Bond Elut Plexa Sample Preparation for LC/MS/MS Determination of Hormones in Pork

Application Note

Food Testing & Agriculture

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Introduction

Food safety is increasingly an important concern of people worldwide, because many chemicals added to food create potential hazards to human health. Hormones are a common food additive. Long term consumption of glucocorticoids can lead to hyperglycemia, hyperglycemia, osteoporosis, birth defects, and immune function decline. Other hormones, such as estrogen, androgen, and progesterone, are carcinogenic and can lead to breast cancer, ovarian cancer, and cell carcinoma. Many countries' regulations clearly define residual limits for these compounds in food. Maximum residue limits for these compounds can vary worldwide, but are generally in the low ppb concentration range. At these concentrations, the analysis of hormones in products such as meat is often very challenging due to the complexity of the sample.

In this application note, pork was prepared and analyzed for hormones at the ppb level, using a straightforward SPE methodology with Agilent Bond Elut Plexa polymeric SPE, efficient separation with an Agilent Poroshell 120 LC column, and sensitive detection with an Agilent 6460 Triple Quadrupole LC/MS system.

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Materials and Methods

LC conditions

Columns:	Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (p/n 699775-902)
Eluent:	A, water; B, acetonitrile
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Gradient:	20% B, linear to 40% B in 5 min, linear to 90% B in 3 min, hold for 10 min
Temperature:	Ambient
Sample vials:	Agilent Certified Vials (p/n 5183-2072)
System:	Agilent 1260 Infinity LC

MS conditions

Ionization mode:	ESI + Agilent Jet Stream
Gas temperature:	325 °C
Gas flow:	10 L/min
Nebulizer:	50 psi
Sheath temperature:	400 °C
Sheath gas flow:	12 L/min
Capillary:	4,500 V (ESI+), 3,500 V (ESI-)
Nozzle voltage:	1,500 V (ESI+), 1,500 V (ESI-)
System:	Agilent 6460 Triple Quadrupole LC/MS

The MRM transitions, fragmentors, and collision energies optimized for the hormones in this study are shown in Table 1.

Table 1. MRM transitions and other conditions for hormones.

Compound name	Precursor ion	Product ion	Fragmentor	CE	Polarity
Triamcinolone	435.4	415.2	100	2	Positive
	435.4	397.2	100	5	Positive
Nandrolone	407.4	257.2	150	10	Positive
phenylpropionate	407.4	105.1	150	25	Positive
Dexamethasone	393.3	373.2	100	2	Positive
	393.3	355.1	100	2	Positive
Methylprednisolone	375.4	357.2	100	2	Positive
	375.4	161.2	100	15	Positive
Hydrocortisone	363.4	327.2	125	8	Positive
	363.4	121.1	125	20	Positive
Prednisolone	361.4	343.2	100	2	Positive
	361.4	147.2	100	20	Positive
Prednisone	359.3	341.2	125	2	Positive
	359.3	147.1	125	20	Positive
Methyltestosterone	303.4	109.1	125	25	Positive
	303.4	97.2	125	25	Positive
Estriol	287.3	171.2	125	30	Negative
	287.3	145.3	125	40	Negative
Trenbolone	271.4	253.2	150	15	Positive
	271.4	199.1	150	20	Positive
Hexestrol	269.3	134.1	125	4	Negative
	269.3	119	125	35	Negative
Diethylstilbestrol	267.3	251.2	150	15	Negative
	267.3	237.2	150	20	Negative

Sample preparation

To pretreat the sample, 5 g ground pork was placed into a 50 mL centrifuge tube, followed by 5 mL methanol and 20 mL water. The tube was then vortexed vigorously for 1 minute. Next, the tube was centrifuged for 5 minutes at 5,000 rpm at 4 °C, and all of the supernatant was transferred into another tube for SPE cleanup.

