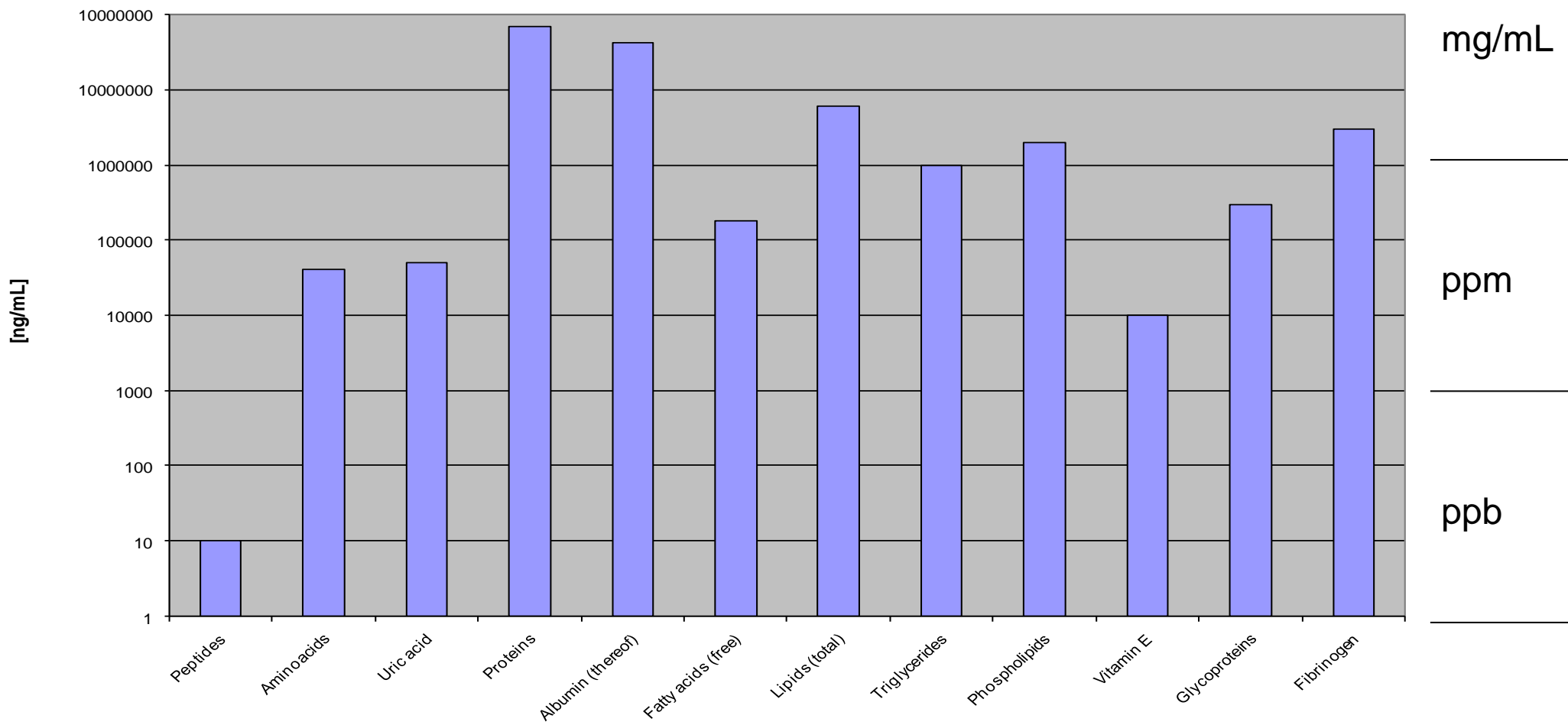


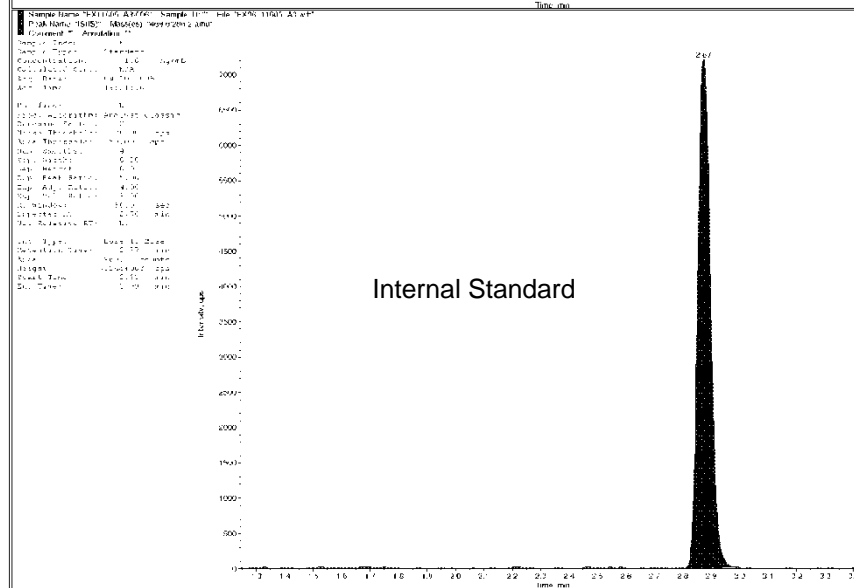
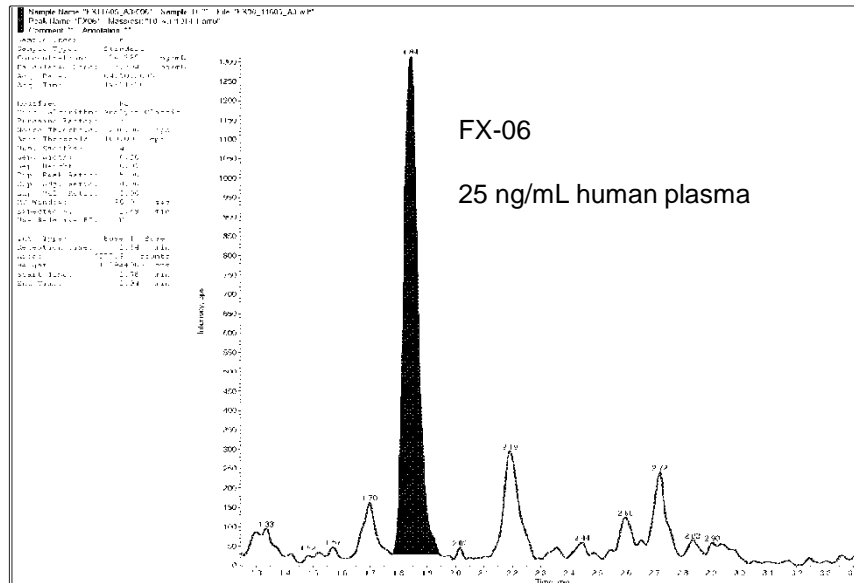
Quantification of Selected Peptides in Biological Fluids e.g. Plasma

