

Development of an HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of a Polypeptide Proteinase Inhibitor from Plasma

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Overview

Purpose – Develop an HPLC/MS/MS method to determine concentrations of Eglin C, a 70 amino acid polypeptide in plasma when typical liquid-liquid and precipitation methods are unsuccessful

Methods – 96 well-plate extraction and HPLC/ESI/MS/MS (API3000)

Results – Range from 0.25 to 100 µg/mL with accuracies and precision better than ±10%

Introduction

Eglin C is a polypeptide of 70 amino acids. Eglin C is a member of the family of serine proteinase inhibitors that have been shown to have therapeutic value. The use of peptides as therapeutic agents or as delivery agents for drugs and drug-peptide conjugates is becoming more common. Thus the quantitative measurement of the peptide from biological fluids is important to obtain pharmacokinetic data at low levels of detection. However, unlike small molecules, traditional liquid-liquid or precipitation methods often lead to poor recovery and precision for the extraction and HPLC/MS/MS analysis of peptides from plasma. Therefore, to obtain quality data, significant method development was warranted to develop a successful extraction technique used in combination with HPLC/MS/MS.

Sequence and Structure of the Polypeptide, Eglin C

TEFGSELKSFPEVVGKTVDDQAREYFTLHYPOYDV
YFLPEGSPVTLDLRINRVRVFPNGTINVVNHVPRVG

Approximate Molecular Weight
8090 amu



Methods

Extraction

Extraction of Eglin C or other polypeptides from 200 µL of rat plasma in 96 well plates
Investigate several methods of extraction including acetonitrile precipitation and 96 well solid phase extraction

- o Acetonitrile
- o 96 well plate SPE extraction: Strata-X

(Phenomenex) with various elution solvents
Extract, transfer aliquot, evaporate and reconstitute in aqueous mobile phase

HPLC

Gradient from 30% to 80% organic in 2.5 minutes and ramp to 90% organic at 3 minutes. Hold for 2.5 minutes.

Flow rate = 0.25 mL/minute
1% formic acid in MeOH and Water
Waters, Xterra MS C18, 5 µm 2.1 x 50 mm column
Thirty µL injections

Mass Spectrometry

Sciex API3000 operating in MRM mode
Turboionspray (400 °C)
Positive ion mode
MRM transitions for Eglin C–
•1350 (M+6H)⁶⁺ →578

Table 1. Development of an Extraction Method for the HPLC/MS/MS Analysis of Eglin C from Plasma

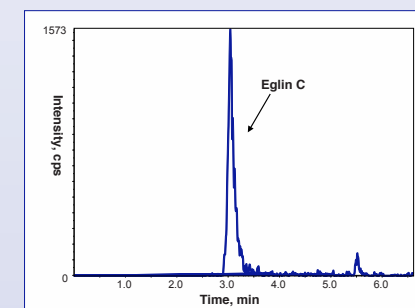
Extraction Procedure	% Recovery
Acetonitrile Precipitation	<10
Strata X elution with acetonitrile	61
Strata X elution with 1% formic acid in acetonitrile	69
Strata X elution with 5% formic acid in acetonitrile	81

* Final SPE Method: Condition plate with 1% formic acid/MeOH followed by 1% formic acid/water. Add 200 µL of plasma with 200 µL of 1% formic acid in water to well. Allow to gravity drip for one minute and vacuum for one minute. Elute into a clean 96 well plate with 0.75 mL of 5% formic acid in ACN (2 times).

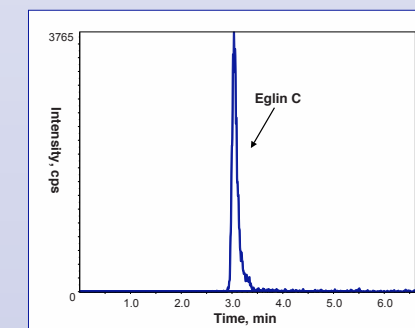
Table 2. Standard Curve Results for the HPLC/MS/MS Analysis of Eglin C from Plasma.

Standard Curve Level (µg/mL)	Calculated Concentration (µg/mL)	% Accuracy
0.250	0.245	97.9
0.250	0.262	105
0.500	0.607	121
0.500	0.410	81.9
1.00	0.811	81.1
1.00	1.03	103
2.5	2.38	95.1
2.5	2.44	97.5
5.0	5.32	106
5.0	5.08	102
7.5	7.23	96.4
7.5	6.96	92.8
10	10.9	109
10	10.1	101
25	26.2	105
25	27.5	110
50	53.0	106
50	49.1	98.2
100	101	101
100	83.7	83.7

HPLC/MS/MS Chromatogram from the Analysis of a Standard Fortified with Eglin C at 0.25 µg/mL and Extracted from Rat Plasma



HPLC/MS/MS Chromatogram from the Analysis for Eglin C from a Rat Plasma Sample 2 Hours Post-Dose



Conclusions

Developed HPLC/MS/MS method to quantify Eglin C from rat and mouse plasma
Found that a polymer type SPE 96 well plate extraction (Strata X) gave best recoveries compared to other extractions
Method supports PK studies for Eglin C and other similar polypeptides