

LC-MS/MS for the quantification of Peptide biomarker and mixture of closely related Protein in formulation

Luc-Alain SAVOY

- **Part I: SGS overview**
- **Part II: Peptides in biological matrices**
- Peptides analysis by mass spectrometry
 - Biomarker peptides (Angiotensins)
 - Therapeutic peptides
- **Part III: Protein in formulations**
- Chorionic gonadotropin (CG) proteins

A circular icon with a gradient from orange to yellow, containing the word 'DELIVER' in white, bold, uppercase letters.

DELIVER

Analytical
Development

Biologics
Characterization
Potency,
Efficacy,
Biosafety

Quality Control

Clinical Research
for Phase I to IV
Trials

A circular icon with a gradient from dark grey to black, containing the word 'ENSURE' in white, bold, uppercase letters.

ENSURE

Quality, Safety and
Effectiveness of
Bio/pharmaceutical

« High value for
money » services

Reduction
time-to-market

LIFE SCIENCE SERVICES OVERVIEW

**SGS MEANS
EXPERIENCE**

- Over 35 years experience - 1.500 full time employees with 28 facilities in 15 countries
- Global drug development partner from Molecule to Market with unique international analytical laboratory network
 - across America, Europe, Asia with Centers of Excellence matching Biopharmaceutical and Small molecules needs
- Expert biopharma analytical services
 - **Research, QC, regulatory**
 - Scientific consultancy
 - Biosimilars
 - Comparability
 - Bioanalysis & bioassays
 - Proteomics, glycomics
 - Extracables & leachables
 - Virus detection and identification
 - Molecular biology assays – q-pcr
 - **Product analysis**
 - Glycoproteins, proteins & peptides
 - Antibodies & vaccines
 - Gene & cell therapies
 - Oligonucleotides & polysaccharides
 - Small molecules & antibiotics
- Strong commitment to clinical and laboratory Quality and Operational Excellence in many areas
 - Harmonized QMS and Validation & Transfer methods, LIMS, Lean

ADVANTAGES OF LC-MS/MS

- Better selectivity between structurally or chemically similar peptides
- Better precision and accuracy
- Antibodies not required
- Low sensitivity (pg/mL)



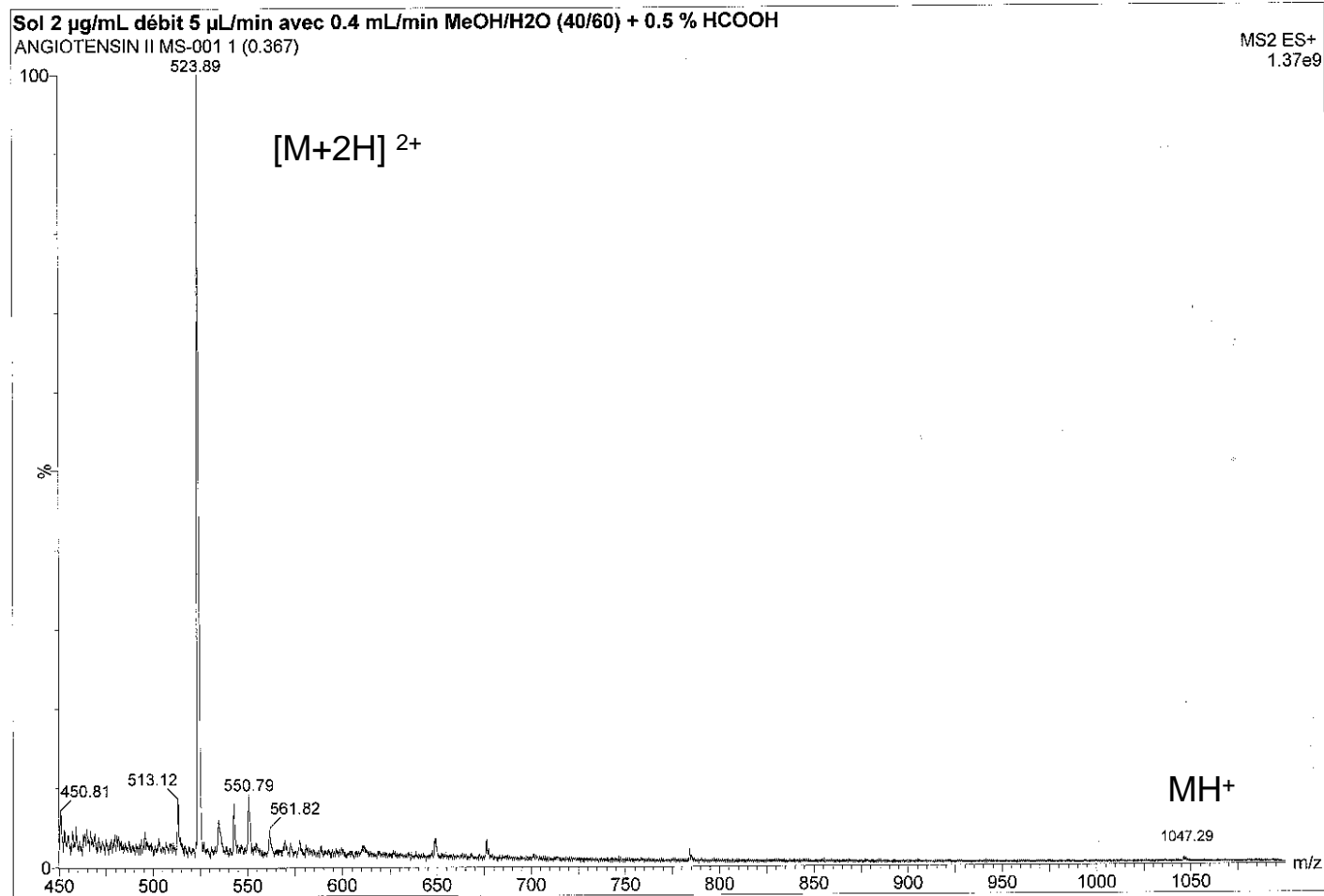
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AMINO ACID SEQUENCES OF ANGIOTENSIN PEPTIDES