EBF-Meeting, Brussels, 21st June 2011

Hermann Mascher

Concentration of Various Compounds in Plasma = ENEMIES





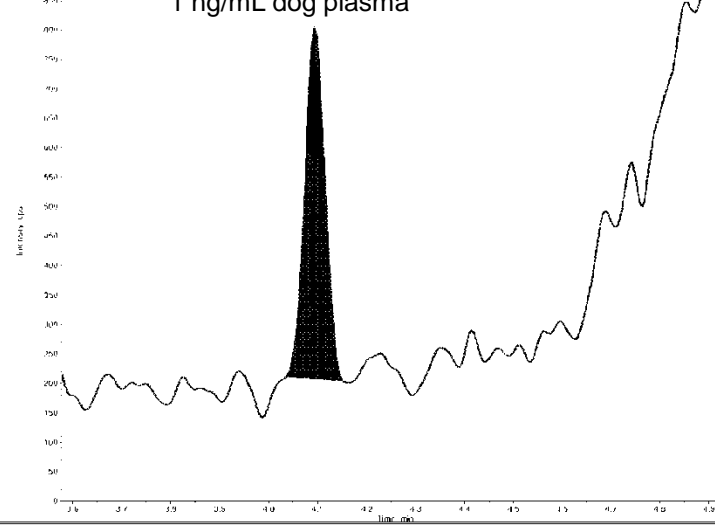
Published in Critical Care Medicine 2007

