



Sensitive And High-Throughput Bioanalysis of Octreotide in Human Plasma Using LC/MS/MS

Authors

Yao Shi, Laixin Wang, Bradley Bessette, Spencer Carter, Scott Reuschel, Min Meng

Introduction

Octreotide is a cyclic-peptide with broad pharmaceutical applications. An LC-MS/MS method has been previously reported for the quantitative analysis of octreotide in human plasma, but the sample preparation procedure includes a manual protein precipitation (PPT) step followed by a manual liquid-liquid extraction (LLE)[1] and is not well suited for high-throughput applications. The purpose of this presentation is to demonstrate a sensitive, rugged and high-throughput method for the quantitative determination of octreotide in human plasma using LC-MS/MS to support clinical studies.

Methodology

SAMPLE PREPARATION AND EXTRACTION

1. Aliquot 300 μL of sample to corresponding wells of a 96-well deep well plate
2. Add 50.0 μL working internal standard [500 ng/mL desmopressin]
3. Add 300 μL of extraction buffer and mix well.
4. Transfer samples from the 96-well plate to an Oasis® WCX $\mu\text{Elution}$ plate which is pre-conditioned with 200 μL MeOH and 200 μL water.
5. Wash with 300 μL 5% NH_4OH and 300 μL Water.
6. Elute samples with 2 x 25.0 μL of elution buffer.
7. Dilute samples with 150 μL of water.



Methodology (continued)

CHROMATOGRAPHIC CONDITIONS

Column: Acquity UPLC® Phenyl, 1.7µm, 2.1 x 50 mm
Mobile Phase: A: 0.1% FA in water
B: MeOH
Injection volume: 20 µL
Column temperature: 35 °C
Flow rate: 0.500 mL/min
AS Temperature: Ambient

MASS SPECTROMETER CONDITIONS

Instrument: Sciex API 5000™
Ionization mode: Turboionspray, Positive ion mode
Source Temperature: 500 °C
SRM transitions: Octreotide m/z 510.5 → 120.0
Desmopressin m/z 535.5 → 328.2

Results and Discussion

METHOD DEVELOPMENT

- Both octreotide and desmopressin are cyclic peptides with molecular weights of 1019.24 and 1069.22, respectively. Each peptide contains a disulfide bond in the molecule (Figure 1).
- Octreotide and desmopressin essentially co-elute under the optimized LC conditions. However, there is no cross-contribution from one to the other in the selected MRM transitions (Figure 2 and Figure 3).



Results and Discussion (continued)

FIGURE 1. Chemical Structures of Octreotide (top) and Desmopressin (bottom)