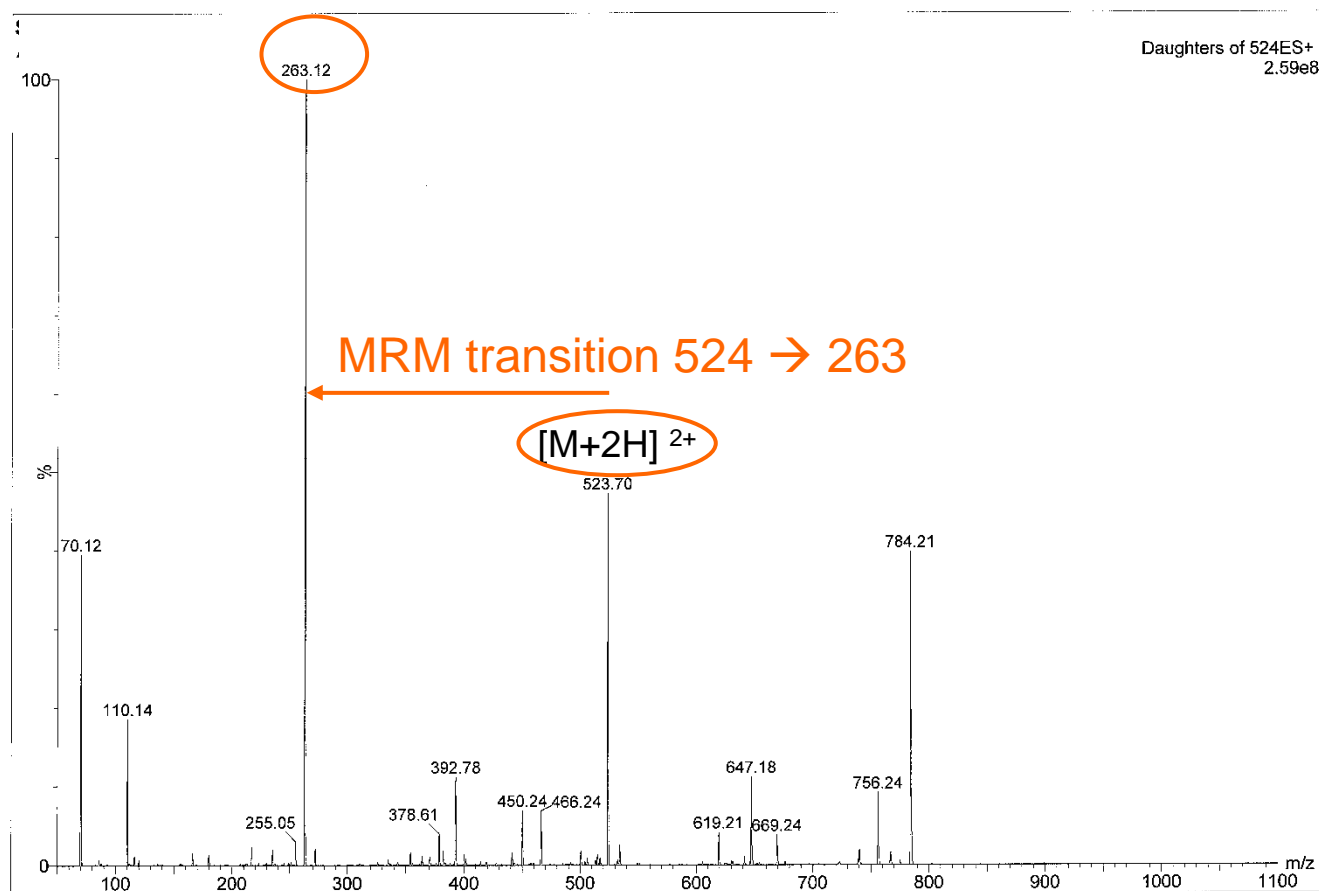
	Sequences	MW
Angiotensin I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu	1296,5
Angiotensin II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	1046,2
Angiotensin III	Arg-Val-Tyr-Ile-His-Pro-Phe	931,1
Angiotensin IV	Val-Tyr-Ile-His-Pro-Phe	774,9
Internal Standard	Sequences	MW
Angiotensin I	Asp-Arg-Val-Tyr-Ile*-His-Pro-Phe-His-Leu	1303,5
Angiotensin II	Asp-Arg-Val-Tyr-Ile*-His-Pro-Phe	1053,2
Angiotensin III	Arg-Val-Tyr-Ile*-His-Pro-Phe	938,1
Angiotensin IV	Val-Tyr-Ile*-His-Pro-Phe	781,9
[Ile* = I(13C6,15N)]		