- **Chromatographic Conditions:** YMC Pack Pro C8 3 μm , 100 x3, 20 mM TFA in H_2O and acetonitrile, gradient: 0.0 – 1.1 min: linear: 15 % B \rightarrow 28 % B, 1.1 – 3.4 min linear: 28 % B \rightarrow 57 % B; 3.4 – 3.9 min isocratic: 90 % B, 3.9 – 4.9 min isocratic: 15 % B; flow: 1 mL/min; Inj. Vol.: 5 μL ; Inj. (abs): 125 pg; column temp: 40°C
- **MS/MS Parameters:** API4000, ESI, pos., MRM, Temp: 590°C, Ionisation voltage: 5.2 kV, Transitions: FX06: 1014.1 \rightarrow 1014.1 m/z, IS (Ipep 87) : 489.8 \rightarrow 286.2 m/z

Peak Name	Retention Time (min)	Area	Height
1	2.03	1000	100
2	2.11	1000	100
3	2.21	1000	100
4	2.31	1000	100
5	2.41	1000	100
6	2.51	1000	100
7	2.61	1000	100
8	2.71	1000	100
9	2.81	1000	100
10	2.91	1000	100
11	3.01	1000	100
12	3.11	1000	100
13	3.21	1000	100
14	3.31	1000	100
15	3.41	1000	100
16	3.51	1000	100
17	3.61	1000	100
18	3.71	1000	100
19	3.81	1000	100
20	3.91	1000	100
21	4.01	1000	100
22	4.11	1000	100
23	4.21	1000	100
24	4.31	1000	100
25	4.41	1000	100
26	4.51	1000	100
27	4.61	1000	100
28	4.71	1000	100
29	4.81	1000	100
30	4.91	1000	100
31	5.01	1000	100

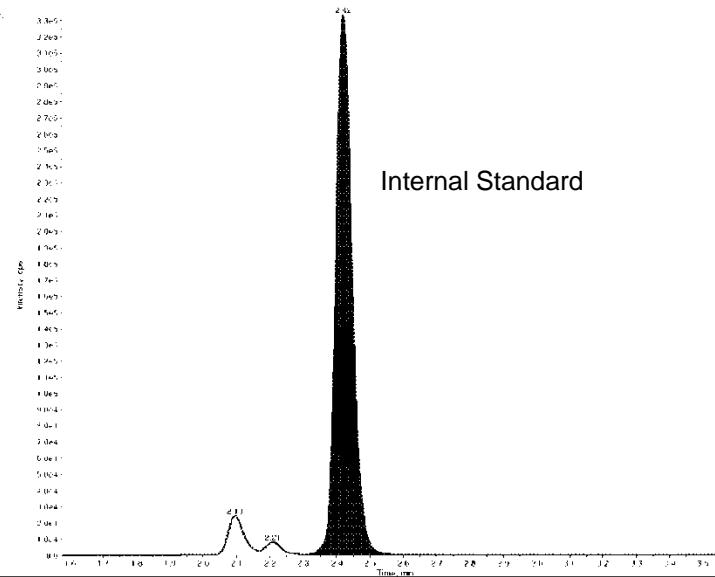
Duramycin

1 ng/mL dog plasma



Peak Name	Retention Time (min)	Area	Height
1	2.03	1000	100
2	2.11	1000	100
3	2.21	1000	100
4	2.31	1000	100
5	2.41	1000	100
6	2.51	1000	100
7	2.61	1000	100
8	2.71	1000	100
9	2.81	1000	100
10	2.91	1000	100
11	3.01	1000	100
12	3.11	1000	100
13	3.21	1000	100
14	3.31	1000	100
15	3.41	1000	100
16	3.51	1000	100
17	3.61	1000	100
18	3.71	1000	100
19	3.81	1000	100
20	3.91	1000	100
21	4.01	1000	100
22	4.11	1000	100
23	4.21	1000	100
24	4.31	1000	100
25	4.41	1000	100
26	4.51	1000	100
27	4.61	1000	100
28	4.71	1000	100
29	4.81	1000	100
30	4.91	1000	100
31	5.01	1000	100

