Application Note: 480

Detection of Six Zeranol Residues in Animal-derived Food by HPLC-MS/MS

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Key Words

- Food residue analysis
- Veterinary drugs
- TSQ Quantum
- SRM (Selective Reaction Monitoring)

Introduction

Zeranol is a non-steroidal estrogenic growth stimulator that is widely used in food-producing animals in the United States and other countries. A synthetic derivative of the mycotoxin zearalenone, zeranol is a potential endocrine disrupter that may have adverse effects on humans, such as birth defects and reproductive disorders, because of its hormone-like properties.[1] In addition, zeranol residues in animal-derived food may increase human breast cancer risks by acting like estrogen in the body and stimulating estrogen-modulated genes that promote carcinogenesis.[2] The use of zeranol in the livestock industry may also cause secondary pollution and environmental contamination of drinking water and foods.

Because of the potential health concerns, the use of zeranol and other anabolic growth promoters in food animals is banned in the member states of the European Union (EU)[3], China[4], and other countries. Therefore, highly sensitive and specific methods with low levels of detection are needed to analyze zeranol residues in edible tissues. Here an LC-MS/MS method is established to detect zeranol (α-zearalanol) and its main metabolites, β-zearalanol and zearalanone, and the mycotoxin zearalenone and its metabolites, α-zearalenol and β-zearalenol, in animal-derived foods, such as pork, beef, lamb, chicken liver, milk, and eggs.

Goal

To develop an effective LC-MS/MS method to detect six zeranol residues in animal-derived foods.

Experimental Conditions

Sample Preparation

A 5 g tissue sample, taken from about 500 g muscle or liver, 10 eggs, or 500 mL milk, was weighed into a 50 mL centrifuge tube and 10 mL acetonitrile was added. The sample was vortexed for 1 minute and then centrifuged at 5000 rpm for 10 minutes. The supernatant was transferred into another 50 mL centrifuge tube. The sample was extracted again with 10 mL acetonitrile. The two supernatants were combined and evaporated to dryness at 50 °C. The residue was dissolved in 3.0 mL 0.1 M NaOH and the pH was adjusted to 11.0. The solution was loaded to the MCX cartridges SPE column. The SPE column was conditioned with 2 mL of both methanol and water. After drying, the analytes were eluted with 3 mL 5% formic acid methanol solution and evaporated to dryness under nitrogen at 50 °C. The residues were reconstituted in 1.0 mL 20% acetonitrile aqueous solution. The resulting solutions were vortexed for 1 minute and then centrifuged at 10,000 rpm for 10 min. The upper clear solutions were transferred to another sample vial for LC-MS/MS analysis.

LC

HPLC analysis was performed using the Thermo Scientific Surveyor HPLC system. Each 10 µL sample was injected onto a Thermo Scientific Hypersil GOLD 150 mm x 2.1 mm, 5 µm column. A gradient LC method used mobile phases A (water with 0.1% formic acid) and B (acetonitrile) at a flow rate of 250 µL/min. Table 1 illustrates the gradient LC method.

Table 1. Gradient details

Retention Time (min)	A (%)	B (%)
0	70	30
5	70	30
8	10	90
12	10	90
12.1	70	30
14	70	30

MS

MS analysis was carried out on a Thermo Scientific TSQ Quantum triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) probe. The MS conditions were as follows:

Ion source polarity:	Negative ion mode
Spray voltage:	3500 V
Sheath gas pressure (N_2):	40 units
Auxiliary gas pressure (N_2):	8 units
Capillary temperature:	350 °C
Collision gas pressure (Ar):	1.5 mTorr

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The SRM transitions that were monitored are summarized in Table 2.

Table 2. SRM transitions

Drug	Parent Ion (m/z)	Product Ions (m/z) [Collision Energy (V)]
α-zearalanol	321	277 [24], 303 [24]
β-zearalanol	321	277 [24], 303 [24]
α-zearalenol	319	205 [22], 275 [24], 301 [26]
β-zearalenol	319	205 [22], 275 [24], 301 [26]
Zearalanone	319	205 [22], 275 [24], 301 [26]
Zearalenone	317	175 [26], 273 [20]

Results and Discussion

Figure 1 displays the SRM chromatograms for the six zeranol residues. The limits of detection (LOD) for all six zeranol residues in animal-derived foods are 0.1 µg/kg. The limits of quantitation (LOQ) of these residues are 1.0 µg/kg. These LOQs easily meet the specified MRLs of the European Union and China of 2 µg/kg in meat and 10 µg/kg in liver.[5]

The extraction recovery of zeranol is between 65% and 115% and achieves the minimum detection requirements. Thus, the qualification method is accurate and reproducible.

Conclusion

The LC-MS/MS method described here is able to detect zeranol residues from animal-derived foods, such as pork, beef, lamb, chicken liver, milk, and eggs. The method yielded high recovery rates and enabled the accurate quantification of the residues. The sensitivity, extraction recovery, and reproducibility of this method meet international regulation and detection requirements.

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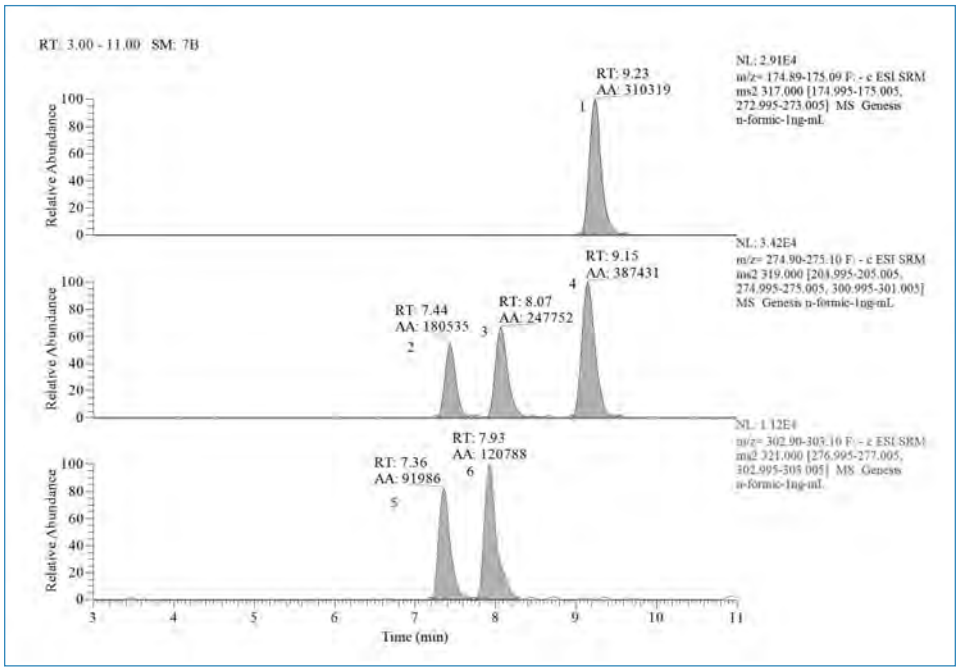

Figure 1. SRM chromatograms for the six zeranol residues. The LODs for all six zeranol residues in animal-derived foods are 0.1 µg/kg.

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AN62968_E 10/09S