Stable isotope labeled peptides are used as Internal Standards (IS) to correct variabilities during the entire bioanalytical process (extraction, dilution, adsorption, evaporation, degradation...).

POSITIVE ESI MASS SPECTRUM OF ANGIOTENSIN II (Precursor ion)



POSITIVE ESI MASS SPECTRUM ANGIOTENSIN II (PRODUCT IONS)



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MASS SPECTROMETER SETTINGS FOR ANGIOTENSIN MRM TRANSITIONS

Angiotensin	Pecursor ion (m/z)	Product ion (m/z)
Angiotensin I	433.2 (3+)	110,1
Angiotensin I (IS)	435.4 (3+)	110,1
Angiotensin II	523.8 (2+)	263,1
Angiotensin II (IS)	527.4 (2+)	263,1
Angiotensin III	466.4 (2+)	263,1
Angiotensin III (IS)	469.9 (2+)	263,1
Angiotensin IV	388.4 (2+)	235,1
Angiotensin IV(IS)	391.8 (2+)	235,1

The selection of specific MRM transitions results in a highly sensitive and selective detection of the peptides.

MRM: Multiple Reaction Monitoring

UPLC-MS/MS CONDITIONS

- Column: Acquity (Waters)
 - Length: 100 mm
 - Internal diameter: 2.1 mm
 - Particule size: 1.7 μm
- Flow rate : 0.5 mL/min
- Mobile phase
 - A : H₂O (+ formic acid)
 - B : MeOH (+ formic acid)
- Gradient table:

