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Stability applications in food, cosmeceuticals and pharma

M. D'Hondt , J. Boonen and B. De Spiegeleer
Drug Quality & Registration (DruQuaR) group

1. Outline
2. Small molecules
   - β-artemeter (2)
   - Triple IT
3. Peptides
   - Buserelin
   - Peptide mixture
4. Fused-core modelling
5. Extra column volume

[O/ref.: 2012-338b]

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1. Introduction

Outline

Buserelin

β-artemether

Casein hydrolysate

Use of fused-core and sub-2 µm particles HPLC

Triple IT

BBB peptides
(Fused-core retention model)

Spilanthol – plant extract in cosmeceutical
(Fused-core injection volume)

2. Small molecules Artemether (1/2)

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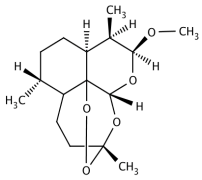
Analytical stability study:

→ no peak area mass balance in stability samples

Approach: RF determination degradants by dry heat stress of β-artemether under different environments



β-artemether

Protocol

Reductive	Temp (°C)	125	125	130	130	130	140	140	145	145
	Time (min)	30	60	30	60	90	15	45	15	30
Oxidative	Temp (°C)	125	125	130	130	130	140	140	145	145
	Time (min)	30	60	15	45	90	15	45	15	30
Neutral	Temp (°C)	130	130	140	140	140	145	145	150	150
	Time (min)	30	60	15	30	60	15	30	10	20

+ blanks, refs. → **Approx. 50 samples**

***Ref.**: De Spiegeleer, D'Hondt et al. Journal of Pharmaceutical and biomedical Analysis 70 (2012) 111-116*

2. Small molecules Artemether (1/2)

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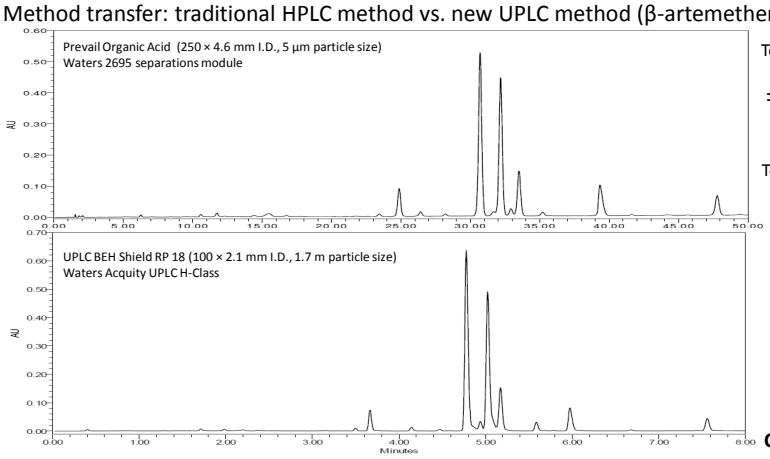
Need for fast, efficient separation chromatography

Method transfer: traditional HPLC method vs. new UPLC method (β-artemether 140°C, 15 min.)



Total run time: 85 min

±71 hrs / 50 samples

Total run time: 17.5 min

±14 hrs / 50 samples

Significant reduction

Analysis time
Solvent use/cost

HIGH THROUGHPUT

Concl.: RRF up to 42

***Ref.**: De Spiegeleer, D'Hondt et al. Journal of Pharmaceutical and biomedical Analysis 70 (2012) 111-116*

2. Small molecules Artemether (2/2)

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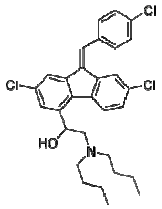
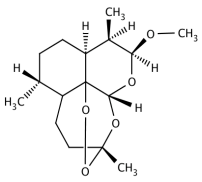


Lumefantrine



β-artemether

inherently **unstable**

Controlled distribution and storage ⟺ African setting

Need: Fast stability-indicating simultaneous assay of lumefantrine and β-artemether

Ref.: *Suleman, Vandercruyssen, Wynendaele, D'Hondt et al. Manuscript in preparation*

