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QuEChERS Sample Preparation for LC-MS/MS Determination of Steroid Hormones in Meat and Milk

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Abstract

In this application note, milk and ground beef are prepared and analyzed for steroid hormones at the ppb level, using QuEChERS methodology and UPLC-MS/MS.

Benefits

· Simple, high throughput preparation of meat and milk samples using QuEChERS methodology

· High sensitivity UPLC-MS/MS analysis of steroids in food products with fast and easy sample preparation

· Achieve parts per billion (ppb) level detection of steroids in complex livestock matrices

· Sample preparation using DisQuE products conforms to CEN method 15662

Introduction

Progesterone and testosterone are naturally occurring substances that are used worldwide as growth

promoters in livestock. Dexamethasone is an anti-inflammatory glucocorticoid steroid drug that is commonly administered simultaneously with natural hormones. Maximum residue limits (MRL) for these compounds can vary worldwide, but are generally in the low ppb concentration range. At these concentrations, the analysis of steroids in products such as milk and meat is often very challenging due to the complexity of the samples.

QuEChERS is a simple and straightforward sample preparation technique that involves a salting-out liquid extraction followed by optional dispersive solid phase extraction (d-SPE). Sample preparation using QuEChERS allows for fast throughput and high sensitivity in food analysis. Although QuEChERS is commonly used for multi-residue pesticide analysis in fruits and vegetables, it is also applicable in the analysis of steroids in livestock products. In this application note, milk and ground beef are prepared and analyzed for steroid hormones at the ppb level, using QuEChERS methodology and UPLC-MS/MS.

Experimental

LC conditions

System:	ACQUITY UPLC System
Column:	ACQUITY BEH C_{18}, 2.1 x 100 mm (1.7 μm)
Part Number:	186002352
Injection Volume:	3 μL
Temperature:	40 ° C
Mobile Phase:	A: Water
	B: Methanol
Flow Rate:	0.40 mL/min

| Gradient: | 30% B initial, linear gradient to 97% B in 5 minutes, hold until 8 minutes, back to 30% B at 8.1 minutes. Hold and re-equilibrate until 10 minutes. |

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Sample Vials: Maximum Recovery Vial

Part Number: 600000670 CV

MS conditions

System: Xevo TQ-S Mass Spectrometer

Ionization Mode: Electrospray positive (ESI+)

The MRM transitions, cone voltages, and collision cell energies optimized for steroid hormones in this study are presented below.

Compound Name	MRM Transition (m/z)	Cone (V)	Collision (eV)
Testosterone	289 > 97	15	23
Progesterone	315 > 97	20	20
Dexamethasone	393 > 373	10	7

Table 1: MRM transitions.

Sample preparation

Initial Extraction (QuEChERS):

Place 10 mL whole milk (pasteurized) or 10 g ground beef (85% lean) into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE pouch salts for European Committee for Standardization (CEN) QuEChERS (part number: 186006813) and shake vigorously for 1 minute. Centrifuge for 3 minutes @ 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2 mL d-SPE cleanup tube that contains 150 mg of magnesium sulfate, 50 mg PSA sorbent and 50 mg C_{18} sorbent (part number: 186004830) and shake vigorously for 1 minute. Centrifuge for 3 minutes at 4000 rpm and take a 0.5 mL aliquot as a sample for UPLC-MS/MS analysis.

Results and Discussion

Recovery was measured for each steroid hormone at both low and high concentration levels (Table 2). Recovery was calculated by comparing the MRM peak area for samples spiked prior to QuEChERS extraction (pre-spiked samples) with the MRM peak area for samples spiked after QuEChERS extraction (post-spiked samples). Table 3 shows the calculated matrix effect for each compound. Matrix effects were calculated by comparing the MRM peak area of post-spiked samples with the MRM peak area for equivalent standards prepared in acetonitrile. Figures 1 and 2 show UPLC-MS/MS chromatograms obtained from the analysis of meat and milk samples, respectively, spiked with the low levels of steroid hormones.

| Conc. Level | Concentration Range (ppb) | | Average % Recovery (%RSD) n=5 | | | |
| | | | Ground Beef | | Whole Milk | |
	Low Level	High Level	Low Level	High Level	Low Level	High Level
Testosterone	0.5	5	87(2.5)	89(2.2)	89(5.4)	95(1.1)
Progesterone	50	100	91(1.9)	92(3.7)	94(1.4)	95(0.8)
Dexamethasone	10	50	86(4.3)	91(3.2)	90(3.9)	91(4.4)

Table 2. Recoveries of steroid hormones from ground beef and whole milk samples.

| Conc. Level | Concentration Range (ppb) | | Matrix Effect (%)* n=5 | | | |
| | | | Ground Beef | | Whole Milk | |
	Low Level	High Level	Low Level	High Level	Low Level	High Level
Testosterone	0.5	5	103	98	94	92
Progesterone	50	100	105	95	87	83
Dexamethasone	10	50	104	102	98	97

Table 3. Matrix effects of steroid hormones from ground beef and whole milk samples.