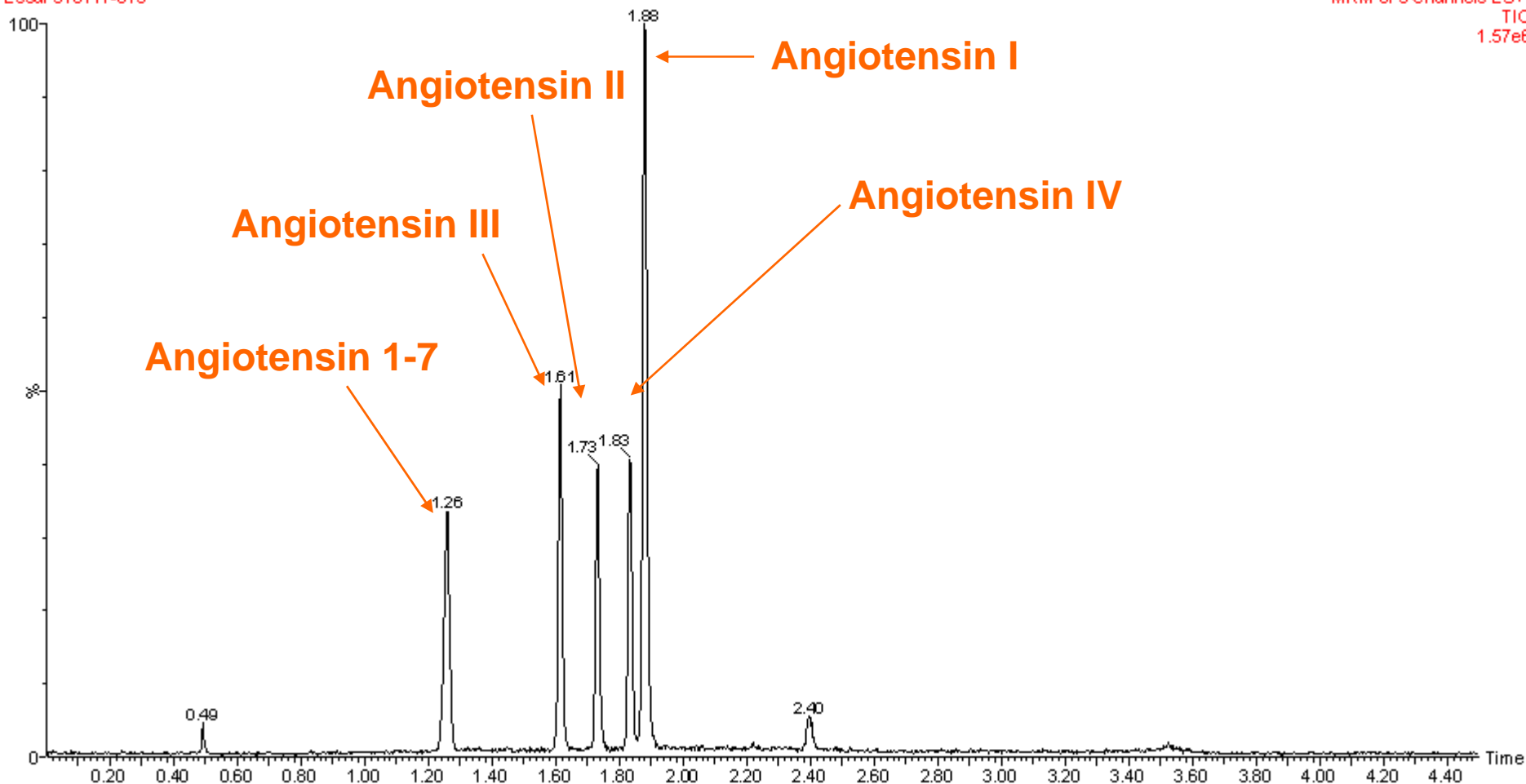
Time (min)	%A	%B
0	100	0
2,9	50	50
3,4	50	50
3,5	100	0
5	100	0

LC-MS/MS CHROMATOGRAM FOR ANGIOTENSINS IN SOLUTION

Sol 250 pg/mL angiotensin I,II,III, IV et 1-7

Essai 310111-019

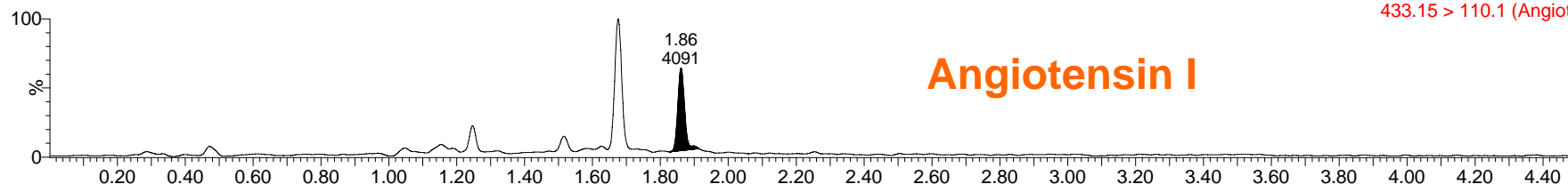
MRM of 5 Channels ES+
TIC
1.57e6



LC-MS/MS CHROMATOGRAM FOR ANGIOTENSINS IN HUMAN PLASMA

Plasma humain

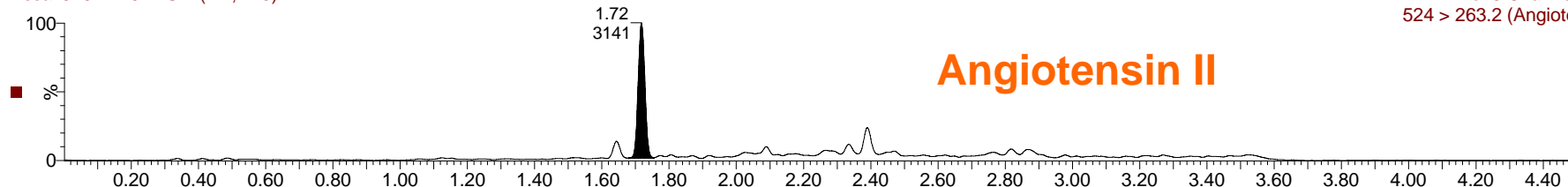
Essai 310111-027 Sm (Mn, 2x3)