2. Small molecules Artemether (2/2)

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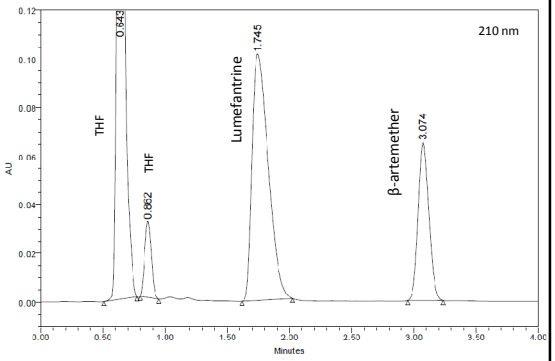
Method:

- **Column**: HALO RP-amide (50 × 4.6 mm I.D., 2.7 µm particle size)
- **Mobile phase**: Acetonitrile / 1 mM phosphate buffer pH 3.0 (52:48, V/V)
- **Flow rate**: 1.0 ml/min
- **Detection**: 210 nm (β-artemether) and 335 nm (lumefantrine)
- **Column temperature**: 30°C
- **Injection volume**: 3 µl
- **Total run time**: 4 min
- **Sample solvent**: THF



- ✓Simultaneous assay
- ✓Stability-indating
- ✓Isocratic HPLC
- ✓ Dual λ
- ✓ Fast (low cost)

Concl.: ideal, esp. for **resource limited countries**

Ref.: *Suleman, Vandercruyssen, Wynendaele, D'Hondt et al. Manuscript in preparation*

2. Small molecules Triple IT

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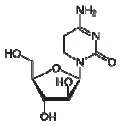
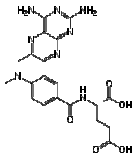
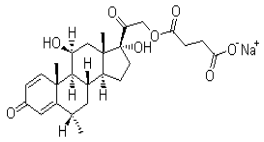
Triple IT solution:



Cytarabine



Methotrexate



Methylprednisolone (21) sodium succinate

Treatment of acute lymfoblastic leukemia ⟶ in-situ prepared before **each!** administration

In-use stability protocol:
- 3 different batches
- 3 different packaging materials
- 3 different storage conditions
- Time points (n=6): 0, 4, 8, 24, 32, 48 hrs.

#Samples ↑↑↑

need for **fast!** simultaneous stability-indicating assay method

Ref.: *D'Hondt et al. American Journal of Health-System Pharmacy 69 (2012) 232-240*

2. Small molecules Triple IT

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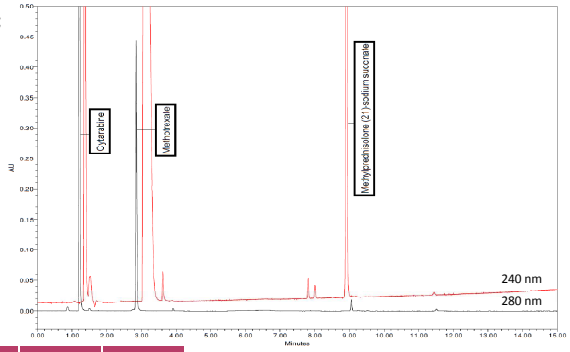
Method:
- **Column**: Halo C18 (150 × 4.6 mm I.D.; 2.7 µm particle size)
- **Mobile phase**:
  A: 0.1% V/V glacial acetic acid in H_2O
  B: 0.1% V/V glacial acetic acid in ACN
- **Gradient**:
  0 ➔ 15 min: 90 ➔ 10% A
- **Flow rate**: 1 ml/min
- **Column temperature**: 30°C
- **Injection volume**: 10 µl
- **Detection**: 240 nm (MPSS) and 280 nm (CB and MT)



Method verification

Parameter	CB	MTX	MPSS
Linearity (R^2; 80-100-120% l.c.)	1.0000	0.9992	1.0000
Repeatability (%RSD; 100% l.c.; n = 3)	0.464	1.352	0.155
LoQ (% l.c.)	0.03	0.07	0.05

Fit for in-use stability protocol

Concl.: stable for 12 hrs at 5°C

Ref.: *D'Hondt et al. American Journal of Health-System Pharmacy 69 (2012) 232-240*

3. Peptides Buserelin

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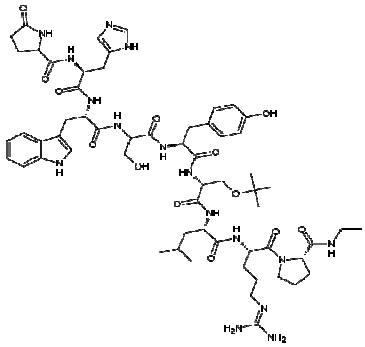

