



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

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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 μ L of sample to corresponding wells of a 96-well deep well plate
2. Add 50.0 μ L working internal standard [500 ng/mL desmopressin]
3. Add 300 μ L of extraction buffer and mix well.
4. Transfer samples from the 96-well plate to an Oasis® WCX μ Elution plate which is pre-conditioned with 200 μ L MeOH and 200 μ L water.
5. Wash with 300 μ L 5% NH₄OH and 300 μ L Water.
6. Elute samples with 2 x 25.0 μ L of elution buffer.
7. Dilute samples with 150 μ L 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).

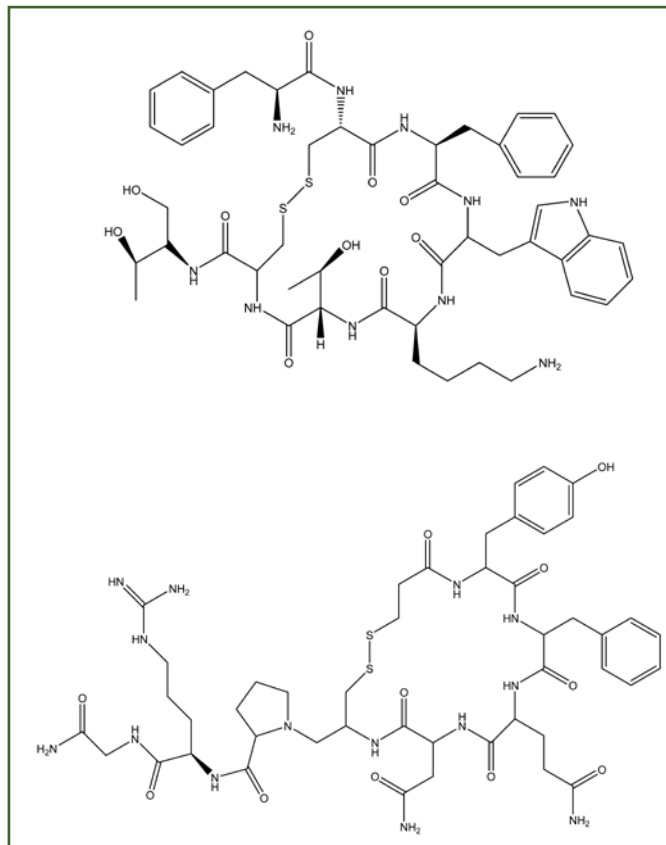


Results and Discussion (continued)

MS/MS transition 262 m/z – 102 m/z

- Mass 262 m/z – 102 m/z has good specificity
(See extracted blank - Figure 2)
- Analyte has retention in reversed phase
(See extracted LLOQ- Figure 3)

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





Results and Discussion (continued)