Angiotensin I

MRM of 5 Channels ES+
433.15 > 110.1 (Angiotensin I)
2.95e5
Area

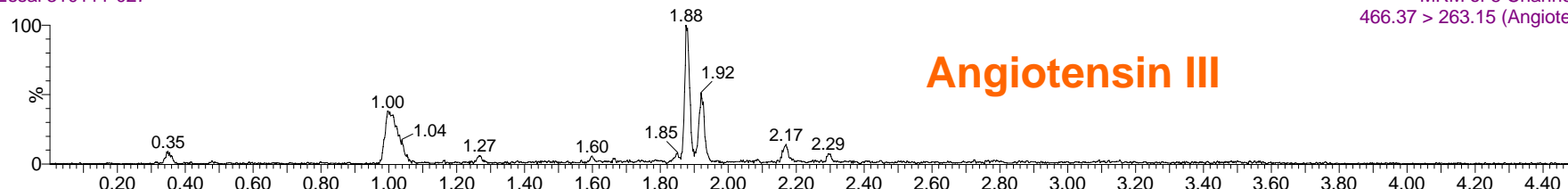
Essai 310111-027 Sm (Mn, 2x3)



Angiotensin II

MRM of 5 Channels ES+
524 > 263.2 (Angiotensin II)
1.41e5
Area

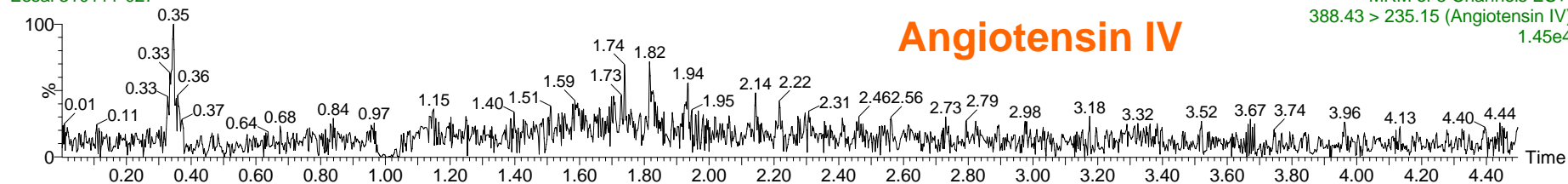
Essai 310111-027



Angiotensin III

MRM of 5 Channels ES+
466.37 > 263.15 (Angiotensin III)
2.21e5

Essai 310111-027

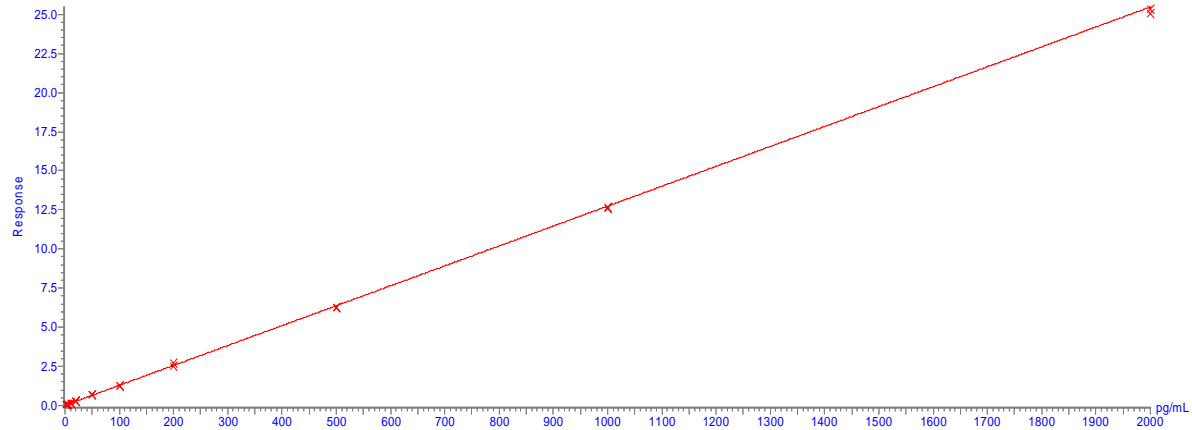


Angiotensin IV

MRM of 5 Channels ES+
388.43 > 235.15 (Angiotensin IV)
1.45e4

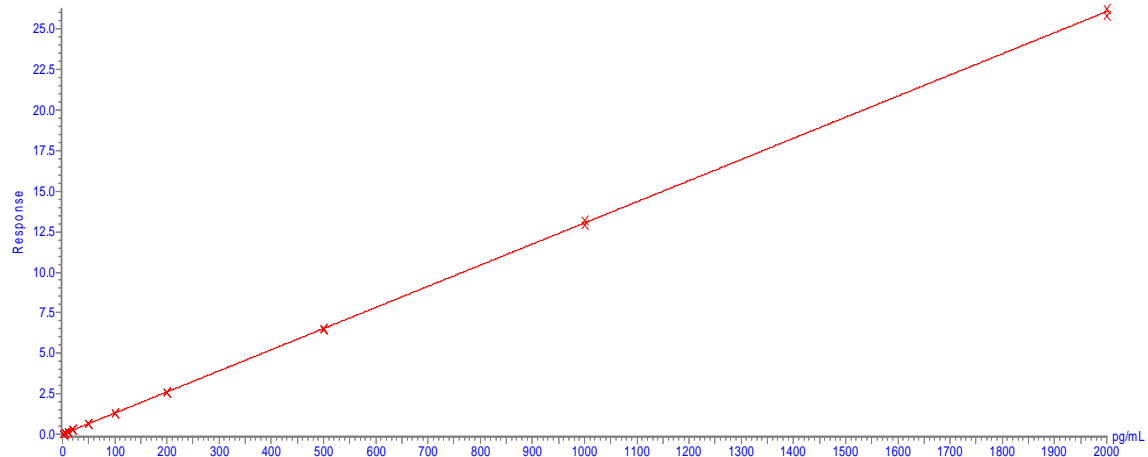
Time

Angiotensin I 2 to 2000 pg/mL



Compound name: Angiotensin I
 Correlation coefficient: $r = 0.997474$, $r^2 = 0.994955$
 Calibration curve: $0.0130392 \cdot x + 0.00329752$
 Response type: Internal Std (Ref 3), Area * (IS Conc. / IS Area)
 Curve type: Linear, Origin: Exclude, Weighting: 1/x², Axis trans: None

Angiotensin II 2 to 2000 pg/mL



INTRA-RUN ASSAY PERFORMANCE AT THE LLOQ (2 pg/mL)

