TRISKELION

QUANTITATIVE ANALYSIS OF THERAPEUTIC PEPTIDE TRIPTORELIN IN HUMAN PLASMA USING SPE AND UPLC-MS/MS

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INTRODUCTION

THE QUANTITATIVE BIOANALYSIS OF (THERAPEUTIC) PEPTIDES IS VERY CHALLENGING. PEPTIDES TEND TO ADSORB TO MATERIALS (SAMPLE VIALS, LC TUBING, PIPET TIPS, ETC), THEY CAN HAVE LIMITED STABILITY IN STOCK SOLUTIONS AND PLASMA SAMPLES, AND CANNOT ALWAYS EASILY BE EXTRACTED FROM PLASMA DUE TO THEIR STRONG PLASMA-PROTEIN-BINDING PROPERTIES. THIS CAN RESULT IN LOSS OF PEPTIDES DURING SAMPLE PRE-TREATMENT AND LOW EXTRACTION RECOVERIES. ON THE OTHER HAND, EXTREMELY LOW PEPTIDE LEVELS IN LOW-VOLUME PLASMA SAMPLES MUST BE QUANTIFIED TO SUPPORT PHARMACOKINETIC AND TOXICOKINETIC STUDIES. THE AIM OF THIS RESEARCH WAS TO DEVELOP AND VALIDATE AN ANALYTICAL METHOD FOR A (STICKY) THERAPEUTIC PEPTIDE, TRIPTORELIN, IN HUMAN PLASMA SAMPLES. TRIPTORELIN (MOLAR MASS 1311.47 G/MOL), A SYNTHETIC ANALOGUE OF GONADOTROPIN-RELEASING HORMONE (GNRH), IS USED TO TREAT PROSTATE CANCER. SAMPLE PRE-TREATMENT WAS BASED ON SOLID-PHASE EXTRACTION (MICRO-ELUTION PLATES, WATERS); LHRH WAS USED AS THE INTERNAL STANDARD. THE EXTRACTS WERE ANALYZED USING UPLC-MS/MS (XEVO TQ-S, WATERS).

EXPERIMENTAL I: TRIPTORELIN AND INTERNAL STANDARD

Peptide	Mass (Da)	Amino acid Sequence
Triptorelin	1311.47	pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2
LHRH (internal standard)	1182.31	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

EXPERIMENTAL II: SAMPLE PRE-TREATMENT

Solid phase extraction

- 10 µl of IS solution (20 ng/mL) was added to 100 µL human plasma (EDTA) sample.
- the plasma sample was 1:1 diluted with H3PO4 (4%).

SPE procedure (WCX micro-elution plates, Waters).

SPE step	Solvent/volume
Conditioning I:	200 µL methanol
Conditioning II:	200 µL MilliQ water
Sample application:	Plasma sample (spiked with IS and diluted with H_3PO_4)
Wash step I:	200 µL NH_4OH (5%)
Wash step II:	200 µL acetonitrile/ MilliQ water (20/80, V/V)
Elution:	2 x 25 µL 1% TFA in acetonitrile/ MilliQ water (75/25, V/V)

- The eluate was 1:1 diluted with MilliQ water.

EXPERIMENTAI III: UPLC-MS/MS EQUIPMENT AND CONDITIONS

Parameter	Value
UPLC:	Acquity (Waters)
UPLC column:	Acquity C18 BEH; 100 x 2.1 mm; 1.7 µm (Waters)
Column temperature:	50 °C
Autosample temp:	10 °C
Mobile phase A:	0.1 % formic acid in MilliQ water
Mobile phase B:	0.1 % formic acid in acetonitrile
Injection volume:	10 µl
Flow:	400µL/min
Mass spectrometer:	XEVO TQ-S (Waters)
Ionization mode:	Electrospray; positive mode; MRM

RESULTS I. MASS CHROMATOGRAMS

Figure 1. Mass chromatogram of Triptorelin (2.5 min) in aqueous solution (50 pg/mL; 38 pmol/L).

