Determination of Octreotide In Human Plasma By LC-MS/MS

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Introduction

Octreotide is a synthetic cyclic octapeptide which structurally and pharmacologically resembles Somatostatin (Figure 1). Therefore it is a potent inhibitor of growth hormone, glucagon and insulin. Octreotide acetate has been used to treat the symptoms associated with metastatic carcinoid tumors or to reduce growth hormone levels in patients with acromegaly.

Octreotide quantitation in human plasma is analytically challenging because of the low abundance of this drug in plasma. Here we describe the development of an LC-MS/MS method for the determination of Octreotide in human plasma (K3EDTA) based on current analytical technologies like micro-elution solid phase extraction, coupled with enhanced chromatographic separation on an Ultra Performance Liquid Chromatography (UPLC) instrument and detection on a high-sensitivity triple quad ABSCIEX 6500 mass spectrometer.

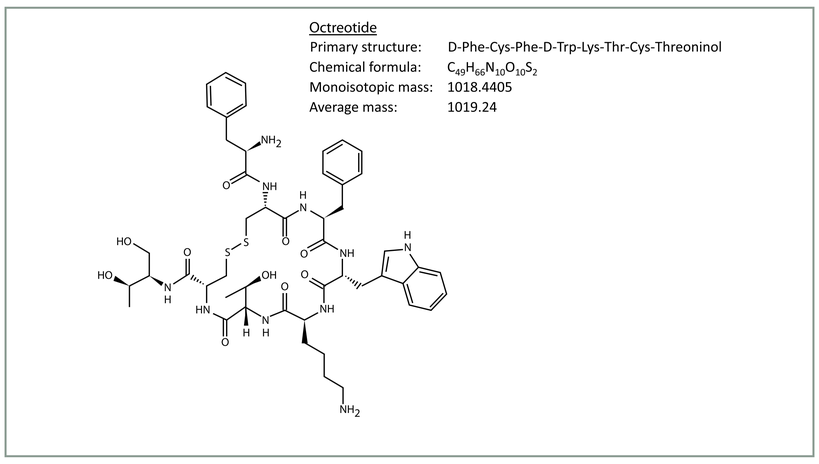

Figure 1: Main Octreotide characteristics.

Method Description

Extraction Procedure

The bioanalytical method allows the quantification of Octreotide in human plasma (K3EDTA) between 25 pg/mL and 25 ng/mL. The developed procedure is described in Figure 2. Notably, it has been found that Octreotide working solutions must be prepared in polypropylene tubes. This might be a consequence of non-specific binding.

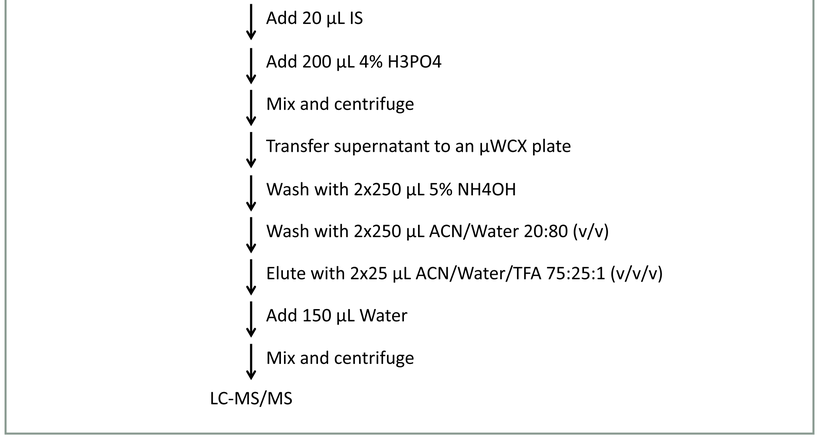

Figure 2: Procedure used to quantify Octreotide in human plasma.

LC-MS Conditions

When the doubly charged Octreotide ion is subjected to fragmentation, two main fragment ions together with the molecular ions are detected (Figure 3). The first ion corresponds to a fragment of phenylalanine (transition 510.3 → 120.2), whereas the second one corresponds to transition 510.3 → 872.1, which is almost the intact molecular ion with loss of phenylalanine. Additionally, this second ion could be also detected after water loss (510.3 → 853.9).

