**In vitro* Caco-2 cell monolayer experiment[1]
**In vivo* oral gavage experiment with rats[1]
→penetrate the intestinal barrier

-Topical:

**In vitro* transdermal Franz diffusion cell experiment using human skin and pig mucosa[2-4]
→penetrate the stratum corneum

1: Veryser *et al.* (2015). Gut and blood-brain barrier pharmacokinetics of the plant N-alkylamide spilanthol using *in vitro* and *in vivo* rodent models. *Manuscript in preparation.*
2: Boonen *et al.* (2010). LC-MS profiling of N-alkylamides in Spilanthes acmella extract and the transmucosal behaviour of its main bio-active spilanthol. *J. Pharm. Biomed. Anal.* 53(3), 243-249.
3: Boonen *et al.* (2010). Transdermal behaviour of the N-alkylamide spilanthol (affinin) from Spilanthes acmella (Compositae) extracts. *J. Ethnopharmacol*. 127, 77-84.
4: Veryser *et al.* (2014). Quantitative transdermal behavior of pellitorine from Anacyclus pyrethrum. *Phytomedicine.* 21(14), 1801-1807.

INTRODUCTION
OBJECTIVE
METHODS
RESULTS
CONCLUSIONS

NAAs in blood → brain ???

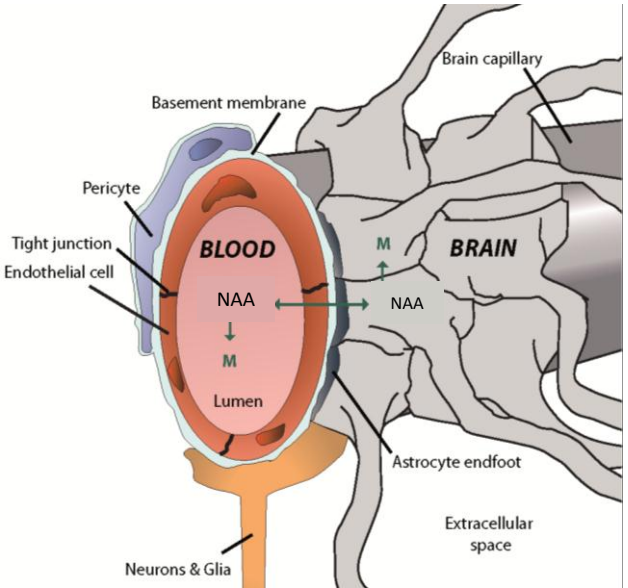
To investigate the permeability of spilanthol and pellitorine through blood-brain barrier (BBB)

1. Influx: from blood-to-brain

   a) Multiple time regression (MTR) experiment
   b) Capillary depletion experiment (brain distribution)

2. Efflux: from brain-to-blood

Investigation using the *gold-standard* ***in vivo*** **method**



In vivo blood-brain barrier (BBB) experiment with mice

1. **Influx** (blood-to-brain transport)
   - Dose= spilanthol: 2.77 mg/kg mouse, pellitorine: 1.23 mg/kg mouse
   - IV injection

   a) **Multiple time regression** (MTR) experiment
   - Collection of serum and isolation of brain at specified time points

   b) **Capillary depletion** (brain distribution) experiment
   - Collection of serum and isolation of brain after 10 min.

2. **Efflux** (brain-to-blood transport)
   - Dose= spilanthol: 0.14 mg/kg mouse, pellitorine: 0.06 mg/kg mouse
   - Intraventricular injection
   - Collection of serum and isolation of brain at specified time points