Buserelin

Stability study
- Kinetic degradation profile of buserelin
- Degradation profile for MS identification

Stability protocol
- 5 temperature settings
- 4 time points / temperature
- Duplicate
- + refs, blanks

Approx. 60 samples

Need for fast stability-indicating assay method

Ref.: *D'Hondt et al. Manuscript in preparation*

3. Peptides Buserelin

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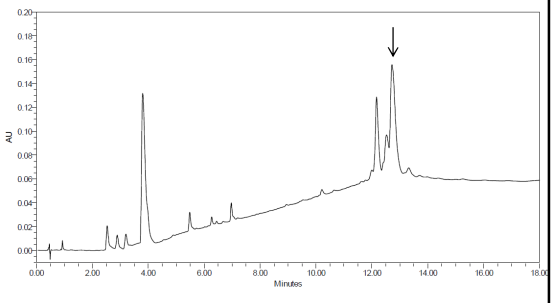
Method:

•**Column**: Acquity UPLC BEH 300 RP 18 (100 × 2.1 mm I.D., 1.7 µm particle size)
•**Mobile phase**:
MP A: 95% water and 5% acetonitrile with 0.1% formic acid (m/V)
MP B: 95% acetonitrile and 5% water with 0.1% formic acid (m/V)
•**Gradient**:
0 → 1.5 min: 100% A
1.5 → 11 min: 100% A → 79% A
11 → 18 min: 79% A
•**Flow rate**: 0.6 ml/min
•**Column temperature**: 30°C
•**Injection volume**: 2 µl
•**Detection**: 220 nm
•**Total run time:** 35 min



Buserelin 165°C for 60 min

Method verification

•Linearity (1% to 100%): R^2 = 0.9966
•Repeatability (6x 100%): %RSD = 0.99
•LoD: 0.04% relative to 100% solution

✓**Buserelin assay → kinetics and Ea**
✓**Separation and identification of degradants (MS)**

Ref.: *D'Hondt et al. Manuscript in preparation*

3. Peptides
Peptide mixture

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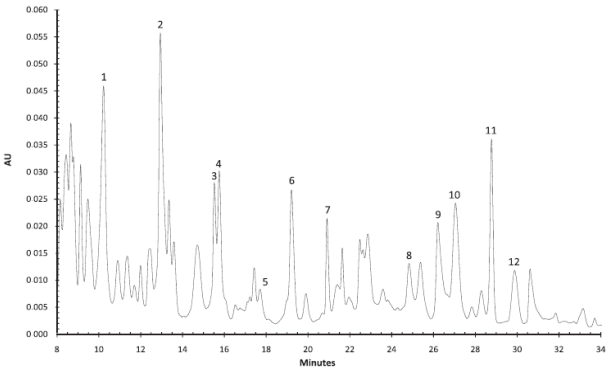

Pancreatic casein digest
(peaks indicated by number were identified by means of MS)

Method:

- **Column**: HALO Peptide ES-C18 (75× 3 mm I.D., 2.7 µm particle size)
- **Mobile phase**:
  MP A: 0.1% formic acid (m/V) in H2O
  MP B: 0.1% formic acid (m/V) in ACN
- **Gradient**:
  0 ➔ 40 min: 95% A ➔71% A
- **Flow rate**: 0.6 ml/min
- **Column temperature**: 30°C
- **Injection volume**: 10 µl
- **Detection**: 220 nm
- **Total run time:** 60 min