Both ions were initially selected to quantify Octreotide in extracted plasma samples. To reduce sample complexity, the extracts are separated on an ACQUITY UPLC BEH C18 column (2.1 x 50 mm, 1.7 um) kept at ambient temperature using a UPLC I-Class instrument. The LC gradient consisted of Water and Acetonitrile containing 0.1% Formic acid at a flow rate of 0.3 mL/min. Under such conditions, Octreotide and IS elute at approximately 2.10 and 1.95 min, respectively.

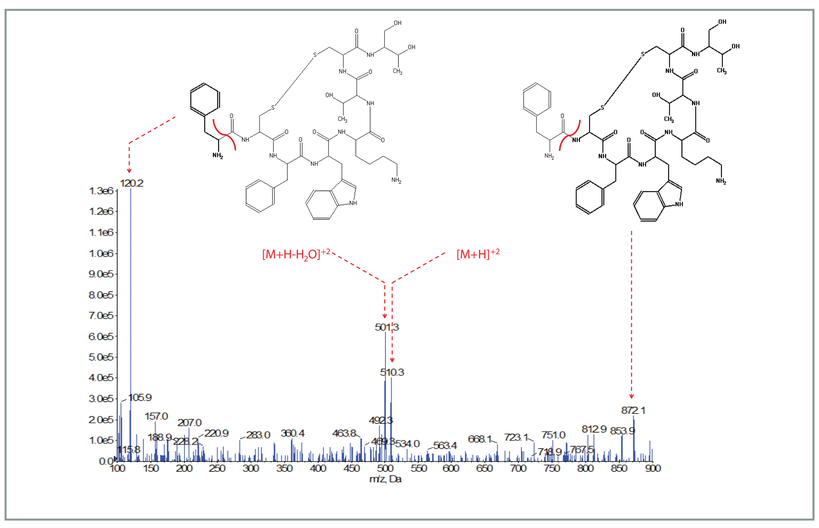

Figure 3: Fragmentation spectrum for doubly charged Octreotide ion, m/z 510.3. Two intense singly-charged fragments are observed at m/z 120.2 and m/z 872.1.

When extracted plasma samples were loaded on the BEH C18 column, transition 510.3 → 120.2 was not selective enough for a reliable quantitation, while the second transition, 510.3 → 872.1, could be resolved from interfering peaks (Figure 4A). Making the gradient slope shallower allowed to better resolve transition 510.3 → 872.1 but did not significantly improve the selectivity of transition 510.3 → 120.2 (Figure 4B). Even with a longer gradient the resolution was not sufficient to separate the two peaks at the baseline. This observation might explain why previous LC-MS methods to quantify Octreotide in plasma described in the literature, all made use of 100 or 150 mm C18 columns. Since transition 510.3 → 120.2 showed two collision energy (CE) optima (at 30 and 60 V), an attempt was made to increase CE from 30 to 60 V as this may improve the selectivity of this transition. No significant improvement could be obtained by moving to this higher CE optimum.

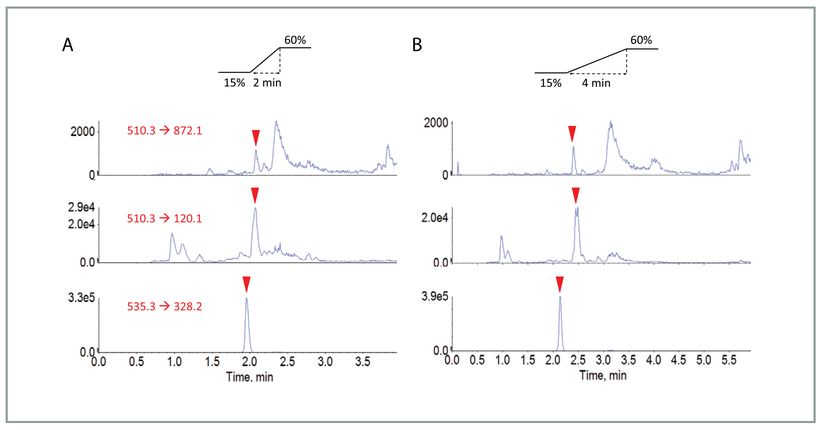

Figure 4: Impact of the LC gradient slope on the selectivity of transition 510.3 → 120.1 and 510.3 → 872.1.