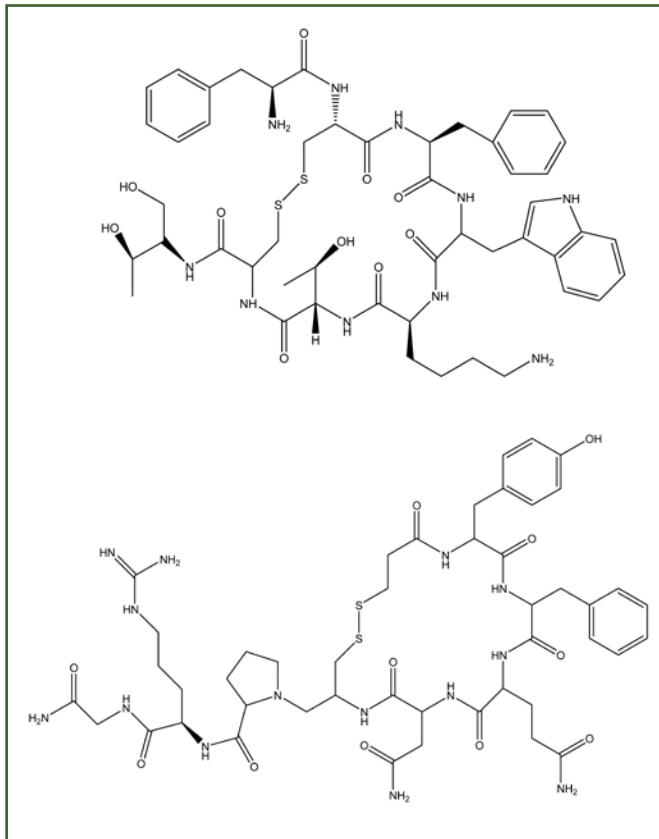
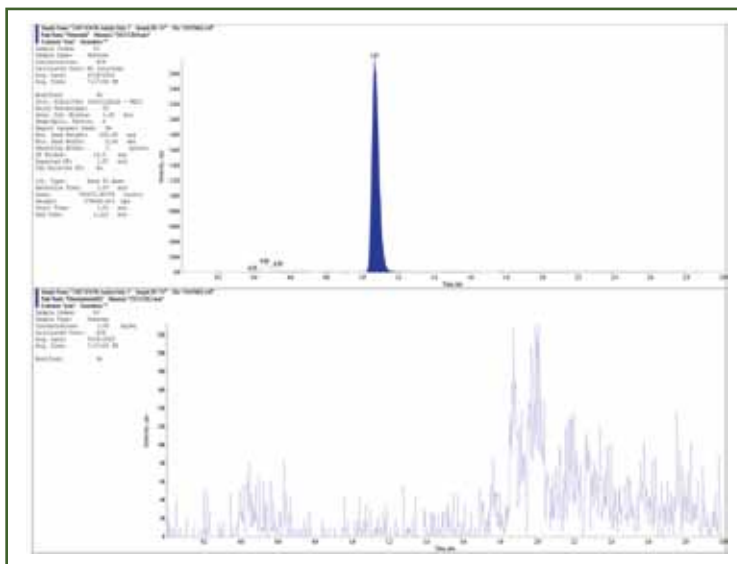


FIGURE 2. High Standard Without Internal Standard (20.0 ng/mL)





Results and Discussion (continued)

FIGURE 3. Internal Standard Only (83.3 ng/mL)