To extract the sample, the procedure shown in Figure 1 was used. Agilent Bond Elut Plexa cartridges (60 mg, 3 mL, p/n 12109603) were preconditioned with 3 mL methanol then 3 mL water. The extract (equivalent to 5 g sample) was passed through the cartridge at a rate of 1 mL/min. After the sample passed through completely, the cartridge was washed with 3 mL 35% methanol in water, and the entire effluent was discarded. The cartridge was dried under negative pressure (below 2.0 kPa) for 5 minutes. The sample was eluted with 5 mL of methanol. The eluate was collected and dried under nitrogen below 40 °C. The sample residue was then dissolved and brought to a constant volume of 1.0 mL using 20% methanol in water (v:v), filtered through a 0.2 µm filter membrane (Agilent Captiva Polyethersulfone, p/n 5190-5096), and analyzed by LC/MS/MS.

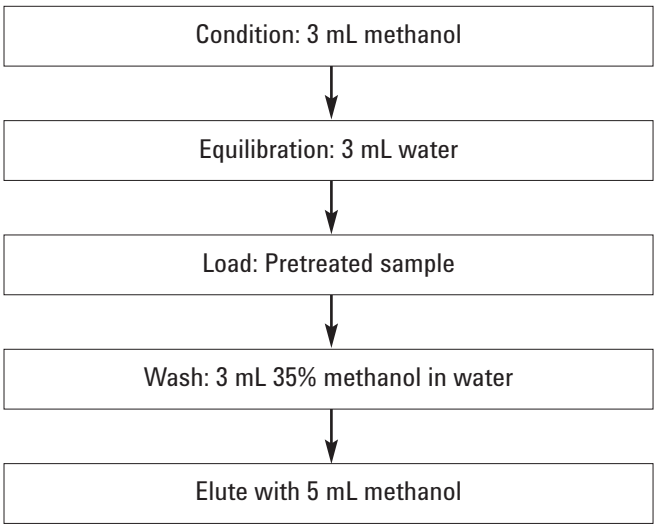

Figure 1. The SPE procedure for a pork sample.

Results and Discussion

Recovery was measured for each hormone at both low and high concentration levels (Table 2). Recovery was calculated by comparing the MRM peak area for samples spiked prior to SPE extraction, with the MRM peak area for samples spiked after SPE extraction (post spiked samples). Figure 2 shows a chromatogram obtained from the analysis of pork blank sample spiked with low levels of hormones. Figure 3 is the sample blank.

Table 2. Extraction recoveries of hormones from pork with SPE.

Hormone	% Recovery (%RSD) n = 6 low level (1 ppb)	% Recovery (%RSD) n = 6 high level (10 ppb)
Triamcinolone acetonide	67.2 (5.4)	78.3 (7.9)
Nandrolone phenylpropionate	66.7 (9.8)	70.3 (6.3)
Dexamethasone	86.9 (4.5)	91.4 (3.9)
Methylprednisolone	95.8 (5.2)	94.3 (8.3)
Hydrocortisone	102.3 (7.1)	98.7 (4.4)
Prednisolone	82.8 (3.7)	75.3 (3.9)
Prednisone	77.2 (4.3)	86.9 (1.4)
Methyltestosterone	80.1 (1.7)	87.6 (2.2)
Estriol	53.7 (4.9)	67.5 (8.7)
Trenbolone	98.0 (3.5)	103.5 (3.2)
Hexestrol	34.7 (10.7)	46.8 (8.4)
Diethylstilbestrol	42.3 (5.1)	36.2 (5.8)