Since the low mass transition was characterized by an intense signal (around 5 times more intense than the second transition), some attempts were made to improve its selectivity. For this, alternative chromatographic columns were tested (Figure 4B). Changing the analytical column to CSH C18, led to a better chromatographic separation for both transitions. However, the baseline for transition 510.3 → 120.1 remained high and both signals were generally less intense than using a BEH C18 column (Figure 5). Moreover, Trifluoroethanol (TFE), an additive that is known to slightly alter the selectivity of reverse-phase separations, was added to the mobile phases to check whether under such conditions the analyte peak for transition 510.3 → 120.1 could be separated from the interference. Interestingly, a change in selectivity with addition of 5% TFE to both mobile phases allowed to accurately measure both transitions. However, this was at the expense of IS peak shape (peak fronting).

These results show that a compromise must be taken between sensitivity and selectivity. Since a better S/N is obtained using the second transition, it was decided to continue the method based on this transition.

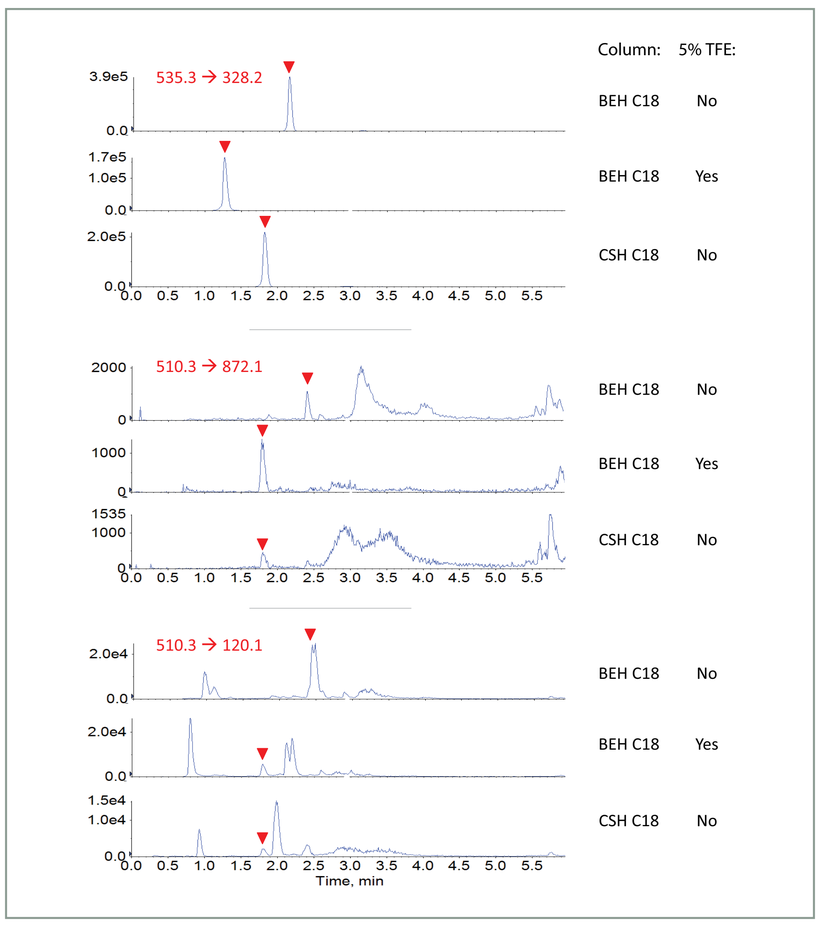

Figure 5: Impact of the use of a CSH C18 column and of the addition of TFE to mobile phases on the selectivity of transitions 510.3 → 120.1 and 510.3 → 872.1.

Method Performance

The method was subjected to an exploratory prevalidation to ensure its "validatability". This is the performance data presented here. Each prevalidation run contained duplicate standard curves and six quality control (QC) replicates spiked at LLOQ, Low, Medium and High level. Calibration curves were constructed using peak area ratios of Octreotide and IS. A linear, 1/concentration squared (1/c2) weighted regression model was applied to the calibration curve data points.

Overall good intrabatch precision and accuracy were obtained using transition 510.3 → 872.1. At LLOQ the precision was between 8.2 and 9.5% and the accuracy varied from 100.7 to 109.6% (Figure 6). No particular issues related to carry-over were observed, being always less than 10% of LLOQ (Figure 6). SPE recovery for both analyte and IS was also assessed by comparing the area response for both compounds spiked before and after SPE extraction. In such an experiment ion suppression losses are disregarded and the recovery can be considered as an "SPE extraction recovery". For IS the recovery was around 70%, while Octreotide recovery was around 55% (Figure 6). This data suggests that there might be some room for improvement of the S/N and LLOQ.

