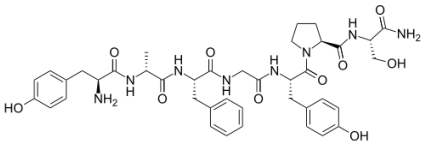
The Analysis of Dermorphin, an Opioid Peptide in Equine Plasma using the EVOQ Elite.

Abstract

Dermorphin, a naturally occurring hepta-peptide, is a potent analgesic, approximately 40x more potent than morphine and a class I prohibited substance in horse racing. Dermorphin was quantified in equine plasma using solid phase extraction cleanup. The linear dynamic range of the assay was 0.5-1000ng/mL and was easily achieved on the EVOQ Elite LC-TQ.

Introduction

Dermorphin is an opioid hepta-peptide derived from the skin secretions of South American frogs (Phyllomedusa sauvagei). It is 30-to-40 times more potent an analgesic than morphine[1] and is unique because it contains D-alanine, a property that makes it highly resistant to protease mediated degradation. While d-amino acid peptides are sought after for their low immunogenic response and in peptidomimetic drug design, it was dermorphin's use in horse racing that catapulted this opioid peptide from obscurity into the headlines. The pain-killing properties of dermorphin were used to drive race horse performance beyond normal physical limits, often injuring the animal. As a result, dermorphin is now a class I prohibited substance as decreed by the Association of Racing Commissioners International (RCI) Model Rules. The analysis of dermorphin in equine plasma is a challenging assay because of the associated complex matrix and lack of a stable labeled internal standard.



Structure of Dermorphin

Experimental

Chromatography (Advance HPLC)

- Column: C18, 3µ, 2.1x100mm (ACE 3µ, C18, 100x2.1mm)
- Injection volume: 40µL
- Flow rate: 0.4mL/min
- Mobile phase A: Water with 0.2% Formic acid
- Mobile phase B: Acetonitrile with 0.2% Formic acid
- Gradient conditions:
  - 0.00min 5% B
  - 0.20min 5% B
  - 8.00min 95% B
  - 8.50min 95% B
  - 8.51min 5% B
  - 12.50min 5% B

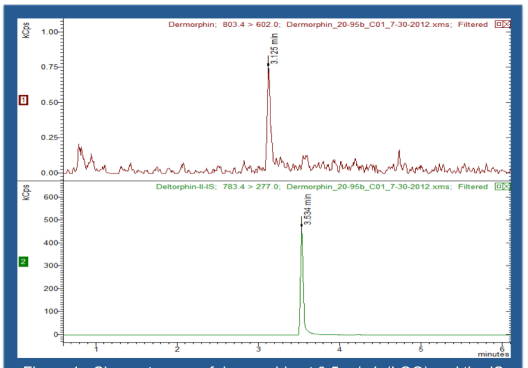


Figure 1. Chromatogram of dermorphin at 0.5ng/mL (LOQ) and the IS deltorphin (green trace) in equine plasma.

Mass Spectrometry (EVOQ Elite)

- VIP Heated-ESI Temp: 350°C
- Heated gas: 70 units
- Nebulizer gas: 70 units
- Cone gas temp: 250°C
- Cone gas: 10 units
- Spray voltage: 4000v
- Active exhaust: On
- Dermorphin ($C_{40}H_{50}N_8O_{10}$) transitions
  - m/z 803→202 (CE:32v, quantifier ion)
  - m/z 803→602 (CE:20v)
  - m/z 803→325 (CE:40v)
  - m/z 803→352 (CE:35v)

- Deltorphin-II (IS) transitions
  - m/z 783→277 (CE:40v)
  - m/z 783→120 (CE:65v)
  - m/z 783→235 (CE:45v)

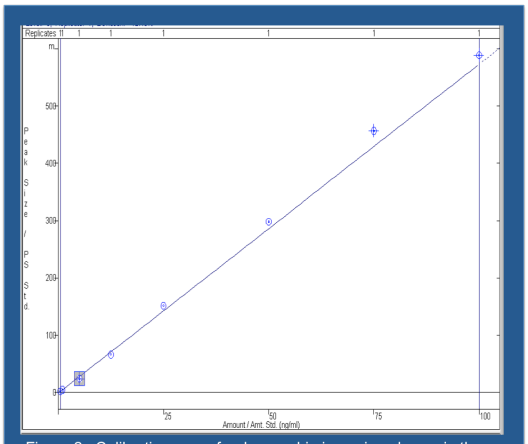


Figure 2. Calibration curve for dermorphin in equine plasma in the range 0.5-100ng/mL.

Results and Discussion

Accurate quantification of dermorphin in equine plasma between the range of 0.5-1000ng/mL in equine plasma was easily achieved on the EVOQ Elite. Figure 1 shows the S/N of the LLOQ of 0.5ng/mL dermorphin in equine plasma. The calibration curve (Fig 2) had a response factor relative standard deviation (RSD) of 12% RSD. The response factors for the QC's had a RSD of 7% RSD are shown in the table 1. The ion source on the EVOQ Elite easily met the LLOQ requirements for dermorphin in equine plasma without the use of a divert valve.

Table 1: Response factor %RSD for the QC

QC	Area Ratio	Response Factor
Low QC	0.01000	0.03335
Low QC	0.01031	0.03438
Mid QC	0.22314	0.03719
Mid QC	0.17834	0.02972
High QC	1.67945	0.03359
High QC	1.62102	0.03242

References:

1. Broccardo, M et. al. Br. J. Pharmac.1981, 73, 625-631.