Liquid Chromatography-Tandem Mass Spectrometry for the Determination of Nortestosterone Glucuronide in Rainbow Trout Muscle

Pak-Sin Chu[1], Eric Evans[2], and Sara Sklenka[1], FDA Center for Veterinary Medicine
Office of Research, Division of Residue Chemistry[1] and Division of Applied Veterinary Research[2], Laurel, MD 20708

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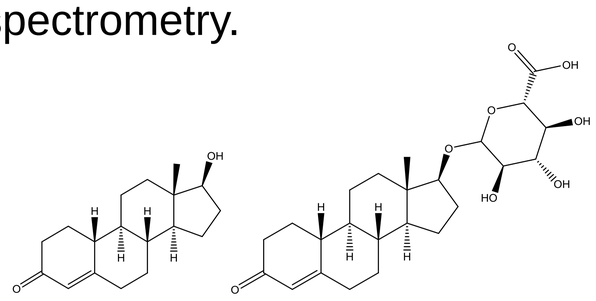
Introduction

- 19β-nortestosterone (NT) is a steroid hormone that may be used in food-producing animals to improve meat quality and feed efficiency.
- It is not approved for use in the US and has been banned for use in many countries.
- Illegal use of NT is of regulatory concern because it may lead to harmful residues in edible tissues destined for human consumption.
- Conventional analytical methods involve analysis of the parent NT, either with or without enzyme deconjugation.
- A faster and more direct method is needed.

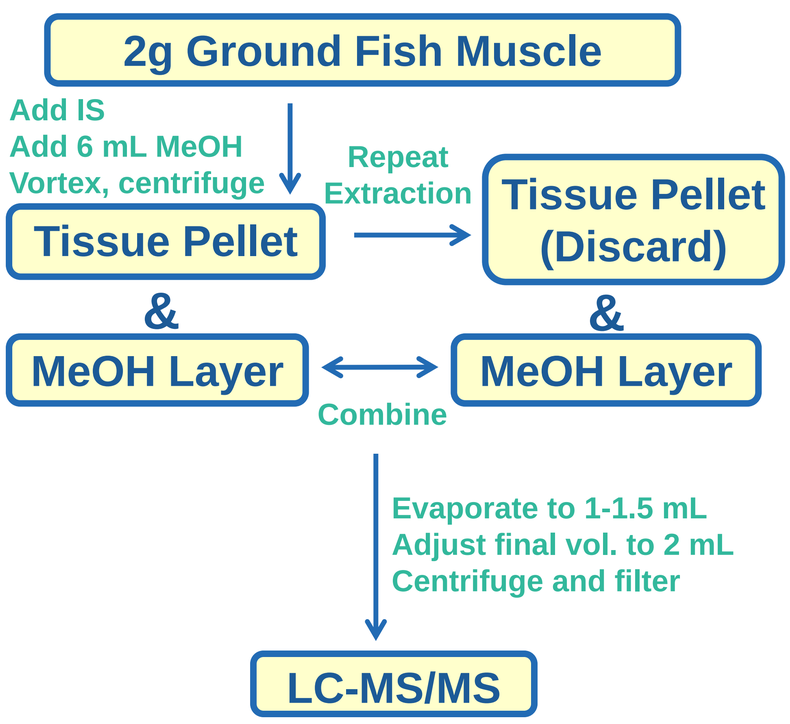
Objective

- To develop an analytical method for the determination of the intact nortestosterone glucuronide (NT-GLU) in rainbow trout muscle at 2 ng/g (ppb) using liquid chromatography-tandem mass spectrometry.



Methodology

Extraction



Instrumentation

LC Conditions

HPLC: Shimadzu Nexera
Flow rate: 0.4 mL/min
Column: Luna C18(2), 3 µm, 100 x 2.0 mm
Mobile phase A (MPA): 0.1% formic acid
Mobile phase B (MPB): acetonitrile

Gradient Table

Time (min)	0	0.5	3.5	5	7	7.1	12
MPA (%)	95	95	60	20	20	95	95
MPB (%)	5	5	40	80	80	5	5

MS Conditions

MS: AB Sciex QTRAP 6500
Probe: TurboIonSpray, Negative Ion
NT-GLU: *m/z* 449 → 85,113*, 271, 273
TEST-GLU: *m/z* 463 → 85, 113*, 285, 287
*Quantitative ion

Relevance to FDA's Mission

- Provides FDA with a fast and direct tool to monitor NT-GLU residues in fish.
- Helps FDA to ensure that the US seafood supply is safe for human consumption.

