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Application of a sensitive liquid chromatography-tandem mass spectrometric method to determination of octreotide in human plasma

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Overview

A fast and sensitive method for octreotide in human plasma by ultra high performance liquid chromatography-tandem mass spectrometry was developed. The lower limit of quantitation was 5.0 pg/mL and the results of other parameters for method validation were good.

Introduction

Analysis of therapeutic peptides in bio-matrices is very important in the pharmaceutical industry especially in the area of oncology therapy. Octreotide (Fig. 1) is a synthetic ling-acting cyclic octapeptide. It is a somatostatin analog that has a longer half life and more selectivity. Radio-immunoassay methods have been used for determination of octreotide in human blood, urine and saliva after dosing octreotide. A LC/MS method has been developed for determination of octreotide in human plasma with a low limit of quantitation (LLOQ) of 500 pg/mL. A LC-MS/MS method has been used for determination of octreotide with LLOQ of 50 pg/mL. However, this method required 400 µL plasma and laborious sample pretreatment. In this study, a rapid, sensitive method using a simple µElution solid phase extraction and ultra high performance liquid chromatography-tandem mass spectrometry was developed and validated.

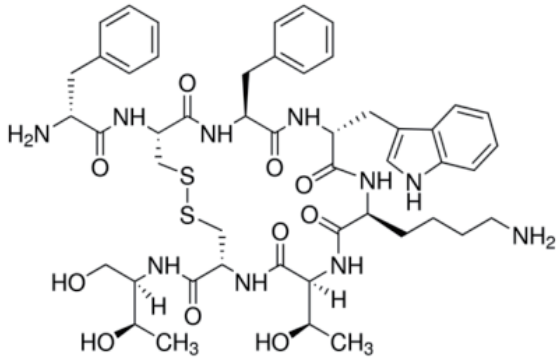

Fig.1 Structure of octreotide

Methods

Sample Preparation

(1) Add 200 µL of plasma into the polypropylene tube, add 50 µL of internal standard (IS) working solution (1.0 ng/mL leuprolide), add 200 µL of 4% phosphoric acid, seal the tube and vortex for approximately 1 minute.
(2) Condition the µElution WCX SPE plate with 200 µL methanol then equilibrate with 200 µL of water.
(3) Load the sample onto the SPE plate, wash with 400 µL of 5% ammonium hydroxide, then with 400 µL of 20% acetonitrile, and finally elute the sample with 50 µL of 1% TFA in 75:25 acetonitrile/water (v/v) into a 96-position, 2.0 mL, polypropylene plate.
(4) Dilute the sample with 150 µL of water and votex for 3 minutes.
(5) Inject the sample into the UHPLC-MS/MS system.

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LC-MS/MS Analysis

The analysis was performed on a Shimadzu Nexera UHPLC system (Kyoto, Japan). A triple quadruple mass spectrometer (Shimadzu LCMS-8050, Kyoto, Japan) was connected to the UHPLC instrument via an ESI interface.

HPLC (Nexera UHPLC system)	
Column	: InertSustain Swift C18 (2.1 mmi.d.. x 100 mmL, 1.9 µm, GL Sciences)
Mobile phase A	: water with 0.1% formic acid
Mobile phase B	: acetonitrile
Flow rate	: 0.4 mL/min
Column temperature	: 40 °C
Mode	: Gradient Elution
MS (LCMS-8050 triple quadrupole mass spectrometer)	
Ionization	: ESI
Polarity	: Positive
Ionization voltage	: +4.0 kV
Nebulizing gas flow	: 3.0 L/min
Heating gas flow	: 15.0 L/min
Drying gas flow	: 5.0 L/min
Interface temperature	: 400 °C
Heat block temperature	: 400 °C
DL temperature	: 200 °C
Mode	: MRM

Table 1 MRM parameters

Compound	Precursor *m/z*	Product *m/z*	Dwell time (ms)	Q1 Pre bias (V)	CE (V)	Q3 Pre bias (V)
Octreotide	510.30	120.00	100	-36.0	-31.0	-21.0
Leuprolide	605.40	249.05	100	-22.0	-29.0	-26.0

Results

Human plasma samples containing octreotide ranging from 5 to 2000 pg/mL were prepared, extracted by µSPE and the final extracts were analyzed by LC-MS/MS. The typical MRM chromatogram of octreotide is presented below (Fig.2). The linear regression for octreotide was found to be >0.999. Excellent precision and accuracy were maintained, demonstrating a linear dynamic range suitable for real-world applications. The LOQ for octreotide was 5 pg/mL, which meet the criteria for precision and accuracy within ±20% both within run and between run. The intra- and inter-day precision and accuracy of the assay were investigated by analyzing QC samples. Intra-day precision values at three concentration levels 15 pg/mL, 150 pg/mL and 1500 pg/mL were between 2.32~7.43% and inter-day precision was between 2.44~8.99%. accuracy was within ±15.0%.

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The recoveries of octreotide were 70.6±7.8%, 71.5±1.8% and 70.1±1.9% at three concentration levels individually. The matrix effect factors for three concentration levels were all larger than 80% and internal standard normalized factors for each levels were larger than 90%. There was no significant carryover after analysis of highest concentration of calibration (Fig. 4).