	Angiotensin I 2pg/mL	Angiotensin II 2pg/mL
Replicate analysis of QC samples spiked at the LLOQ	2.03	2.27
	1.79	1.95
	2.55	1.57
	1.61	2.10
	2.32	2.10
	1.81	2.19
Mean Concentration (pg/mL)	2.02	2.03
Standard Deviation	0.36	0.25
Precision (%CV)	17.7	12.3
Accuracy (Bias %)	0.9	1.5
n	6	6

Precision and accuracy acceptance criteria are within $\pm 20\%$ at LLOQ

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- Triptorelin is a decapeptide (MW 1311.5 Da).
- Triptorelin is an agonist of gonadotropin releasing hormone.
- Treatment of prostate or breast cancer.
- New clinical phase III study with LLOQ 10pg/mL.
- Sequence:
PyroGlu-His-Trp-Ser-Tyr-(D)Trp-Leu-Arg-Pro-Gly-NH₂

- A stable isotope labelled internal standard was used

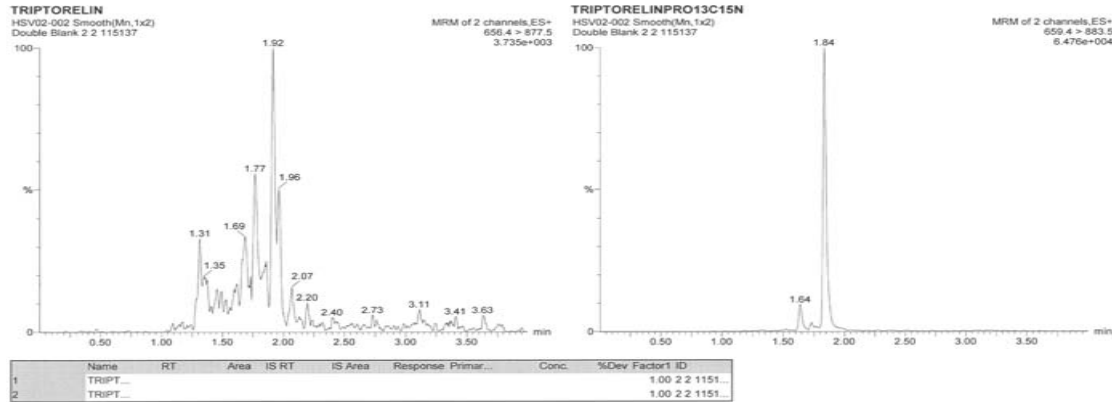
	Sequence	MW
Triptorelin	PGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH ₂	1311.5
Triptorelin (U-¹³C5, ¹⁵N)	PGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-*Pro-Gly-NH ₂	1317.5