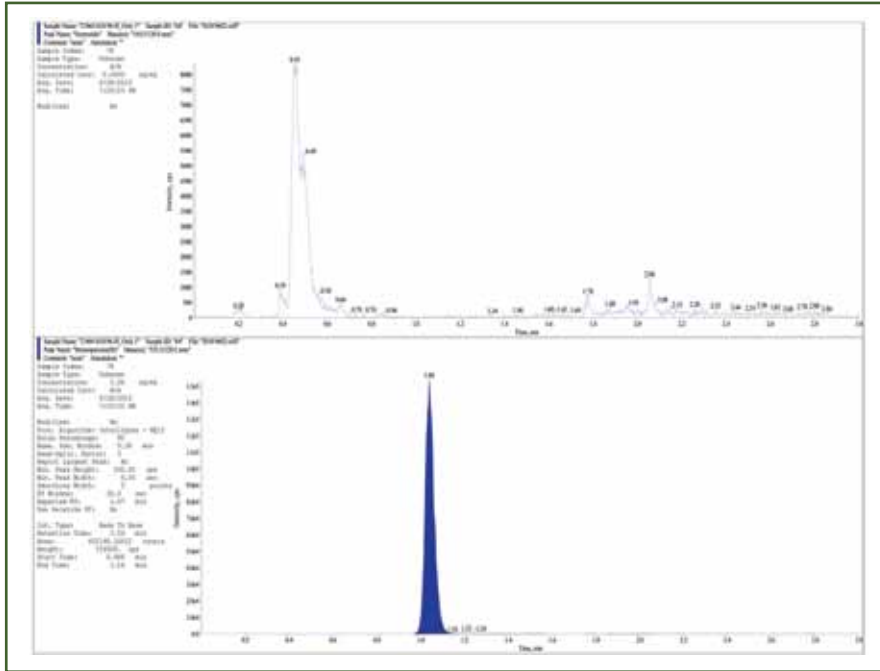
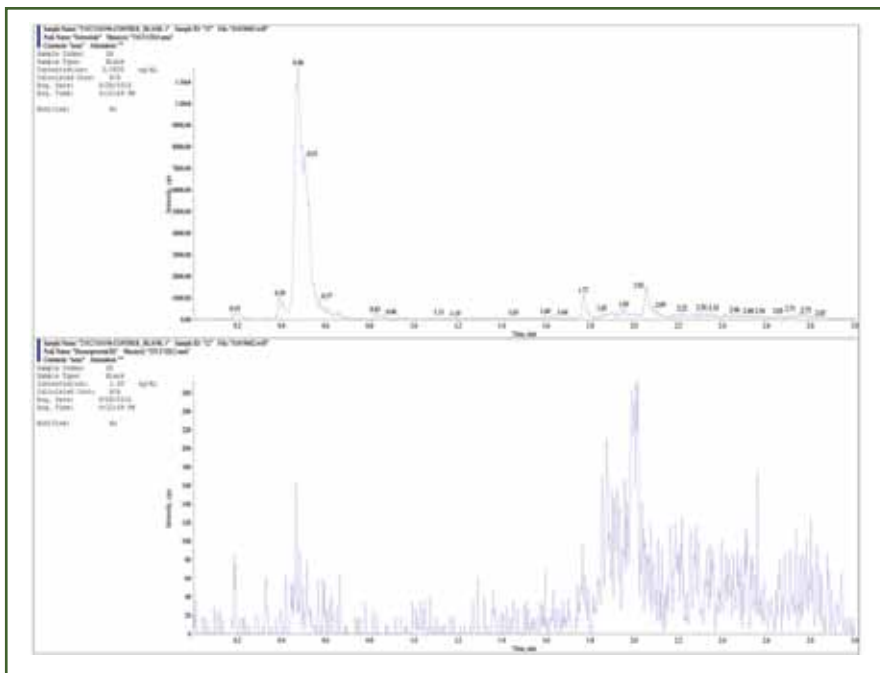


FIGURE 4. Representative Plasma Blank Control





Results and Discussion (continued)

ASSAY VALIDATION

- Accuracy/precision:** Demonstrated at n=6 at LLOQ, Low, Medium, High concentrations over 3 validation runs. (Table 2)
- Selectivity:** Demonstrated with blank and low QC concentrations in six sources of human plasma. (Table 3)
- Ability to dilute:** Demonstrated above ULOQ at DF=10 and DF=50. (Table 4)
- Extraction recovery:** Evaluated for analyte at Low, Medium, High concentrations and for Internal Standard (IS) at working concentration (n=6). (Table 5)
- Matrix factor:** Evaluated for analyte at medium QC concentration and for IS at working concentration (n=6). (Table 6)
- Carryover:** Evaluated in each run. No carryover present.

STABILITY

- In solution:** For both analyte and IS in DMF:
6 hrs at ambient temperature
104 days at 1-8 °C
- In matrix (human plasma):** 4 Freeze/Thaw cycles
6 hrs at ambient temperature
147 days at -70 °C and -20 °C
- Reinjection**
- Reproducibility:** 96 hours at ambient temperature

TABLE 1. Back-Calculated Concentrations of Calibration Standards for Octreotide

(All concentrations are expressed as ng/mL)

| Nominal Conc. | 0.0500 | 0.100 | 0.500 | 2.50 | 5.00 | 10.0 | 18.0 | 20.0 |
|---------------|---------|---------|---------|--------|-------|-------|-------|-------|
| Mean | 0.0500 | 0.0999 | 0.506 | 2.46 | 5.09 | 10.1 | 18.2 | 19.3 |
| S.D. | 0.00285 | 0.00427 | 0.00580 | 0.0818 | 0.157 | 0.295 | 0.616 | 0.339 |
| %CV | 5.7 | 4.3 | 1.1 | 3.3 | 3.1 | 2.9 | 3.4 | 1.8 |
| %Bias | 0.0 | -0.1 | 1.2 | -1.6 | 1.8 | 1.0 | 1.1 | -3.5 |
| n | 5 | 6 | 6 | 6 | 6 | 5 | 6 | 6 |