When 5 different individuals were spiked at Low and High levels, the obtained accuracies were all less than 10% from the theoretical concentration, except for individual 5 were slightly higher accuracies were observed (Figure 6). The fact that for individual 5 at both levels high accuracies were measured might be a consequence of IS ion suppression or caused by an interfering signal at the same retention time where the analyte is eluting. This second option could be excluded because the baseline for individual 5 was completely devoid of interfering peaks.

		LLOQ QC 25 pg/mL	Low QC 75 9g/mL	Medium QC 1000 pg/mL	High QC 20000 pg/mL
Run 1	Accuracy (%)	109.6	104.3	100.6	97.6
	CV (%)	8.2	3.8	1.2	1.5
	N =	6	6	6	6
Run 2	Accuracy (%)	100.7	97.5	99.8	99.1
	CV (%)	9.5	3.4	2.7	2.1
	N =	6	6	6	6
Interbatch	Accuracy (%)	104.2	100.2	100.1	98.5
	CV (%)	9.5	4.9	2.1	1.9
	N =	12	12	11	12

	Low QC		High QC	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Ind. 1	98.7	-	94.5	-
Ind. 2	94.7	-	103.5	-
Ind. 3	98.8	-	104.0	-
Ind. 4	105.7	-	109.0	-
Ind. 5	118.7	-	113.0	-
All	103.3	9.2	104.8	6.6

	Carry-over (%)	
	Min.	Max.
Run 1	3.1	7.8
Run 2	1.7	8.2

	Recovery (%)	
	Low QC	High QC
Run 1	52.1	55.0
Run 2	73.5	71.2

Figure 6: Summary of the main prevalidation tests.

However, when the IS and analyte area for the five individuals were plotted it was evident that for individual 5 the IS signal was lower than in the four other individuals, which might be due to ion suppression. This particular observation, if confirmed in other individual plasma samples, suggests that it might be crucial to incorporate in the existing methodology the use of a stable labeled IS instead of the existing IS.

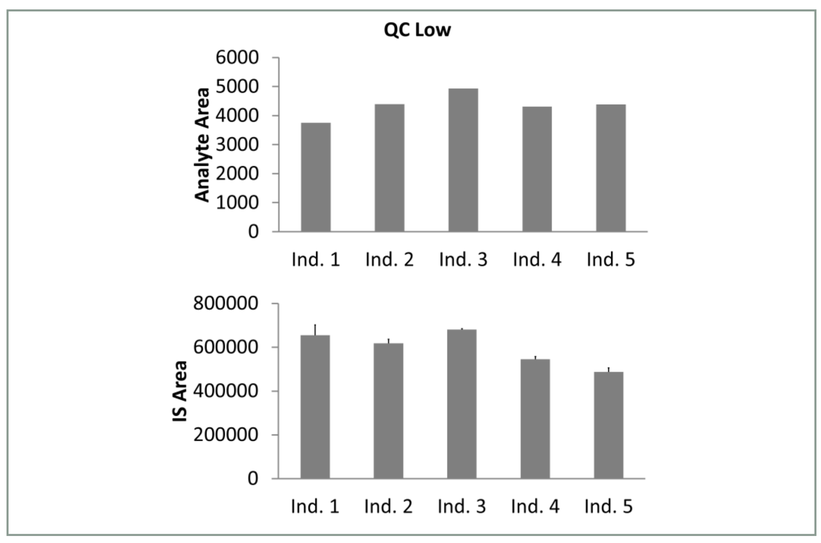

Figure 7: Evidence that IS ion suppression in individual 5 is the cause for the higher accuracy observed during prevalidation.

Conclusions

The use of a high sensitivity MS instrument together with an enhanced chromatographic separation allows to accurately and precisely determine Octreotide in human plasma between 25 pg/mL and 25 ng/mL. There were two possible options to quantify this peptide in plasma, which are based on two different and complementary MS transitions. One transition suffers from selectivity but is more intense once the signal is separated from the background. This can be achieved by using a CSH C18 column or adding TFE to the mobile phases. The second transition, which takes advantage of a high mass fragment, is more selective but not as intense. This second transition was chosen for the final method. Combining current best practice in sample preparation, chromatographic separation and LC-MS/MS detection resulted in a robust, selective and sensitive method suitable for the application to clinical studies.

Poster presentation at EBF 7th Open Meeting, November 19-21 2014