Internal Standard

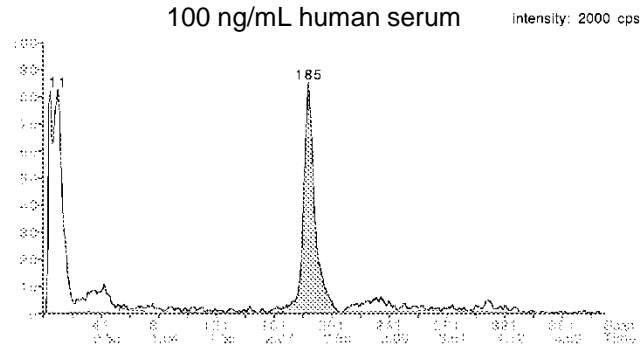


Published in Naumyn Schmiiedebergs Arch. Pharmacol. 2008

- **Chromatographic Conditions:** YMC Pack Pro C8 3 μm , 100 x3, 1 % formic acid in H_2O and acetonitrile, gradient: 0.0 – 0.5 min isocratic: 20 % B, 0.5 – 1.5 min linear: 20 % B \rightarrow 25 % B, 1.5 – 4.4 min linear: 25 % B \rightarrow 37 % B; 4.4 – 5.0 min isocratic: 50 % B, 5.0 – 6.0 min isocratic: 20 % B; flow: 0.8 mL/min; Inj. Vol.: 50 μL ; Inj. (abs): 27.8 pg; column temp: 60°C
- **MS/MS Parameters:** API4000/QTRAP, ESI, pos., MRM, Temp: 600°C, Ionisation voltage: 5 kV, Transitions: Moli1901: 1007.4 \rightarrow 1007.4 m/z, IS (Bacitracin) : 712.0 \rightarrow 119.0 m/z

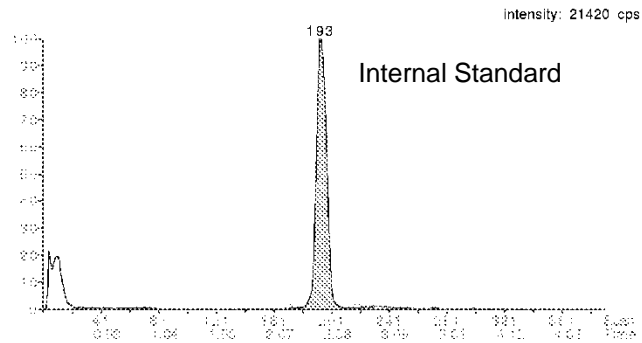
SPC_1B021 SPC_1B021 Die, 27. Mär 2001 11:52 Uhr
No Comment

4.98 in 1 period
r-SPC
Internal Standard: DA-SPC
Use Area
Absolute Retention Time
1: 4.98 MRM, 389 scans
1207.2->71.9
Noise Thres. 2.0
Quant Thres. 1.0
Min. Width 15
Mult. Width 4
Base. Width 50
RT Win. (secs) 20
Smooth 1
Expected RT 2.44
Area 11491
Height 1683
Start Time 2.13
End Time 2.62
Integration Width 0.49
Retention Time 2.37
Integration Type A - VB



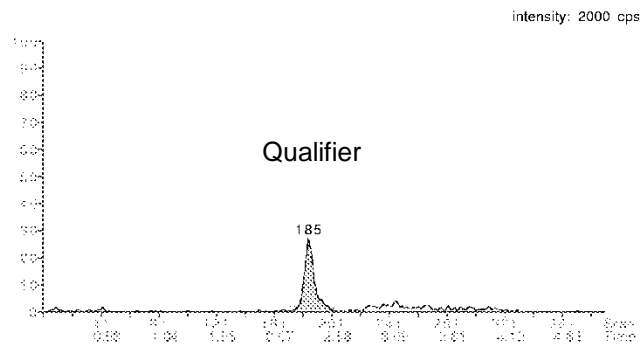
SPC_1B021 SPC_1B021 Die, 27. Mär 2001 11:52 Uhr
No Comment

4.98 in 1 period
DA-SPC
use as Internal Standard
1: 4.98 MRM, 389 scans
1235.5->71.9
Noise Thres. 2.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 20
Base. Width 50
RT Win. (secs) 20
Smooth 1
Expected RT 2.55
Area 135703
Height 21370
Start Time 2.21
End Time 2.81
Integration Width 0.60
Retention Time 2.48
Integration Type A - BV