Concl.: stable up to 3 min at 180°C

Ref.: D'Hondt et al. Food Chemistry 128 (2011) 114-122

4. BBB peptides
Fused-core modelling

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Others:
HILIC, PEC,
PFP,…

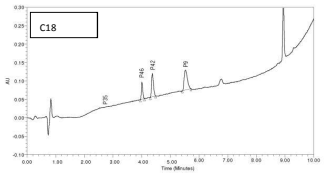
Peptide

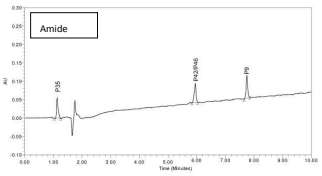
C18

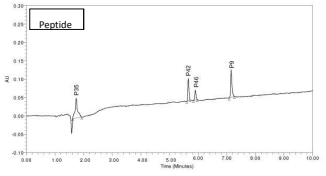
Phenylhexyl

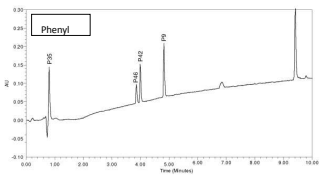
Amide

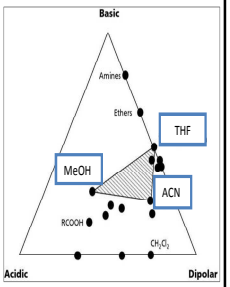
ACN FA
MeOH HAc











Ref.: D'Hondt et al. Journal of Pharmaceutical Analysis, accepted for publication.

4. BBB peptides Fused-core modelling

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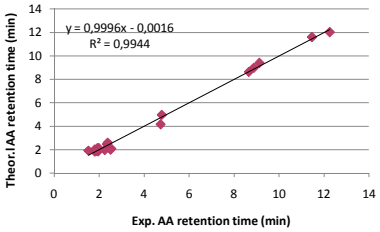
Classic $AA_{descriptor}$ = AA RT

In-silico $AA_{descriptor}$ = – 0.692 + 4.131 AlogP + 1.330 Alog P2 - 17.517 Mor10v + 6.613 Mor10e + 9.302 E1u + 0.240 RDF035e



Name	AlogP	AlogP2	Mor10v	Mor10e	E1u	RDF035e	AA descriptor
Alanine	-0,601	0,361	0,044	0,03	0,473	5,862	2,539767
Arginine	-1,107	1,225	0,09	-0,06	0,572	10,992	2,349747
Asparagine	-1,847	3,413	-0,051	-0,327	0,495	8,225	1,526739
Aspartic acid	-1,245	1,55	-0,093	-0,251	0,545	4,121	2,254253
Cysteine	-0,517	0,268	-0,031	-0,201	0,414	5,521	1,918595
Glutamic acid	-0,924	0,854	-0,026	-0,244	0,538	6,312	1,988002
Glutamine	-1,526	2,329	0,03	-0,035	0,565	7,961	2,510969
Glycine	-0,978	0,957	0,009	0,111	0,518	0,096	1,958558
Histidine	-1,015	1,031	0,029	-0,135	0,549	7,499	1,992075
Isoleucine	0,699	0,488	0,159	0,221	0,466	12,577	8,874091
Leucine	0,631	0,398	-0,05	-0,329	0,567	11,282	9,126088
Lysine	-0,68	0,462	0,168	0,143	0,556	6,342	1,810175
Methionine	-0,273	0,075	-0,012	0,034	0,519	5,179	4,785731
Phenylalanine	0,955	0,913	-0,062	-0,229	0,546	9,758	11,45988
Proline	-0,057	0,003	0,439	0,887	0,51	1,613	2,383431
Serine	-1,489	2,217	0,08	0,359	0,417	3,574	1,814952
Threonine	-1,111	1,235	0,113	0,042	0,598	6,868	1,87025
Tryptophan	1,249	1,56	0,226	0,17	0,56	13,881	12,24835
Tyrosine	0,688	0,473	-0,05	-0,342	0,503	10,771	8,657368
Valine	0,242	0,059	0,096	-0,069	0,477	8,55	4,737297
Aba-Gly	-0,414	0,172	0,126	-0,012	0,438	17,382	3,785984
Dmt	-1,107	1,225	-0,022	-0,605	0,585	7,655	0,027612
D-Arginine	1,66	2,757	-0,036	-0,431	0,433	18,435	16,06485
D-Penicillamine	0,065	0,004	0,009	0,22	0,528	14,375	9,240498
D-Phenylalanine	0,955	0,913	-0,002	-0,463	0,541	11,978	9,347712
D-Tryptophan	1,249	1,56	0,126	-0,234	0,555	18,93	12,49365
Ornithine	-1,136	1,29	0,098	-0,019	0,542	6,517	1,094335
Penicillamine	0,065	0,004	-0,033	-0,071	0,498	12,063	7,217889
PyroGlu	-0,673	0,452	0,401	0,777	0,526	3,756	1,037273

Ref.: *D'Hondt et al. Journal of Pharmaceutical Analysis, accepted for publication.*