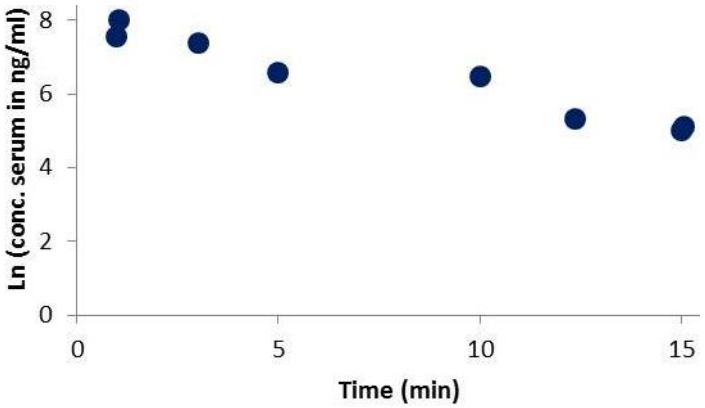
Samples analysed using a bio-analytical UPLC-MS method

1. Influx: blood-to-brain
a) MTR results

- **Serum profiles**

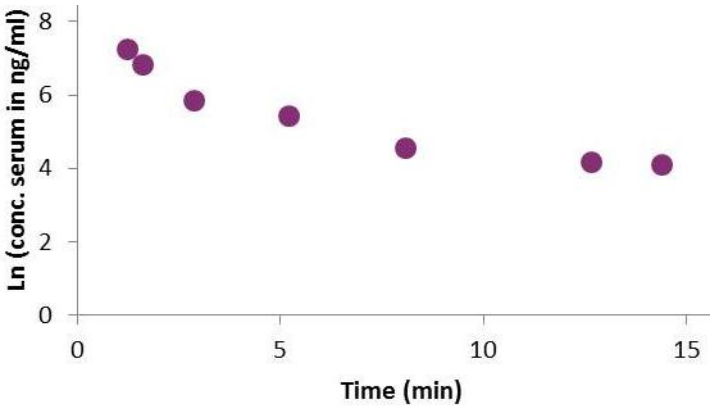
Spilanthol
one-compartmental model

$$C = C_0 \times e^{-k_e \times t}$$



Pellitorine
two-compartmental model

$$C_p = C_1 \times e^{-\alpha \times t} + C_2 \times e^{-\beta \times t}$$



Kinetic parameters of spilanthol

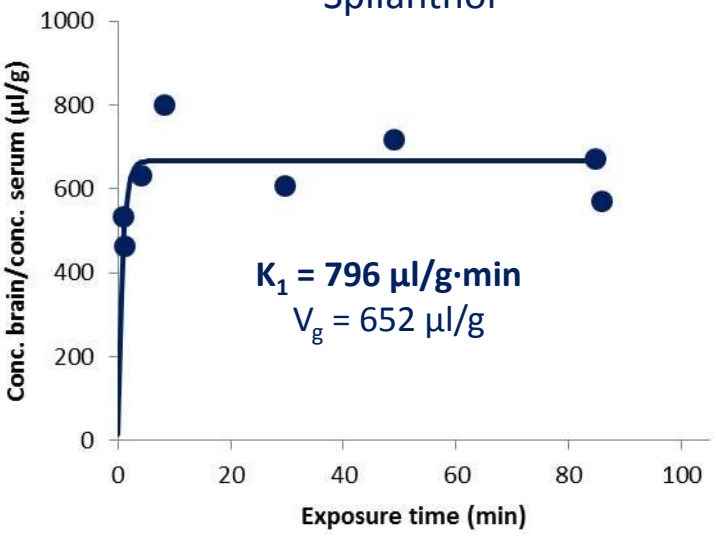
$t_{1/2,elimination}$ = 3.16 min
$k_{elimination}$ = 0.22 min^{-1}
C_0 = 3.05 µg/ml

Kinetic parameters of pellitorine

$t_{1/2,elimination}$ (β) = 4.48 min
α = 1.56 min^{-1}
β = 0.15 min^{-1}
C_1 = 7.02 µg/ml
C_2 = 0.44 µg/ml