Disclaimer

- The views expressed in this poster are those of the authors and do not necessarily reflect the official policy of the Department of Health and Human Services, the US Food and Drug Administration, or the US Government.
- The experimental protocol was approved by the IACUC at the Office of Research, Center for Veterinary Medicine, US Food and Drug Administration, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (2011) and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.
- Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the US Food and Drug Administration.

Results

Ion chromatograms of NT-GLU and internal standard (testosterone-glucuronide, TEST-GLU) extracted from rainbow trout muscle. (a) 2 ng/g standard, (b) control, (c) control fortified at 2 ng/g, and (d) incurred.

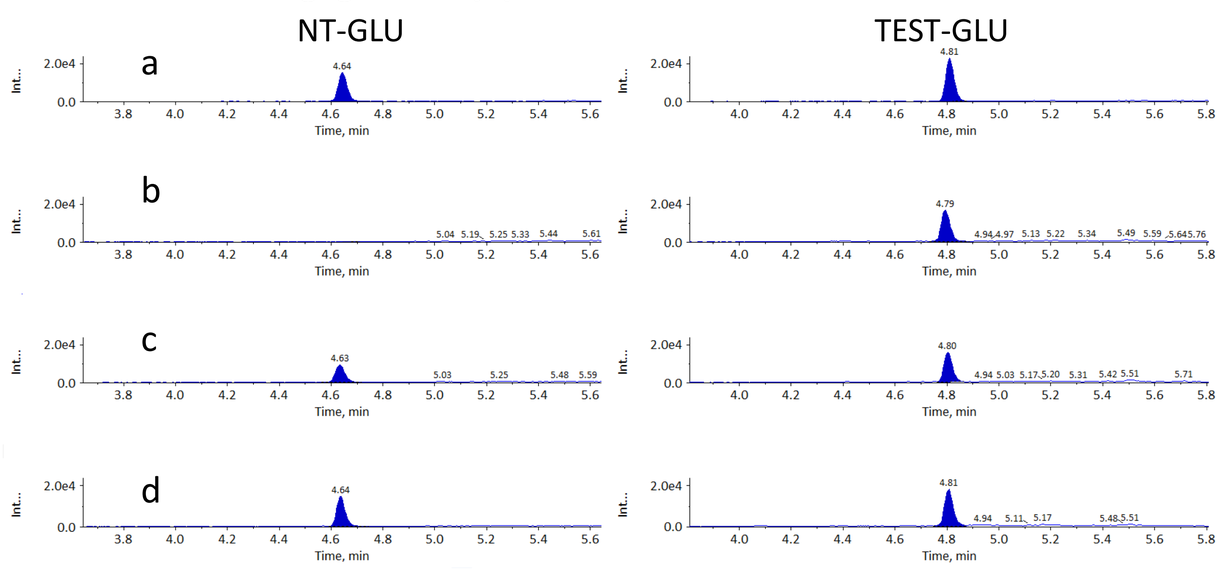


Table 1. Method Performance for Fortified Samples

	External Standard		Internal Standard	
Fortification Level (ng/g)	Recovery (%)	CV (%)	Accuracy (%)	CV (%)
1	75.9	7.9	104	7.4
2	70.5	9.1	102	7.5
4	70.4	9.5	96.9	6.0

N=6

Table 2. Method Performance for Incurred Samples

	External Standard		Internal Standard	
Sample	Conc. Found (ng/g)	CV (%)	Conc. Found (ng/g)	CV (%)
Incurred Level 1	0.68	9.3	1.01	4.0
Incurred Level 2	1.69	4.6	2.21	6.8

Conclusions

- The method meets the accuracy and precision performance criteria set forth by the FDA CVM guidelines for method development.
- NT-GLU is detectable in muscle of rainbow trout dosed with NT.