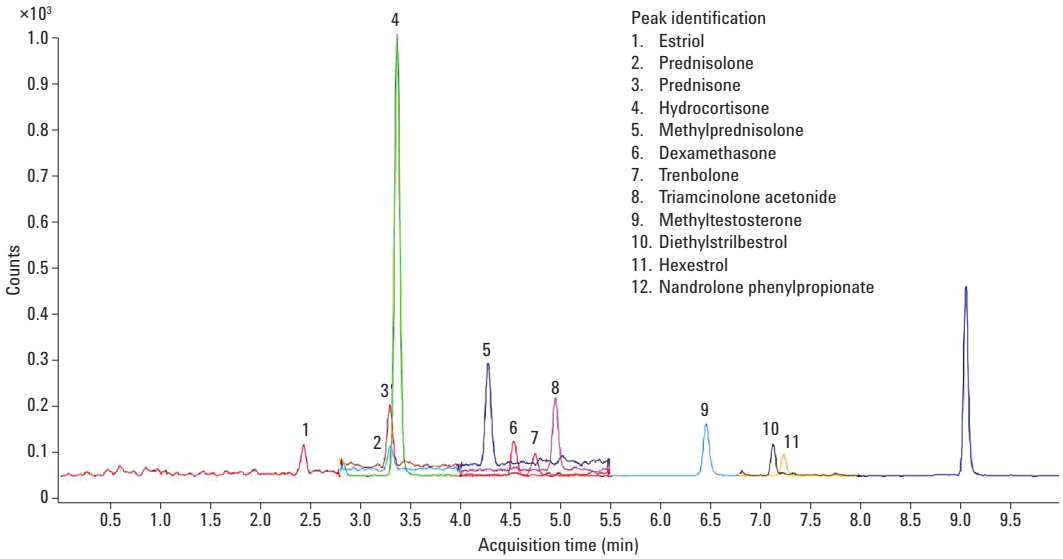

Figure 2. Chromatogram of hormones obtained from pork spiked with a low level sample,

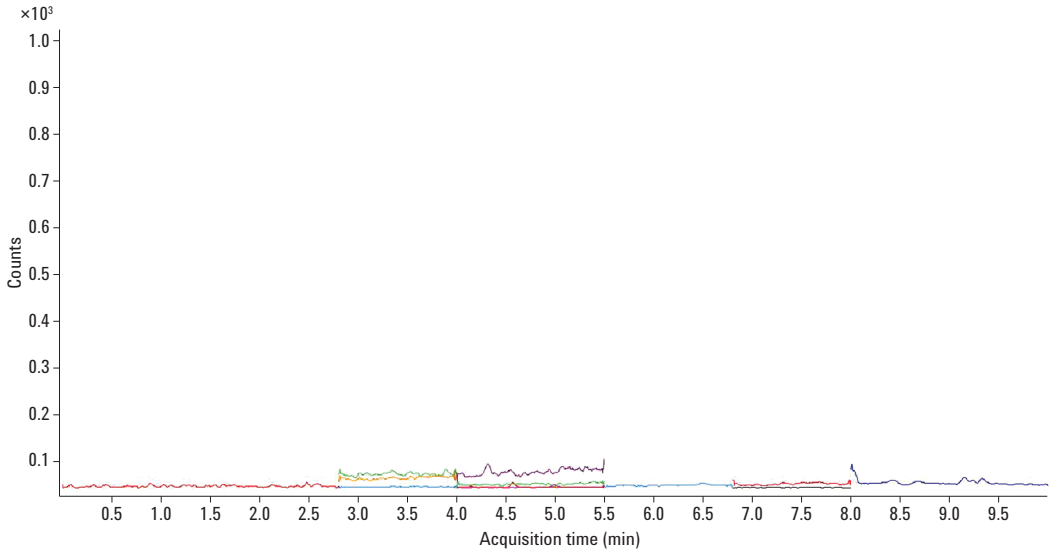

Figure 3. Chromatogram of hormones obtained from a pork blank sample.

Conclusions

Good recovery and reproducibility were obtained with Agilent Bond Elut Plexa SPE for most hormones in a pork matrix. However, for hexestrol and diethylstilbestrol, the results were influenced by matrix effects, and so isotope internal standards should be used for these two compounds to achieve better recoveries.

Bond Elut Plexa SPE combined with LC/MS/MS enables sensitive quantitation of hormones in meat samples at low ppb concentrations.

For More Information

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