Determination of Hormones in Fish (*Carassius Carassius*) by SampliQ-OPT Solid Phase Extraction with High Performance Liquid Chromatography

Application Note

Food Safety

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Abstract

Solid-phase extraction (SPE) coupled with high performance liquid chromatography (HPLC) was optimized for the extraction and determination of sixteen hormones (estriol, prednisolone, hydrocortisone, prednisone, methylprednisolone, betamethasone, dexamethasone, triamcinolone acetate, gestrinone, prednisolone acetate, hydrocortisone acetate, prednisone acetate, estradiol, cortisone acetate, methyltestosterone, estrone) in crucian carp (*Carassius carassius*) meat. Results indicate that SPE using an Agilent SampliQ OPT (60 mg, 3 mL) and HPLC using an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm) is suitable for extraction of these compounds. Recoveries ranged from 76.2 to 106.1 % with relative standard deviations (RSDs) between 1.7 and 8.9 %.

Agilent Technologies

Introduction

Food safety has increasingly become an important concern of people worldwide. Many chemicals added to food create potential hazards to human health. Hormones are a common food additive. Long-term consumption of glucocorticoid can lead to 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.

An Agilent SampliQ OPT SPE cartridge was used to extract 16 kinds of hormones (Table 1) from crucian meat and an HPLC method was established to detect these 16 compounds.

Table 1. Hormones Used in this Study

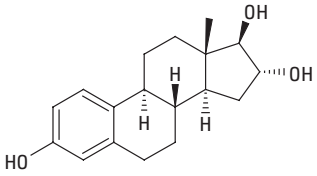
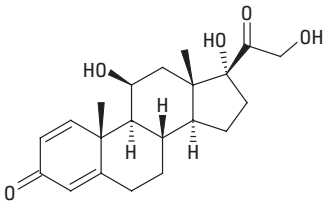
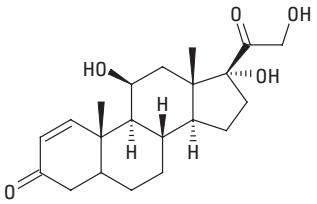
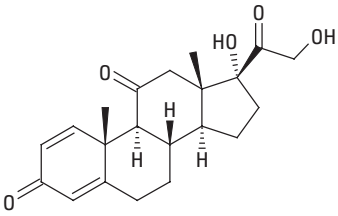
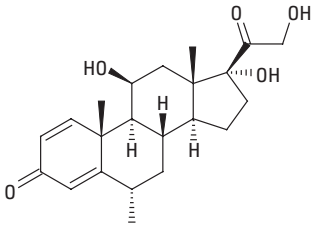
No.	Name	CAS No.	Log P	Structure
1	Estriol	50-27-1	2.45	
2	Prednisolone	50-24-8	1.66	
3	Hydrocortisone	50-23-7	1.79	
4	Prednisone	53-03-2	2.07	
5	Methylprednisolone	83-43-2	2.06	

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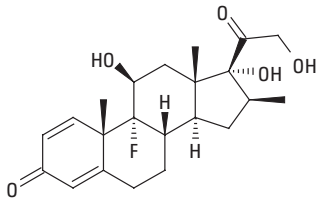
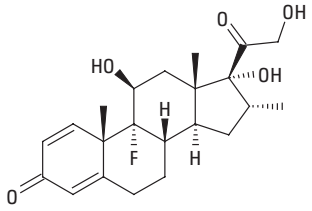
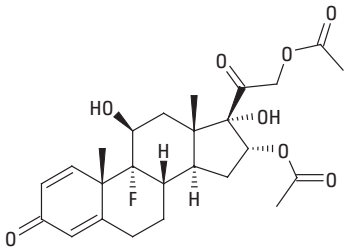
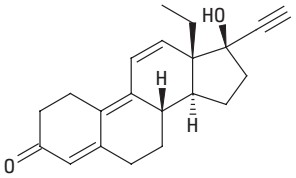
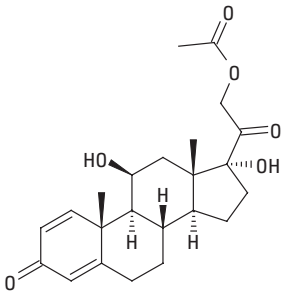
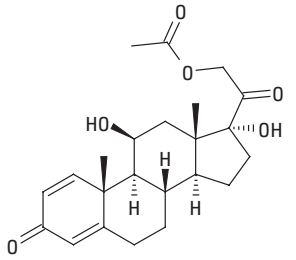
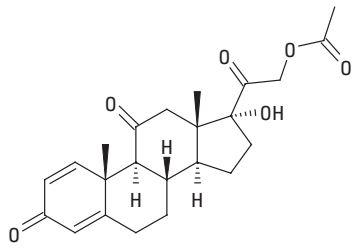
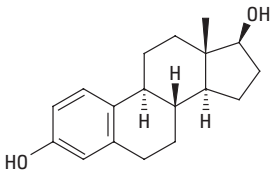
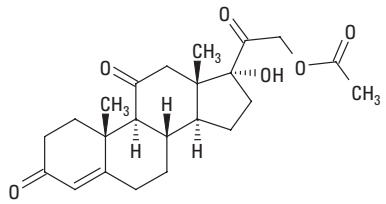
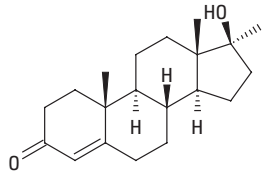
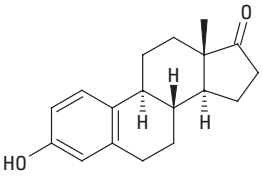
Table 1. Hormones Used in this Study

No.	Name	CAS No.	Log P	Structure
6	Betamethasone	378-44-9	1.93	
7	Dexamethasone	50-02-2	1.93	
8	Triamcinolone acetate	67-78-7	1.9	
9	Gestrinone	16320-04-0	NA	
10	Prednisolone acetate	52-21-1	NA	
11	Methylprednisolone	83-43-2	NA	

(Continued)

Table 1. Hormones Used in this Study

No.	Name	CAS No.	Log P	Structure
12	Prednisone acetate	125-10-0	NA	
13	Estradiol	50-28-2	3.57	
14	Cortisone acetate	50-04-4	2.35	
15	Methylestosterone	58-18-4	NA	
16	Estrone	53-16-7	4.03	

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Hormone standards were purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products). Crucian was purchased from a local market.