* Matrix effect (%) = peak area (post-spiked sample)/peak area (standard) x 100

A value > 100% indicates ionization enhancement

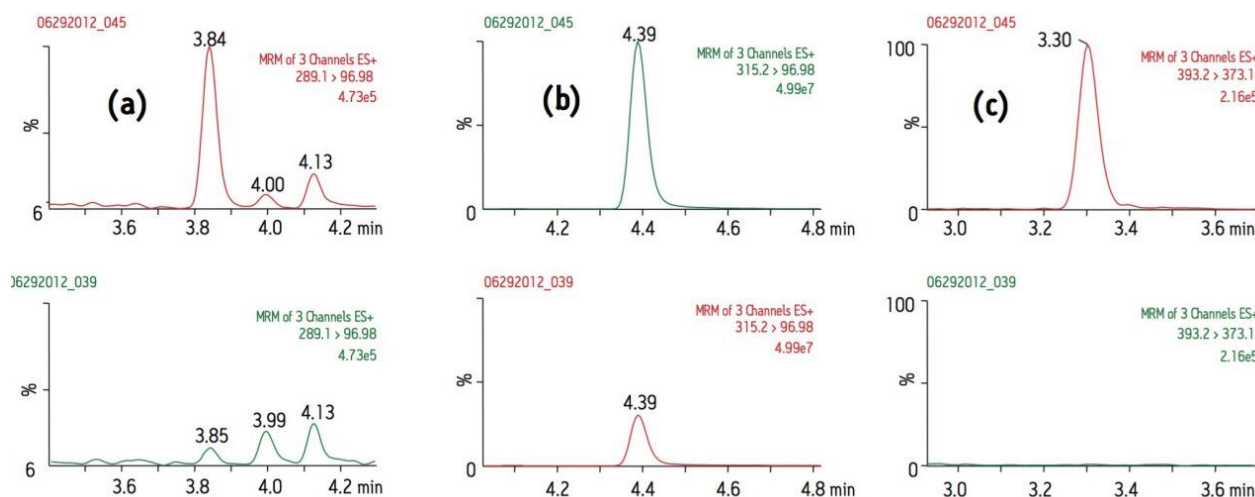A value < 100% indicates ionization suppression

Figure 1. UPLC-MS/MS chromatograms of steroids obtained from ground beef samples; the top trace is the low level spiked sample, the bottom trace is a ground beef blank. (a) testosterone (b) progesterone (c) dexamethasone.
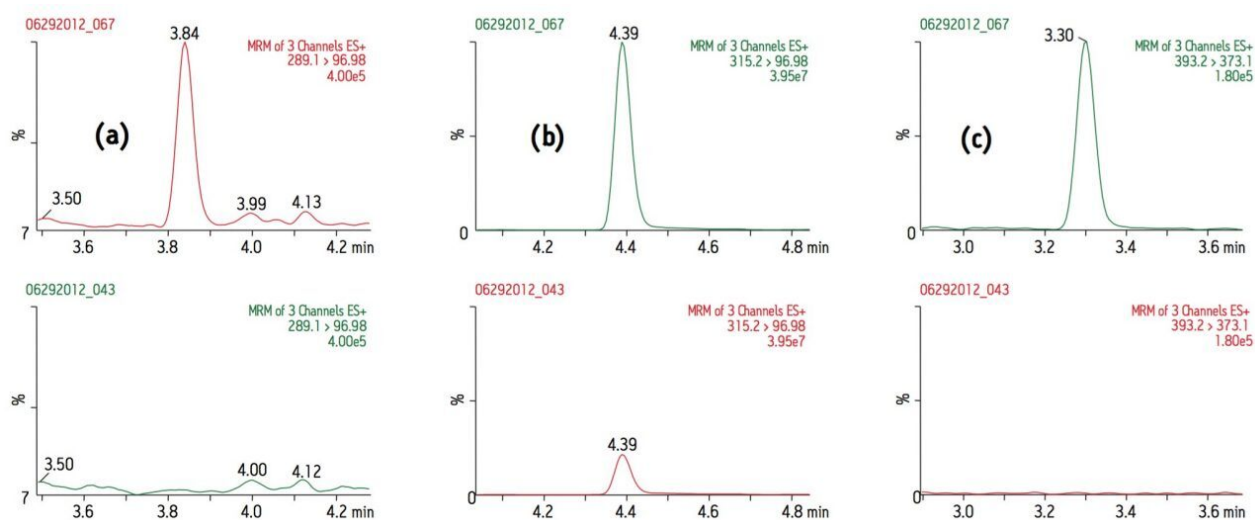
Figure 2. UPLC-MS/MS chromatograms of steroids obtained from milk samples; the top trace is the low level spiked sample, the bottom trace is a milk blank. (a) testosterone (b) progesterone (c) dexamethasone.

Discussion

For the analysis of progesterone, dexamethasone, and testosterone in challenging matrices like whole milk and ground beef, good recovery and reproducibility were obtained with minimal matrix effects using DisQuE pouch sample preparation. QuEChERS extraction is a simple and straightforward method for sample clean-up and has been shown to be effective for the extraction of steroid hormones from milk and meat. The additional step of sample clean-up using d-SPE prior to sensitive UPLC-MS/MS analysis provides more accurate recovery and quantitation of steroids in complex samples, allowing for low ppb range detection.

Conclusion

- The DisQuE pouch (QuEChERS) is effective for extraction of steroid hormones from livestock products.
- DisQuE dispersive sample preparation is an easy and straightforward technique that requires minimal user training, resulting in high throughput sample preparation of complex matrices.
- DisQuE sample preparation combined with UPLC-MS/MS enables sensitive quantitation of steroids in livestock products at low ppb concentrations.

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