

# Detection of Six Zeranols 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

Zeranols are non-steroidal estrogenic growth stimulators that are widely used in food-producing animals in the United States and other countries. A synthetic derivative of the mycotoxin zearalenone, zeranols are potential endocrine disruptors that may have adverse effects on humans, such as birth defects and reproductive disorders, because of their hormone-like properties.<sup>1</sup> In addition, zeranols 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.<sup>2</sup> The use of zeranols 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 zeranols and other anabolic growth promoters in food animals is banned in the member states of the European Union (EU)<sup>3</sup>, China<sup>4</sup>, and other countries. Therefore, highly sensitive and specific methods with low levels of detection are needed to analyze zeranols residues in edible tissues. Here an LC-MS/MS method is established to detect zeranols ( $\alpha$ -zearalanol) and its main metabolites,  $\beta$ -zearalanol and zearalenone, and the mycotoxin zearalenone and its metabolites,  $\alpha$ -zearalanol and  $\beta$ -zearalanol, 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 zeranols 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  $\mu$ L sample was injected onto a Thermo Scientific Hypersil GOLD 150 mm x 2.1 mm, 5  $\mu$ m column. A gradient LC method used mobile phases A (water with 0.1% formic acid) and B (acetonitrile) at a flow rate of 250  $\mu$ L/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 <sub>2</sub> ):	40 units
Auxiliary gas pressure (N <sub>2</sub> ):	8 units
Capillary temperature:	350 °C
Collision gas pressure (Ar):	1.5 mTorr

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)]
$\alpha$ -zearalanol	321	277 [24], 303 [24]
$\beta$ -zearalanol	321	277 [24], 303 [24]
$\alpha$ -zearalenol	319	205 [22], 275 [24], 301 [26]
$\beta$ -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 zearanol residues. The limits of detection (LOD) for all six zearanol residues in animal-derived foods are 0.1  $\mu\text{g}/\text{kg}$ . The limits of quantitation (LOQ) of these residues are 1.0  $\mu\text{g}/\text{kg}$ . These LOQs easily meet the specified MRLs of the European Union and China of 2  $\mu\text{g}/\text{kg}$  in meat and 10  $\mu\text{g}/\text{kg}$  in liver.<sup>5</sup>

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

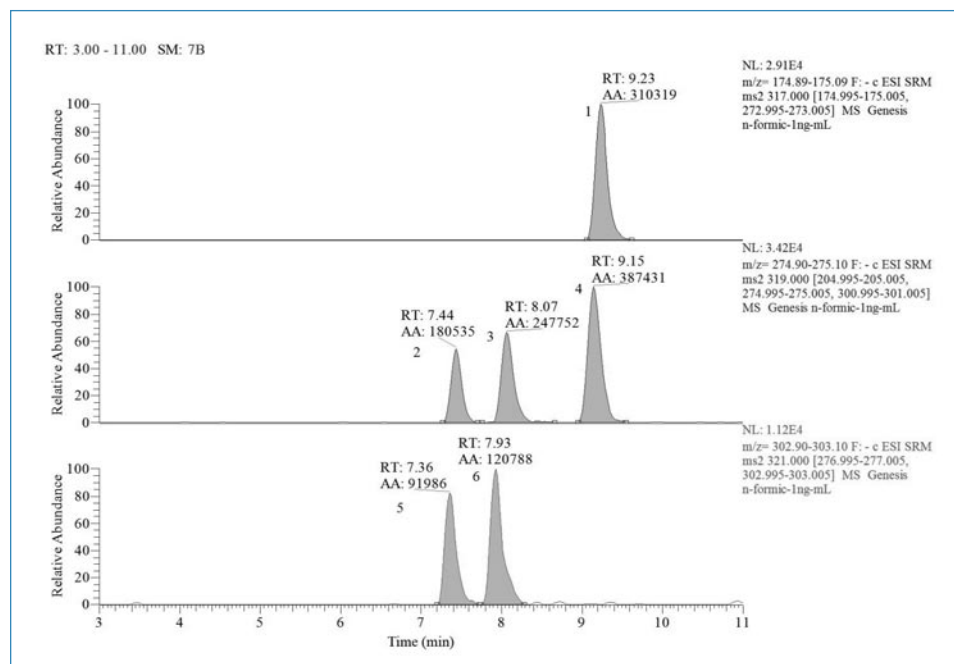


Figure 1. SRM chromatograms for the six zearanol residues. The LODs for all six zearanol residues in animal-derived foods are 0.1  $\mu\text{g}/\text{kg}$ .

## Conclusion

The LC-MS/MS method described here is able to detect zearanol 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.

## References

- Leffers, H; Næsby, M; Vandelbo, B; Skakkebæk, N; Jørgensen, M "Oestrogenic potencies of zearanol, oestradiol, diethylstilboestrol, bisphenol-A and genistein: implications for exposure assessment of potential endocrine disrupters"; *Human Reproduction* 2001, 16(5) 1037-1045.
- Liu, S; Lin, YC. Transformation of MCF-10A human breast epithelial cells by zearanol and estradiol-17beta. *Breast J.* 2004, 10(6), 514-21.
- European Community, Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC, (Official Journal of the European Communities Official Journal L 125, 23 May 1996, pp. 10-32).
- Ministry of Agriculture, P.R. China. Maximum Limit Standard of the Veterinary Drugs in Animals. Regulation 235, 2002.
- European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain on a request from the European commission related to hormone residues in bovine meat and meat products. *The EFSA Journal* 2007, 510, 1-62.

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