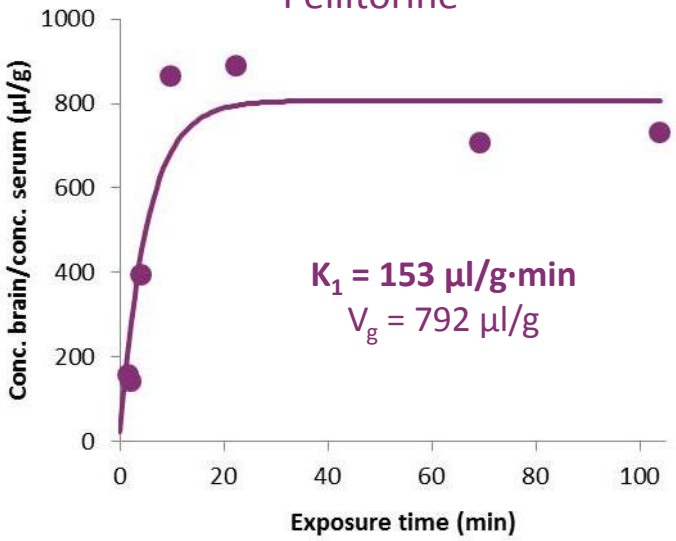
➢ Brain/serum concentration profiles: biphasic model

Spilanthol



(If V_0 and K>0: V_g = 344 µl/g; K_1 = 217 µl/g·min)

Pellitorine



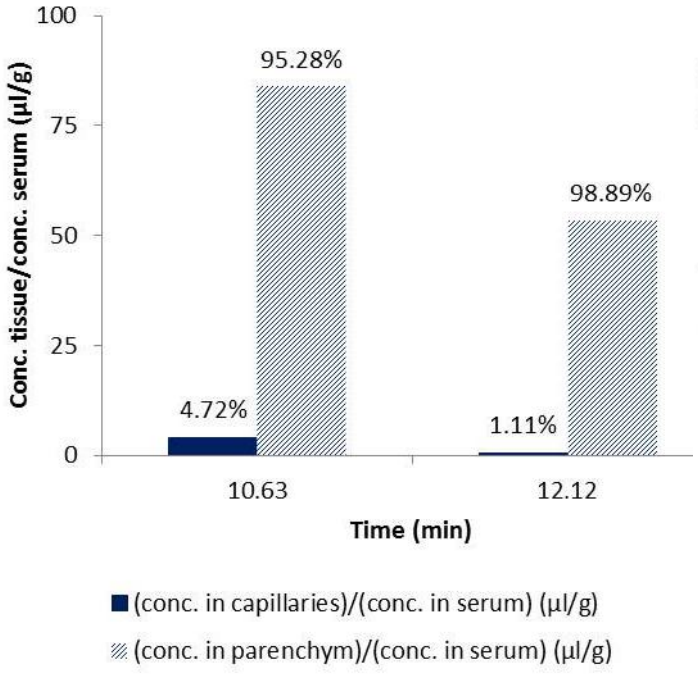
(If V_0 and K>0: V_g = 807 µl/g; K_1 = 159 µl/g·min)

$$\frac{A_m(t)}{C_p(t)} = K\Theta + V_g\left(1 - e^{\left(-\Theta\left(\frac{K_1 - K}{V_g}\right)\right)}\right) + V_0 \overset{K=0}{\simeq} V_g\left(1 - e^{\left(-\Theta\left(\frac{K_1}{V_g}\right)\right)}\right) + V_0$$