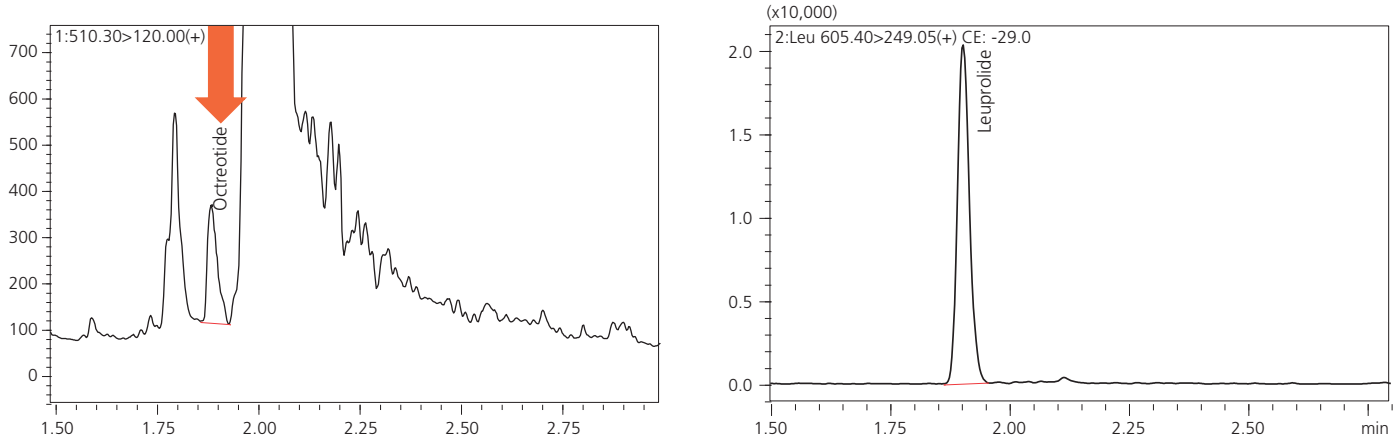

Figure 1 Representative MRM chromatograms of spiked (5 pg/mL) human plasma (left: octreotide, right: IS)

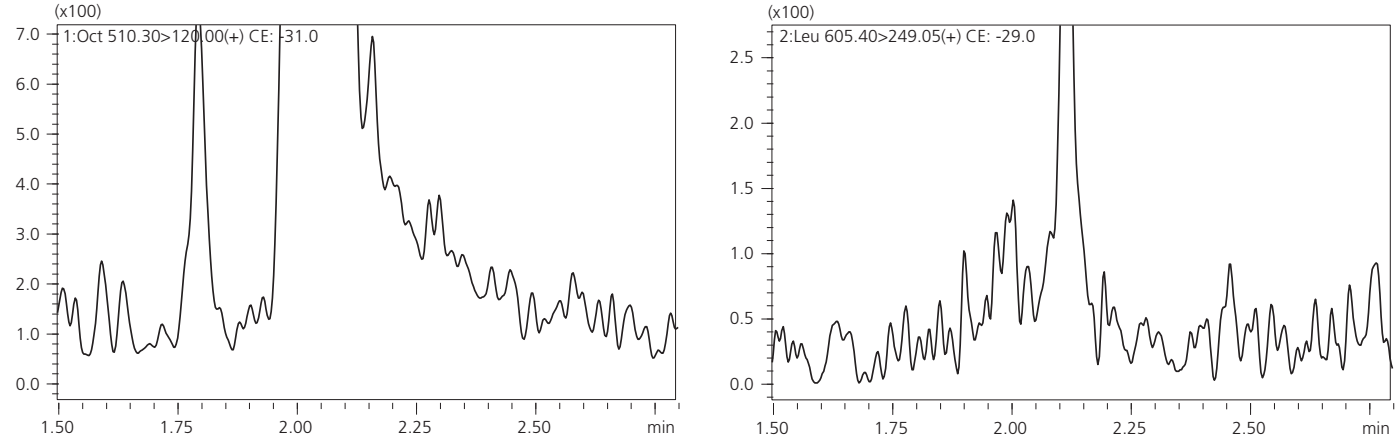

Figure 2 Representative MRM chromatograms of blank plasma (left: octreotide, right: IS)

Application of a sensitive liquid chromatography-tandem mass spectrometric method to determination of octreotide in human plasma