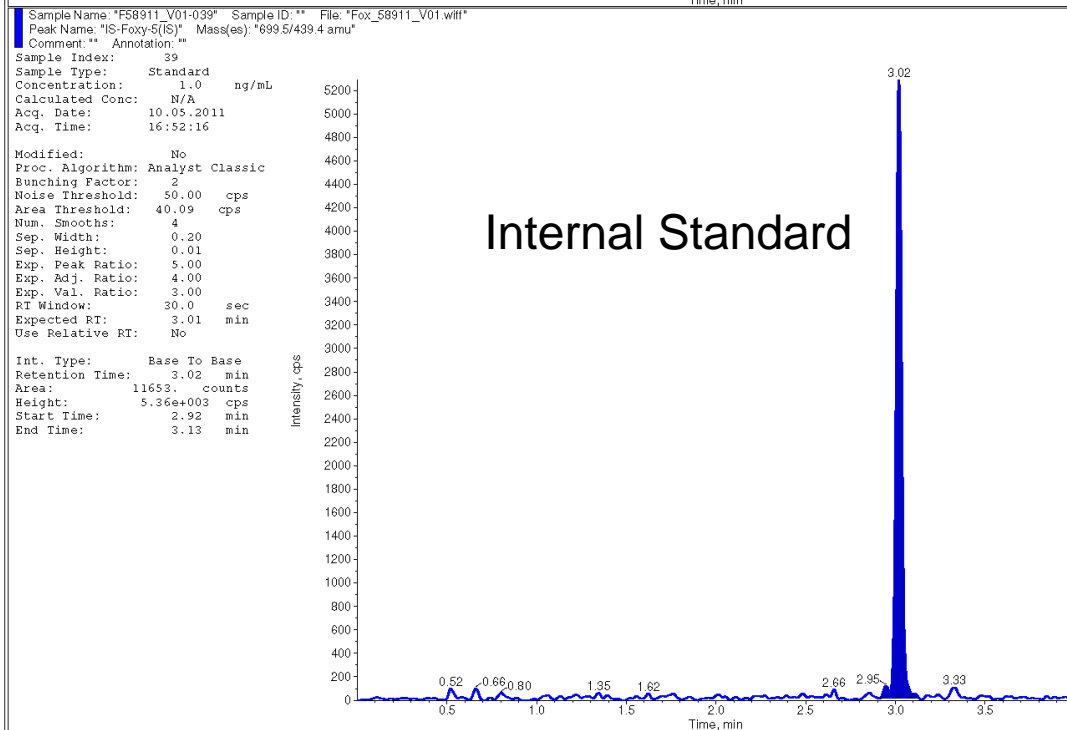
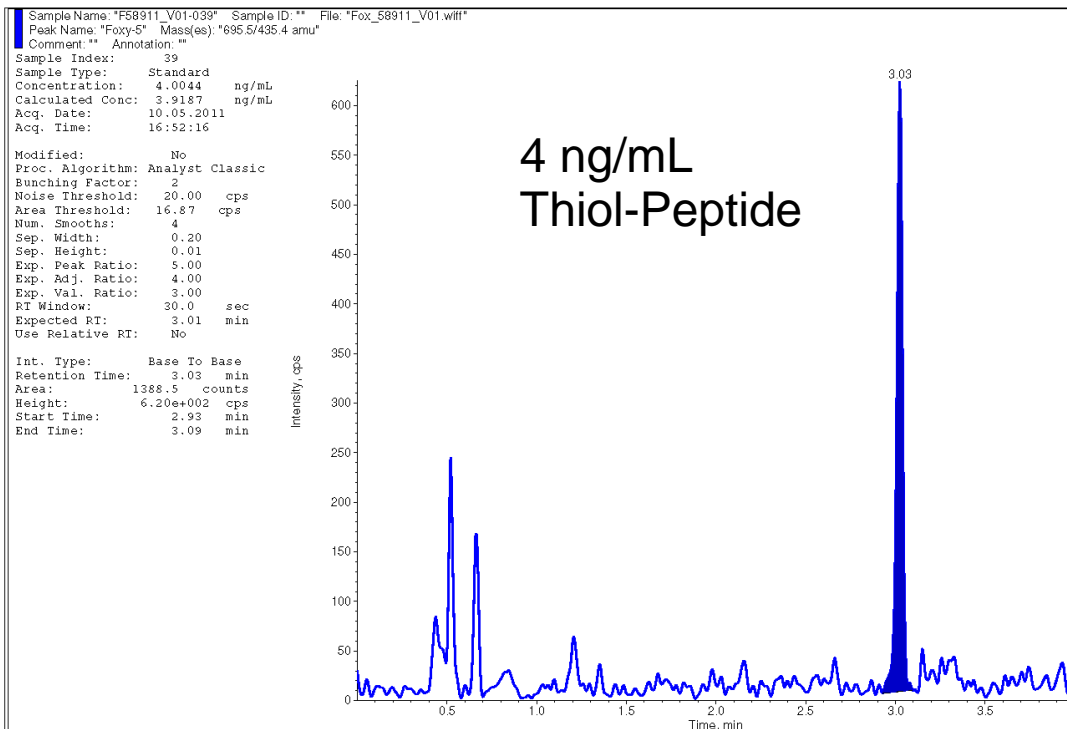