[*Pro= I(¹³C5, ¹⁵N)]

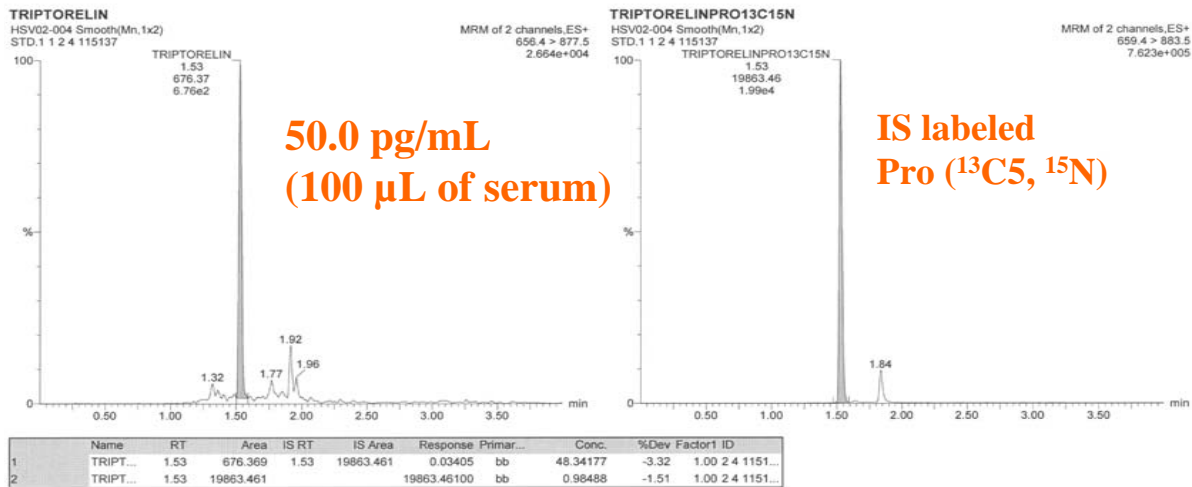
- Assay volume: 100 µL of human serum
- Runtime: 4 minutes.

LC-MS/MS CHROMATOGRAM OF A BLANK HUMAN SERUM AND OF A 50.0 pg/mL QC

Name: HSV02-002, Date: 06-Sep-2011, Time: 14:34:25, ID: 2 2 115137, Description: Double Blank, Instrument: XEVO-TQS#WAA145



Name: HSV02-004, Date: 06-Sep-2011, Time: 14:45:45, ID: 2 4 115137, Description: STD.1 1, Instrument: XEVO-TQS#WAA145



		50.0 pg/mL	150 pg/mL	1500 pg/mL	3500 pg/mL
Intra-run 1	Precision (%CV)	5.53	2.98	1.88	0.53
	Accuracy (Bias %)	2.80	-4.67	2.00	0.00
Intra-run 2	Precision (%CV)	4.29	2.31	0.54	1.88
	Accuracy (Bias %)	-4.00	-7.33	-6.67	-8.86
Intra-run 3	Precision (%CV)	2.13	1.60	0.66	0.71
	Accuracy (Bias %)	5.40	0.67	0.00	-2.29
Inter-run	Precision (%CV)	5.62	4.15	3.89	4.18
	Accuracy (Bias %)	1.40	-3.33	-1.33	3.71

Precision and accuracy acceptance criteria are within $\pm 15\%$

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DOSAGE OF PROTEINS IN FORMULATIONS – CASE STUDY

- Sample : A mixture of closely related proteins (human CG, equine CG)
- Aim : Each variant needs to be quantified individually
- Preparation of experimental procedures : In silico search for specific signature peptide(s) produced by defined proteolysis (enzyme/chemical)

DOSAGE OF PROTEINS IN FORMULATIONS – CASE STUDY

- Sample process : Test of the selected proteolytic procedure on the complex sample
- Synthesis of cold labeled signature peptides
- Establishment of calibration curves