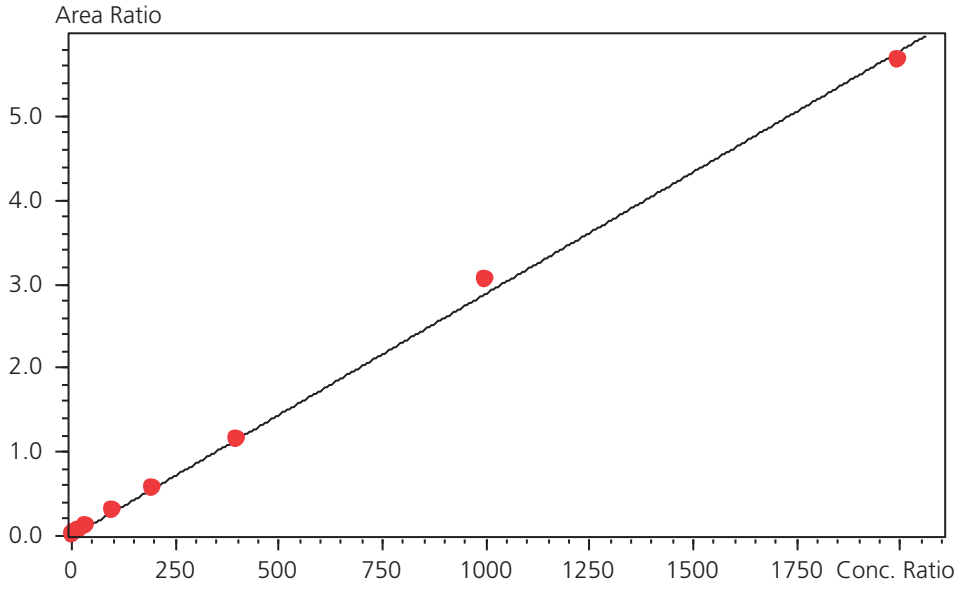

Figure 3 Calibration curve of octreotide in human plasma

Table 2 Calibration curve information

Compound	Calibration curve	Linear range (ng/mL)	Accuracy (%)	r
Octreotide	$Y = (2.89\times10^{-3})X + (-6.34\times10^{-3})$	5~2000	89.6~112.3%	0.9993

Table 3 Accuracy and precision for the analysis of octreotide in human plasma (in pre-study validation, n=3 days, six replicates per day)

Added conc. (ng/mL)	Intra-day precision (%RSD)	Inter-day precision (%RSD)	Accuracy (%)
15	7.43	8.99	87.5~109.4
150	2.41	4.37	95.4~112.1
1500	2.32	2.44	95.4~103.1

Table 4 Recovery for QC samples (n=6)

QC Level	Concentration (ng/mL)	Recovery (%)
LQC	15	70.6
MQC	150	71.5
HQC	1500	70.1

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Table 5 Matrix effect for QC samples (n=6)

QC Level	Added conc. (ng/mL)	Matrix factor	IS-Normalized matrix factor
LQC	15	82.1%	90.5%
MQC	150	87.6%	96.6%
HQC	1500	89.7%	98.9%

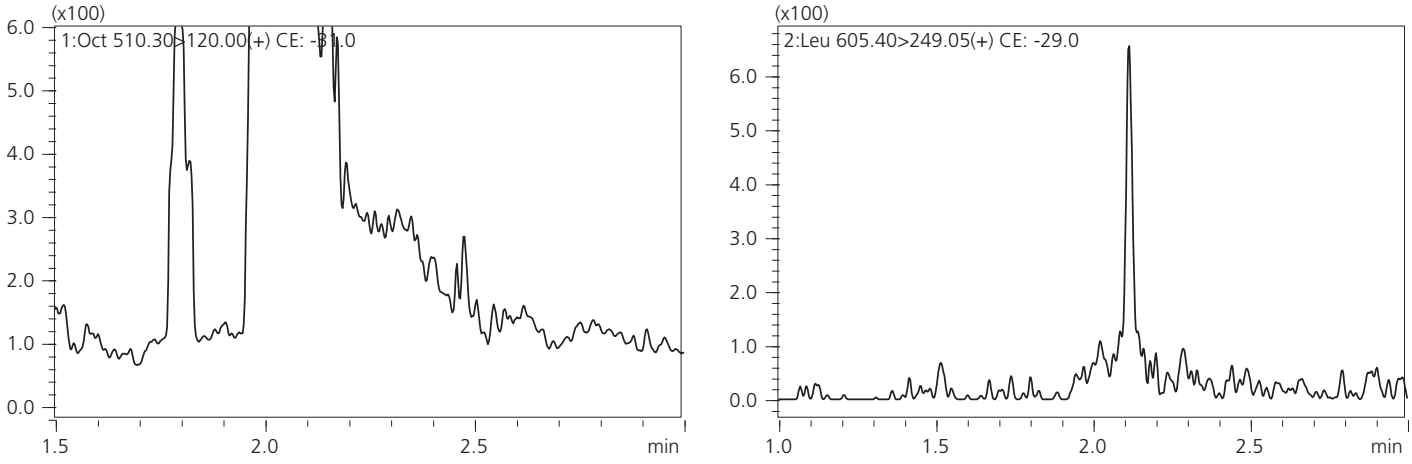

Figure 4 MRM chromatograms of blank plasma after ULOQ sample (Carryover test, left: octreotide, right: IS)

Conclusions

Results of parameters for method validation such as dynamic range, linearity, LLOQ, intra-day precision, inter-day precision, and matrix effect factors were excellent. The sensitive LC-MS/MS technique provides a powerful tool for the high-throughput and highly selective analysis of octreotide in DMPK study.

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