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

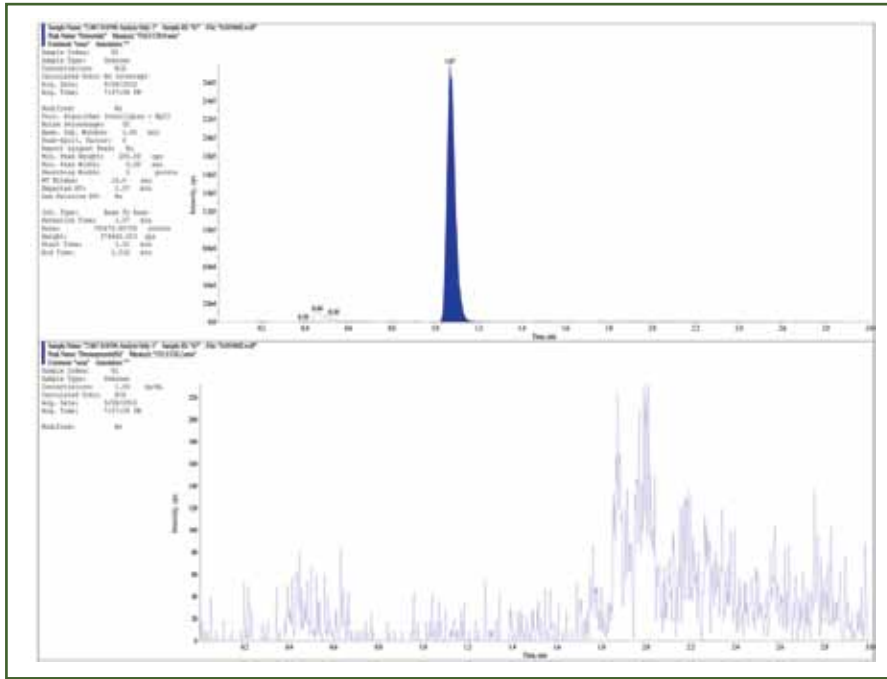
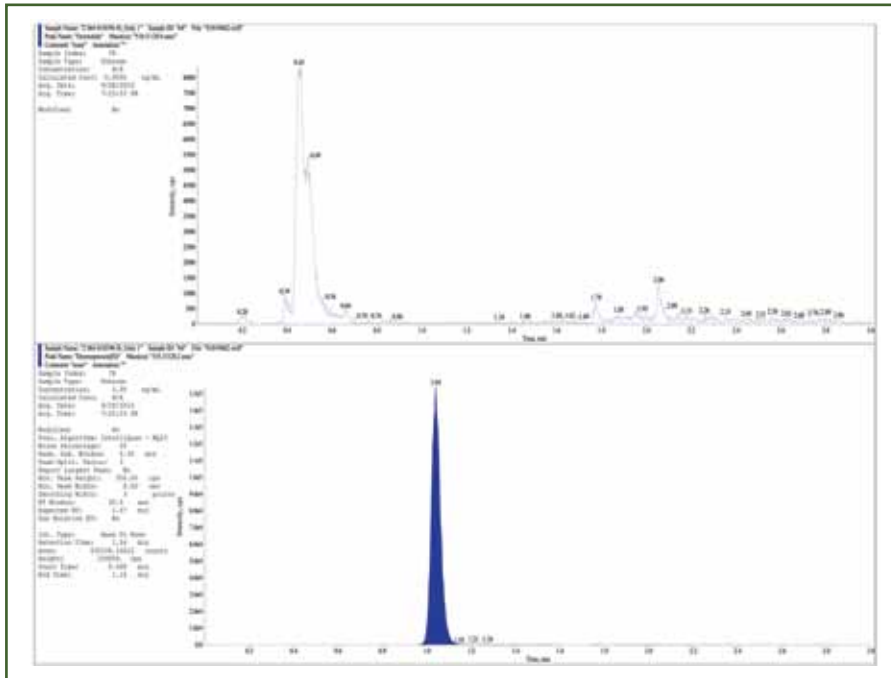


FIGURE 3. Internal Standard Only (83.3 ng/mL)





Results and Discussion (continued)

FIGURE 4. Representative Plasma Blank Control

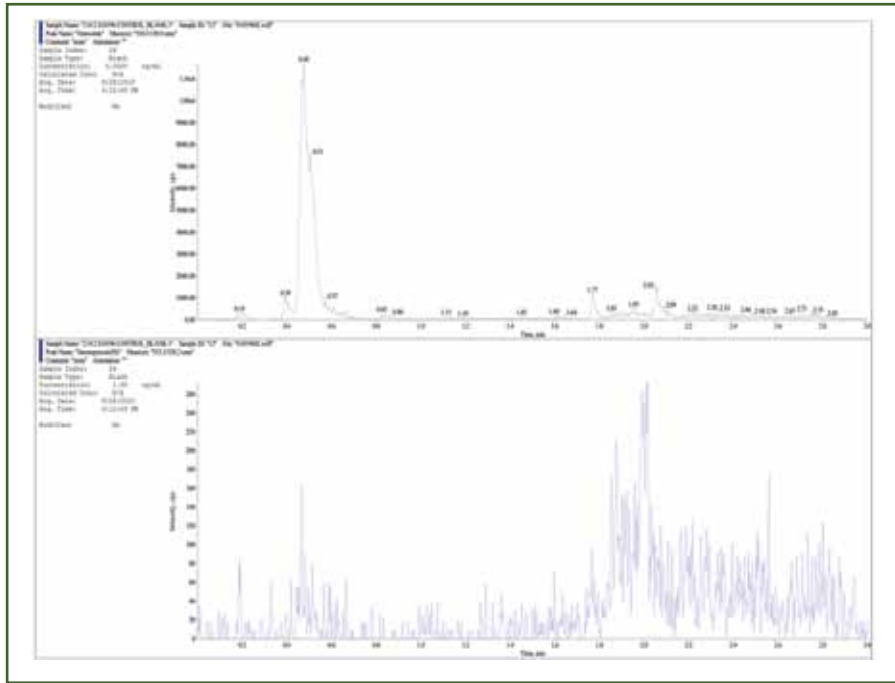
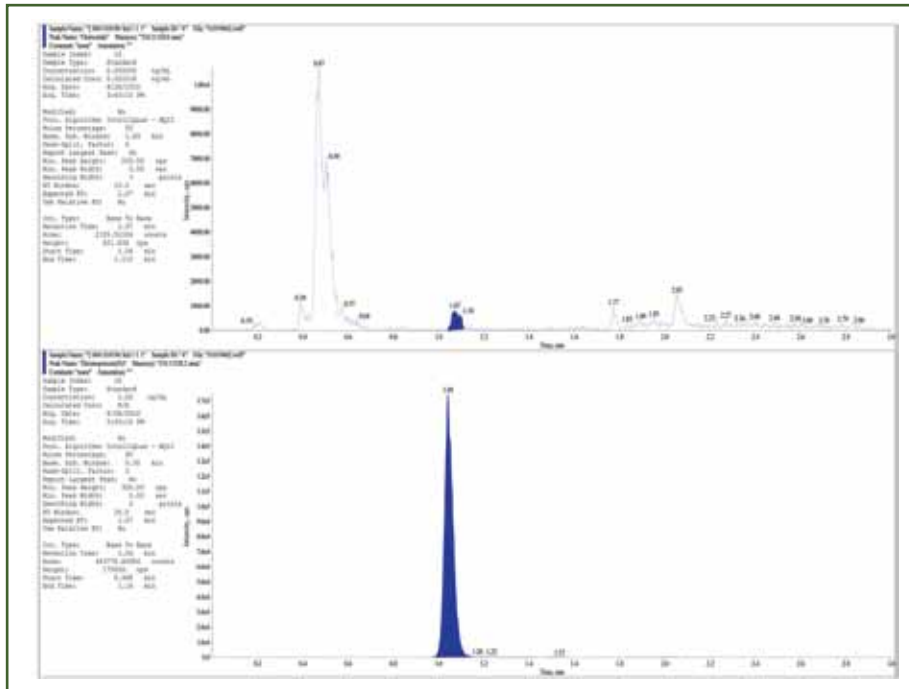