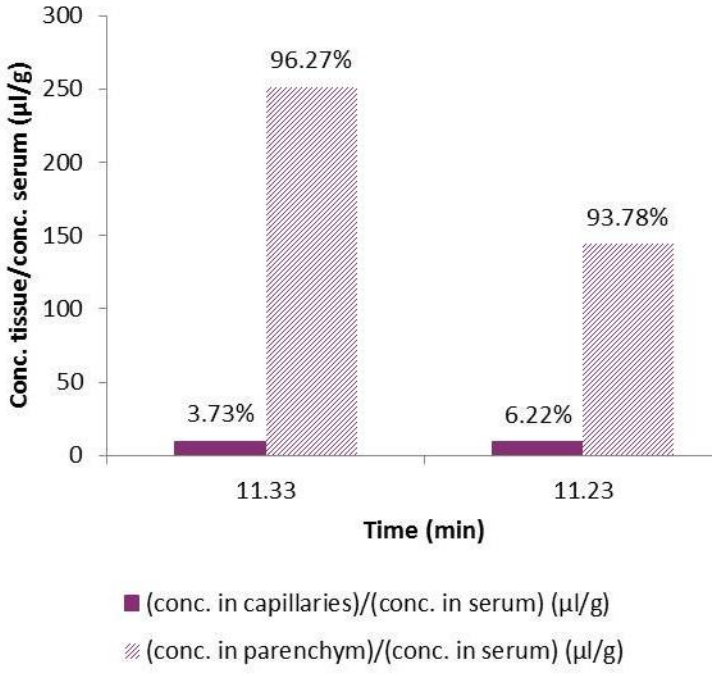
$A_m(t)$ = the concentration of NAA in the brain at time t (ng/g) } y-axis
$C_p(t)$ = the concentration of NAA in serum at time t (ng/µl) }
K_1 = unidirectional clearance (µl/(g·min))
K = the net clearance (µl/(g·min)): 10^{-16} ~ 0
V_g = tissue brain distribution volume (µl/g)
V_0 = vascular brain distribution volume (µl/g): 14.8 (BSA)
Θ = exposure time: x-axis

b) Capillary depletion: brain distribution

Spilanthol

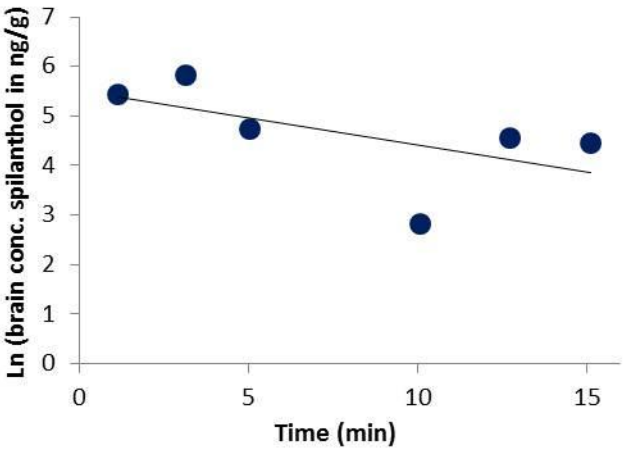


Pellitorine

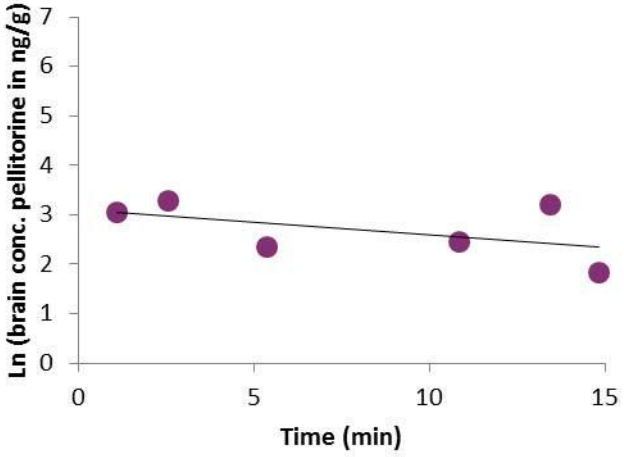


2. Efflux: brain-to-blood

Spilanthol



Pellitorine



Kinetic parameters of spilanthol

k_{out} = 0.11 min^{-1}

$t_{1/2,brain}$ = 6.38 min

Kinetic parameters of pellitorine

k_{out} = 0.05 min^{-1}

$t_{1/2,brain}$ = 13.78 min

Conclusions

- Spilanthol and pellitorine are able to pass the BBB

- Both NAAs show a rapid and high influx rate, with pellitorine somewhat higher BBB influx permeation compared to spilanthol

- Similar K_{in} values were obtained compared to CNS small molecules

- > 95% of NAAs was found in parenchyma of the brains, < 5% in the capillaries
→ possibility to exert CNS effects

- There is also efflux from the brain into the blood

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