DOSAGE OF PROTEINS IN FORMULATIONS – CASE STUDY

- Signature peptides

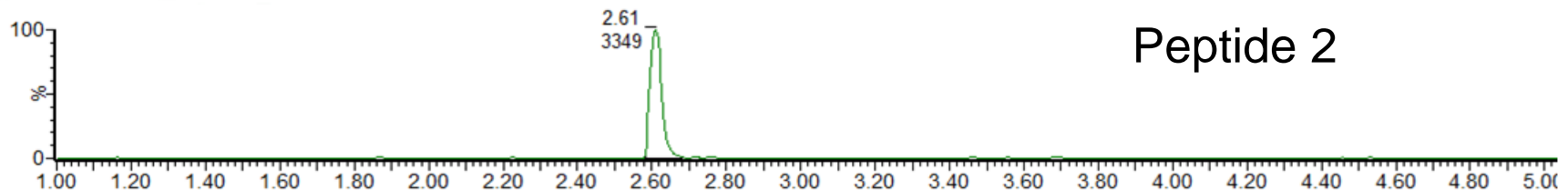
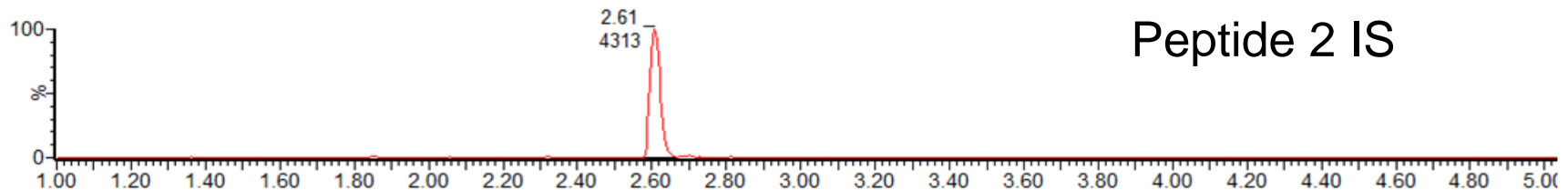
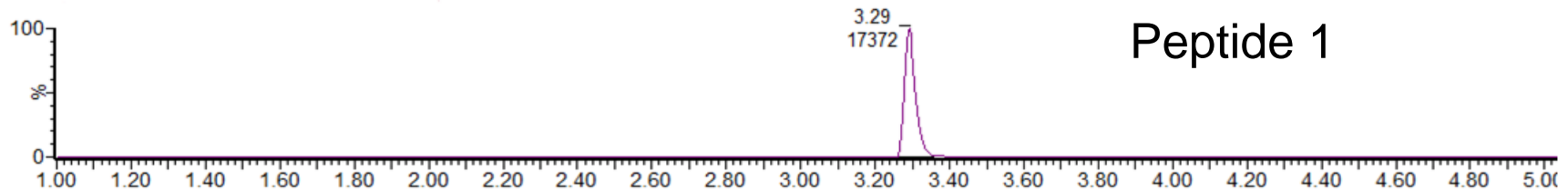
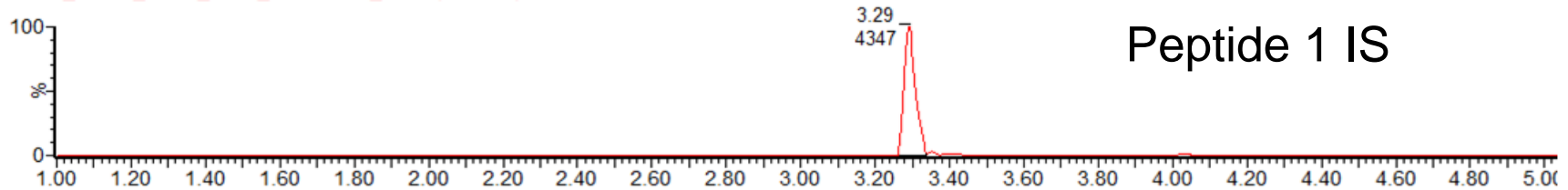
Horse CG R **FASIR** L P G C P
 Human CG R **FESIR** L P G C P

- Signature peptides and IS

Peptide 1	Mw 592.3	Peptide 1 IS	Mw 598.3 (Phe ¹³ C ₆)
Peptide 2	Mw 650.3	Peptide 2 IS	Mw 656.3 (Phe ¹³ C ₆)

- Transitions : Peptide 1 592.3 > 278.2
 Peptide 1 IS 598.3 > 284.2

■ UPLC-MS analysis of processed standard sample spiked with IS



DOSAGE OF PROTEINS IN FORMULATIONS – CASE STUDY

■ Representative results

BATCH No.:	Ratio eCG : hCG
1	8.0 : 1
2	8.3 : 1
3	8.2 : 1
4	5.3 : 1
5	7.9 : 1



- Detailed characterisation of your protein is essential as you will have to make sure that your quantitation assay is targeting:
 - A common feature of all variants if you want total amount (e.g. MAB PK studies).
 - A unique feature of each variant if you want relative amount.



THANK YOU FOR YOUR ATTENTION



M-Scan

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QUESTIONS ?