4. BBB peptides Fused-core modelling

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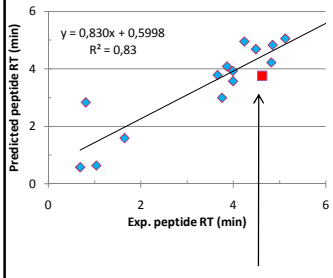
$RT_{peptide} = b_0 + b_1 \log \sum(AA_{descriptor}) + b_2$ cLogP + $b_3 \log$ Sv + $b_4 \log$ nHDon + $b_5 \log$ nHAcc

HALO Peptide (FA/ ACN)



Column	MP	R²	F	Constant	Log Sum AA	cLogP	Log Sv	Log nHDon	Log nHAcc
Peptide	FA/ACN	0,83	9,772	-4,101	7,68	0,085	-2,276	-8,815	7,849
	FA/MeOH	0,93	26,51	-6,252	12,169	0,124	-2,514	-10,761	8,951
	HOAc/ACN	0,829	9,722	-4,096	9,275	0,1	-2,893	-9,787	8,522
	HOAc/MeOH	0,937	29,912	-7,619	11,886	0,145	-1,761	-11,451	9,772
Amide	FA/ACN	0,839	10,391	-8,561	13,271	0,163	-4,062	-17,024	16,039
	FA/MeOH	0,858	12,105	-12,25	29,046	0,267	-10,904	-28,922	26,048
	HOAc/ACN	0,865	12,856	-6,303	17,462	0,163	-7,686	-19,942	18,493
	HOAc/MeOH	0,866	12,95	-16,915	30,853	0,311	-11,525	-38,596	36,06
Phenyl	FA/ACN	0,799	7,965	-1,623	6,852	0,053	-2,688	-5,147	4,071
	FA/MeOH	0,916	21,806	-4,817	13,688	0,112	-4,359	-10,683	8,703
	HOAc/ACN	0,808	8,395	-3,524	9,471	0,099	-3,298	-9,784	8,419
	HOAc/MeOH	0,924	24,234	-6,146	13,229	0,126	-3,083	-11,401	9,122
C18	FA/ACN	0,861	12,376	-8,808	12,671	0,146	-3,544	-17,102	16,136
	FA/MeOH	0,908	19,728	-13,617	27,362	0,242	-8,625	-30,391	27,159
	HOAc/ACN	0,858	12,072	-7,577	17,145	0,151	-7,013	-21,259	19,811
	HOAc/MeOH	0,915	21,517	-16,118	29,241	0,267	-9,966	-35,557	32,719

Buserelin

Includes unnatural AAs

Ref.: *D'Hondt et al. Journal of Pharmaceutical Analysis, accepted for publication.*

5. Spilanthol
(plant extract – cosmeceuticals)

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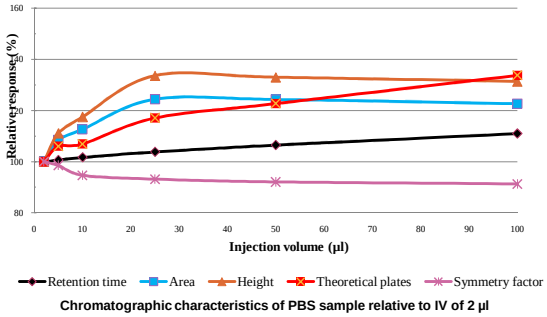
Transdermal: several 100 FDC samples (low concentration ➔ injection volume ↗)

Influence of injection volume on chromatographic responses (extra-column aspect):

Method:
- **Column**: HALO RP Amide (50 × 4.6 mm I.D., 2.7 µm particle size)
- **Mobile phase**: 1% formic acid (m/V) in MeOH/H2O (70/30)
- **Flow rate**: 1.5 ml/min
- **Column temperature**: 30°C
- **Detection**: 237 nm
- **Total run time:** 2 min
- **Injection volume**: 2 to 100 µl
- **Mass on column**: constant

- **Model compound**: spilanthol
- **Sample solvent**: PBS



Chromatographic characteristics of PBS sample relative to IV of 2 µl

injection volume↗

Retention time ↗
Height ↗
Theoretical plates ↗
Symmetry factor ↘

PBS weak solvent

Ref.: *Boonen et al. Journal of Pharmaceutical Analysis, manuscript submitted*

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Thank you for your attention!