SPC_1B021 SPC_1B021 Die, 27. Mär 2001 11:52 Uhr
No Comment

4.98 in 1 period
217
Internal Standard: DA-SPC
Use Area
Absolute Retention Time
1: 4.98 MRM, 369 scans
1207.2->217.3
Noise Thres. 2.0
Quant Thres. 1.0
Min. Width 5
Mult. Width 26
Base. Width 100
RT Win. (secs) 20
Smooth 1
Expected RT 2.41
Area 3245
Height 538
Start Time 2.23
End Time 2.59
Integration Width 0.36
Retention Time 2.37
Integration Type A - BB



- **Chromatographic Conditions:** Phenomenex C18 Security Guard, ODS, 4x3 mm, Solvent A: 10 % Isopropanol / 50 % Acetonitrile / 40 % 0.02 M Trichloroacetic acid (v:v:v)-Solvent B: 25 % Isopropanol / 70 % Methanol / 5 % 0.01 M Hydrochloric acid (v:v:v)-External (post column): 0.02 M Ammonium carbamate, gradient: 0.0 – 1.0 min: isocratic: solvent A, 1.0 – 3.0 min: isocratic: solvent B, 3.0 – 5.0 min: isocratic: solvent A; flow: 0.5 ml / min + 0.1 ml / min (post column); Inj. Vol.: 10 µL; Inj. wash: 2x200 µl post injection of 96 % Isopropanol / 4 % 0.02 M Trichloroacetic acid; Inj. (abs): about 7 ng; column temp: 40°C
- **MS/MS Parameters:** API3000, ESI, pos., Transitions: Peptid: 1207.2 → 71.9 m/z, IS: 1235.5 → 71.9 m/z