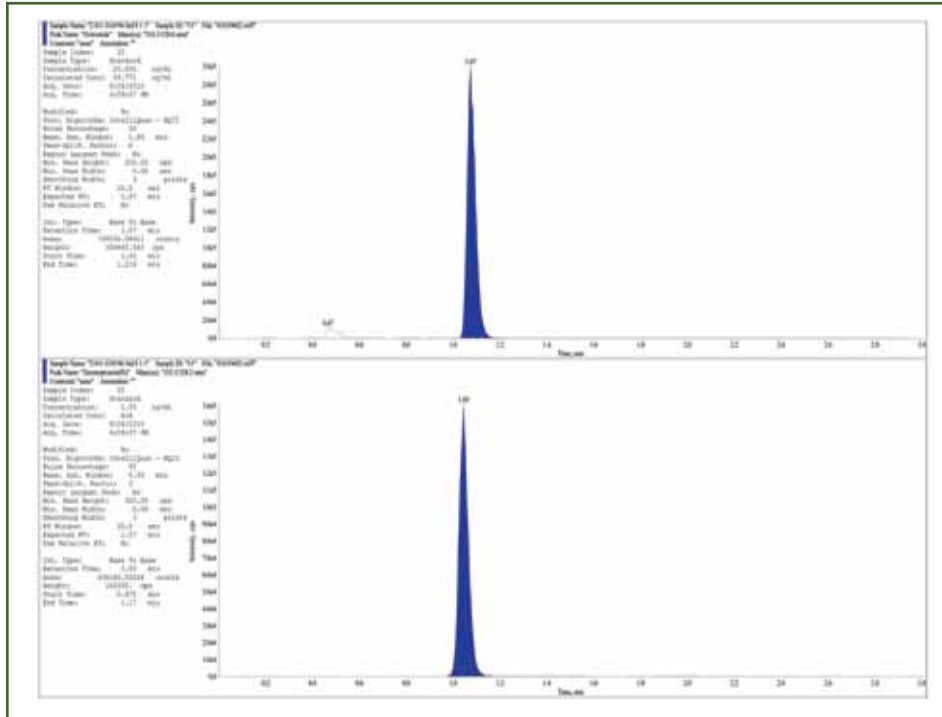
FIGURE 5. Representative Low Standard (0.0500 ng/mL)





Results and Discussion (continued)

FIGURE 6. Representative High Standard (20.0 ng/mL)



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.



Results and Discussion (continued)

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

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



Results and Discussion (continued)

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

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 Octreotide	MF Neat Octreotide	MF Extracted Desmopressin	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.