Results and Discussion (continued)

TABLE 2. Intra- and Inter-Assay Accuracy and Precision Of Quality Control Samples For Octreotide from ANOVA

| Nominal Conc. | LLOQ QC 0.0500 ng/mL | Low QC 0.150 ng/mL | Medium QC 8.00 ng/mL | High QC 16.0 ng/mL |
|-----------------------------|-------------------------|-----------------------|-------------------------|-----------------------|
| Mean Observed Conc. | 0.0526 | 0.150 | 7.70 | 14.8 |
| %Bias | 5.2 | 0.0 | -3.8 | -7.5 |
| Between Run Precision (%CV) | 4.7 | 0.0 | 0.0 | 3.1 |
| Within Run Precision (%CV) | 7.3 | 7.0 | 3.3 | 5.2 |
| Total Variation (%CV) | 8.7 | 6.6 | 3.0 | 6.1 |
| n | 18 | 18 | 18 | 17 |
| Number of Runs | 3 | 3 | 3 | 3 |

TABLE 3. Selectivity at the Low QC Concentration for Octreotide

| | Low QC 0.150 ng/mL |
|--------------|-----------------------|
| | 0.140 |
| | **0.123 |
| | 0.152 |
| | 0.142 |
| | 0.138 |
| | 0.156 |
| Mean | 0.142 |
| S.D. | 0.0116 |
| %CV | 8.2 |
| %Theoretical | 94.7 |
| %Bias | -5.3 |
| n | 6 |
| n | 6 |

** > ±15% deviation from theoretical

TABLE 4. Dilution Quality Control Samples for Octreotide

| | Dilution QC 800 ng/mL DF=50 | Dilution QC 160 ng/mL DF=10 |
|--------------|-----------------------------------|-----------------------------------|
| | 787 | 160 |
| | 752 | 165 |
| | 731 | 156 |
| | 754 | 154 |
| | 753 | 154 |
| | 708 | 159 |
| Mean | 748 | 158 |
| S.D. | 26.4 | 4.24 |
| %CV | 3.5 | 2.7 |
| %Theoretical | 93.5 | 98.8 |
| %Bias | -6.5 | -1.3 |
| n | 6 | 6 |



Results and Discussion (continued)

TABLE 5. Relative Extraction Recovery for Octreotide and Desmopressin (IS)

| | Low (0.150 ng/mL) | Medium (8.00 ng/mL) | High (16.0 ng/mL) |
|---------------------------|----------------------|------------------------|----------------------|
| Recovery for Octreotide | 59.6% | 62.6% | 62.7% |
| Recovery for Desmopressin | 54.6% | | |

TABLE 6. Matrix Factor for Octreotide and Desmopressin (IS)

(Results are expressed as area counts)

| | MF Extracted Oc- treotide | MF Neat Octreotide | MF Extracted Des- mopressin | MF Neat Desmopressin |
|----------------------|------------------------------|-----------------------|--------------------------------|-------------------------|
| Mean | 60903 | 49339 | 221204 | 199261 |
| S.D. | 2841 | 3050 | 11555 | 4246 |
| %C.V. | 4.7 | 6.2 | 5.2 | 2.1 |
| n | 6 | 6 | 6 | 6 |
| Matrix Factor | 1.2 | | 1.1 | |

Conclusion

A robust, sensitive and high throughput method was developed and validated to quantitatively analyze octreotide in human plasma.

Reference

- [1] Jiang Y, Wang J, Wang Y, Du X, Zhang Y, Fawcett JP, Gu J. Determination of long-acting release octreotide, an octapeptide analogue of somatostatin in human plasma by liquid chromatography/tandem mass spectrometry, *Rapid Commun Mass Spectrom.* 2007;21(24):3982-6.