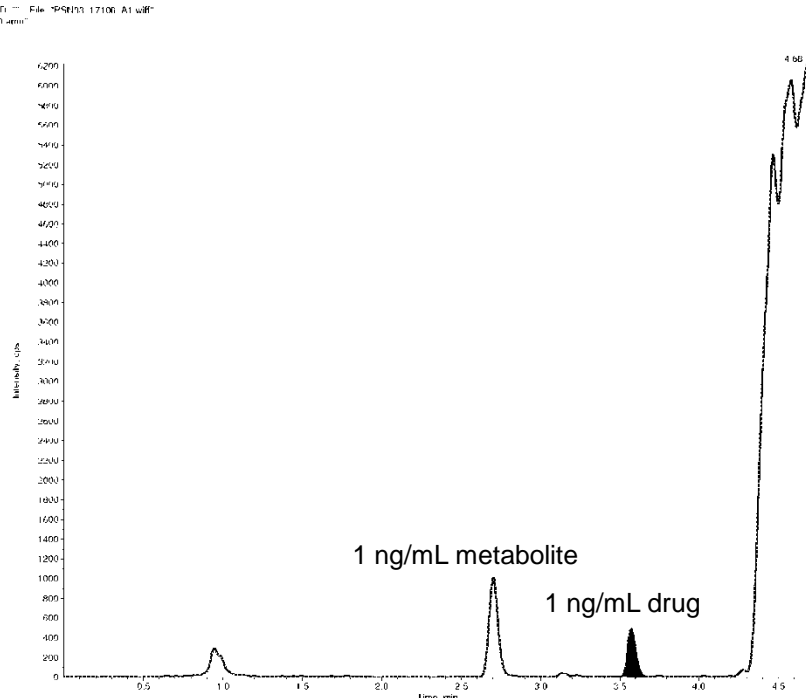


Mobile phase solvent A	1 % FA in Water
Mobile phase solvent B	1 % FA in ACN
Chromatographic run	0.0 – 3.5 min linear gradient: 10 % B → 36 % B 3.5 – 4.1 min isocratic: 90 % B 4.1 – 4.8 min isocratic: 10 % B
Flow	1 mL/min
Injection volume	15 µL
Column	YMC Pack Pro C8, 100 x 3 mm, 3 µm (YMC, Japan)
Column temperature	60°C
Retention time	approx. 3.0 min: Thiol-Peptide and its IS
MS Ionisation mode	ESI
MS polarity	Positive
MS detection mode	MRM
Transitions	696 → 435 m/z: Thiol-Peptide 700 → 439 m/z: IS

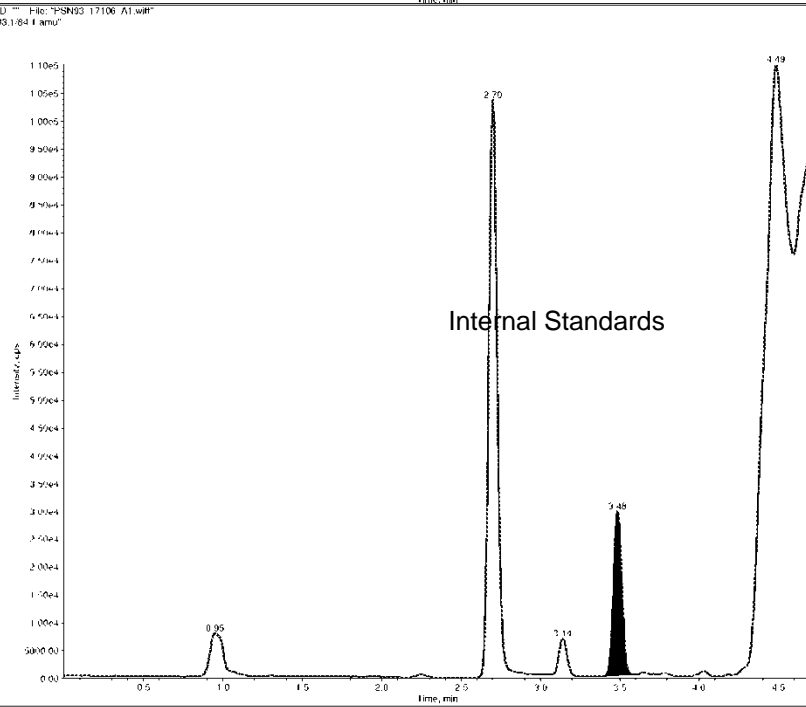
Sample Name: P17106_A1 020

Workstation: RVAL-RE148

Sample Name: P17106_A1 020 Sample ID: File: P8103 17106_A1.wf
 Peak Name: "Peptide" Mass(es): "181.030 1 amu"
 Comment: Annotation:
 Sample Index: 24
 Sample Type: Standard
 Concentration: 1.0 ng/mL
 Acquisition Date: 26.06.2009
 Acquisition Time: 18:35:38
 Acquisition Time: 18:35:38
 Modified: 01
 Method: Analyst Access
 Sampling Rate: 1000 cps
 Noise Threshold: 100 cps
 Area Threshold: 100 cps
 Min. Smooth: 0.20
 Sep. Width: 0.10
 Sep. Height: 0.10
 Exp. Peak Ratio: 0.10
 Exp. Adj. Ratio: 0.10
 Exp. Vol. Ratio: 0.10
 RT Window: 0.10 min
 Stopover RT: 0.10 min
 Tea Retention RT: 0.10