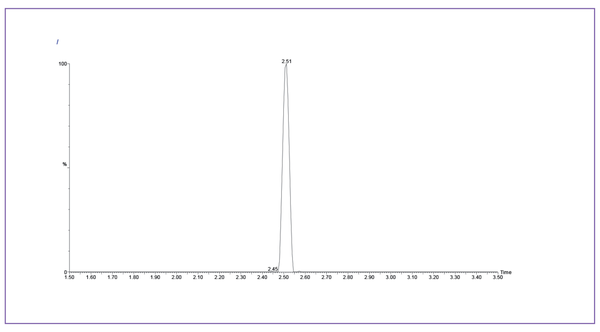


Figure 2. Mass chromatogram of Triptorelin (2.5 min) in human EDTA plasma (100 pg/mL; 76 pmol/L).

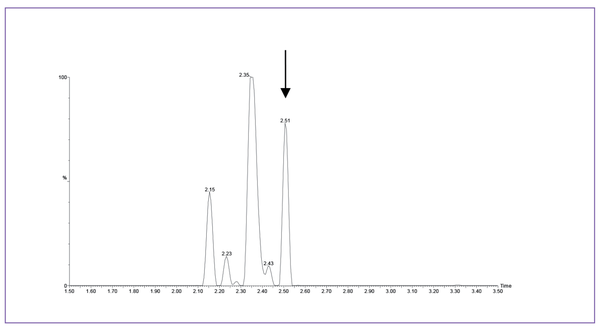
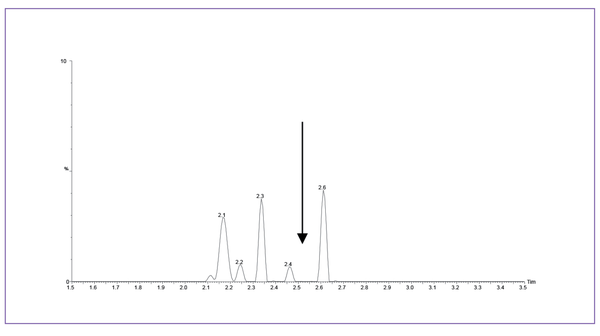
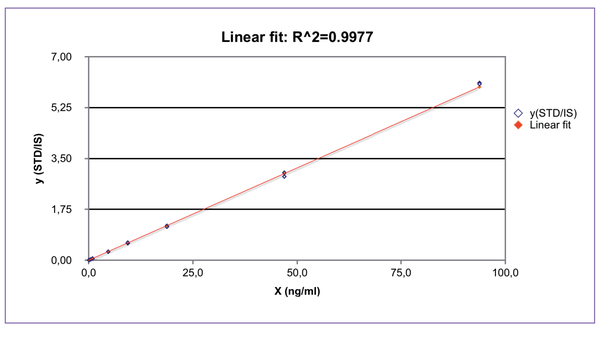


Figure 3. Mass chromatogram of blank human EDTA plasma (no signal at 2.5 min).



RESULTS II. CALIBRATION CURVE

Figure 4. Calibration curve of Triptorelin in human plasma. The calibration range was from approximately 0.1 to 100 ng/ml. Linear regression analysis with weighting factor $1/x^2$ was applied.



CONCLUSIONS

Selectivity Triptorelin was baseline-separated from interferences in human plasma (Figure 3). No signal was observed at the retention time of Triptorelin (2.5 min).

Calibration curve The calibration results of Triptorelin in human plasma were accepted. The calibration range was from approximately 0.1 to 100 ng/ml. Linear regression analysis with weighting factor 1/x2 was applied. The accuracy was within ±15% at all levels (±20% at the lowest level).

Quality control results The accuracy and precision were investigated by the five-fold analysis of four QC samples on three separate days. The accuracy, expressed as the deviation from the actual concentration, was within ±15% at all levels. The intra-day and inter-day precision, expressed as the coefficient of variation (CV, %), were within 10% at all levels.

Lower Limit of Quantification The Lower Limit of Quantification was approximately 100 pg/mL in human plasma (Figure 2).

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