Sample Name: P17106_A1 020 Sample ID: File: P8103 17106_A1.wf
 Peak Name: "IS (Peptide)" Mass(es): "183.184 1 amu"
 Comment: Annotation:
 Sample Index: 24
 Sample Type: Standard
 Concentration: 1.0 ng/mL
 Acquisition Date: 26.06.2009
 Acquisition Time: 18:35:38
 Acquisition Time: 18:35:38
 Modified: 01
 Method: Analyst Access
 Sampling Rate: 1000 cps
 Noise Threshold: 100 cps
 Area Threshold: 100 cps
 Min. Smooth: 0.20
 Sep. Width: 0.10
 Sep. Height: 0.10
 Exp. Peak Ratio: 0.10
 Exp. Adj. Ratio: 0.10
 Exp. Vol. Ratio: 0.10
 RT Window: 0.10 min
 Stopover RT: 0.10 min
 Tea Retention RT: 0.10



- **Chromatographic Conditions:** Luna C8 (2) 3 μm , 100 x 2 mm, 20 mM formic acid containing 5 mM nonafluoropentanoic acid in H_2O and acetonitrile, gradient: 0.0 – 0.1 min: isocratic: 10 % B, 0.1 – 2.0 min linear: 10 % B \rightarrow 30 % B; 2.0 – 3.4 min linear: 30 % B \rightarrow 70 % B, 3.4 – 3.5 min linear: 70 % B \rightarrow 90 % B, 3.5 - 4.1 min isocratic: 90 % B, 4.1 – 4.2 min linear: 90 % B \rightarrow 10 % B, 4.2 – 5.7 min isocratic: 10 % B; flow: 0.4 mL/min; Inj. Vol.: 20 μL ; Inj. (abs): 20 pg; column temp: 25°C
- **MS/MS Parameters:** API4000, APCI, pos., MRM, Temp: 600°C, Corona needle current: 5 μA , Transitions: Peptid and Metabolite: 201.0 \rightarrow 90.0 m/z, IS (Peptid and Metabolite) : 183.1 \rightarrow 84.1 m/z

Quantifizierung verschiedener Peptide in biologischen Flüssigkeiten



Hermann Mascher

WILEY-VCH

Klinische Analytik mit HPLC

Ein Ratgeber für die Praxis



**pharm
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BIOANALYTICS

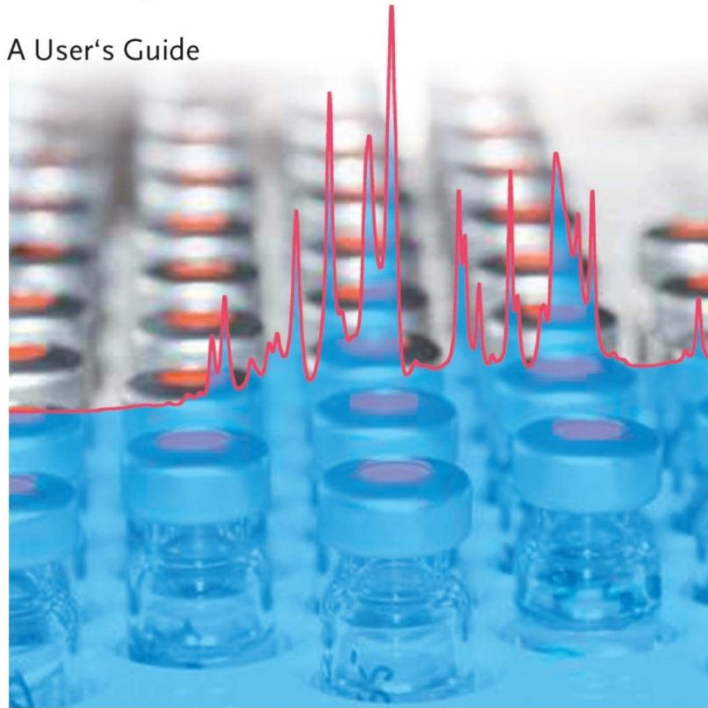
English Version coming soon

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HPLC Methods for Clinical Pharmaceutical Analysis

A User's Guide





Thank you for your attention.