Stock solutions (1 mg/mL) were prepared in methanol and kept in the freezer (–20 °C). Working solutions were prepared using the stock solution diluted with methanol. The working solutions should be prepared every week and need to be stored below 4 °C.

The SPE cartridges were Agilent SampliQ OPT (3 mL, 60 mg, p/n 5982-3036). The analysis was performed on an Agilent 1200 Series HPLC with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 (5 µm 250 mm × 4.6 mm id, p/n 959990-902). An Agilent 0.45-µm PTFE Premium Syringe Filter (p/n 5185-5836) was used to filter the sample solution before HPLC.

HPLC conditions

Column:	ZORBAX Eclipse Plus C18 250 mm × 4.6 mm, 5 µm		
Flow rate:	1.0 mL/min		
Injection volume:	5 µL		
Column temperature:	18 °C		
Detection wavelength:	230 nm		
Mobile phase:	Water-Acetonitrile Gradient		
	Time (minutes)	% Water	% Acetonitrile
	0	70	30
	10	65	35
	23	50	50
	30	20	80

Separation

1. Weigh 200 grams of crucian meat, homogenize, and store in a clean, sealed container at –18 °C.
2. Place 1 g of homogeneous sample (accurate to 0.01 g) into a 10-mL polypropylene centrifuge tube with 5 mL of methanol.
3. Vortex for 1 minute.
4. Extract ultrasonically for 10 minutes in an ice bath.
5. Centrifuge the sample at a speed of 4000 r/min for 5 minutes and remove the 3 mL of supernatant.
6. Save in a clean tube and evaporate with N_2 below 40 °C.
7. Reconstitute the residue in 5 mL of 5 % methanol in water.

SPE Purification

The procedure used for the SPE extraction is shown in Figure 1. Agilent SampliQ OPT cartridges are preconditioned with 3 mL of methanol then 5 mL of water. The 5-mL extract (equivalent to 0.6 g sample) is passed through the SampliQ OPT cartridge at a speed of 1 mL/min. After it effuses completely, the cartridge is washed with 5 mL of 30% methanol in water and the entire effluent is discarded. The cartridge is dried under negative pressure (below 2.0 kPa) for 3 minutes. The sample is then eluted with 6 mL of methanol, and the eluent is collected and dried under nitrogen below 40 °C. The residue is dissolved and brought to a constant volume of 1.0 mL using methanol, filtered through a 0.45 µm PTFE filter membrane, and analyzed by HPLC.

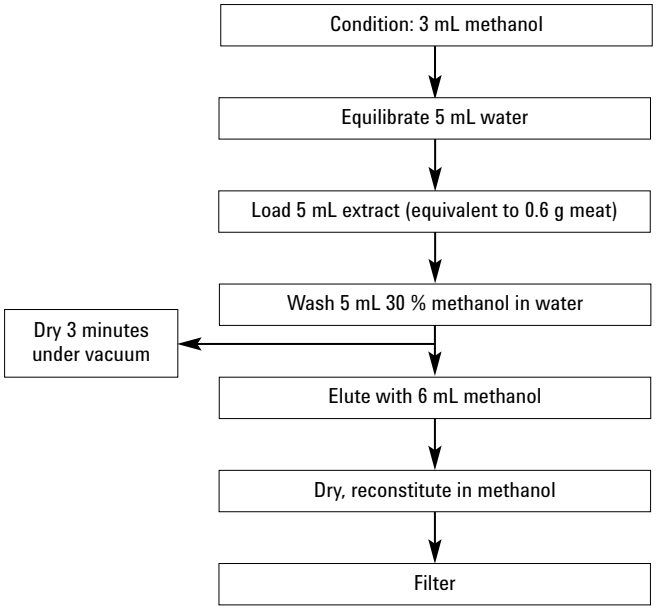


Figure 1. Hormones in crucian meat SPE procedure.

Results and Discussion

Linearity, Limits of Detection

Stock solutions were diluted to different concentrations and analyzed by HPLC. Linear regressions were calculated for the hormones based on the areas and the solution concentrations. Limit of detection (LOD) signifies the injection concentration at which the signal to noise ratio was between 2 and 3. Linear range was between 1–100 mg/kg. The linearity and LOD are shown in Table 2.

Table 2. Linearity and LODs of Hormones.

No.	Compound	Regression equation	Correlation coefficient	LOD (mg/kg)
1	Estriol	Y = 8.096 × –0.824	0.9998	0.5
2	Prednisolone	Y = 17.418 × –2.088	0.9999	0.2
3	Hydrocortisone	Y = 15.746 × –1.518	0.9999	0.3
4	Prednisone	Y = 20.192 × –2.152	0.9998	0.2
5	Methylprednisolone	Y = 16.986 × –1.894	0.9999	0.4
6	Betamethasone	Y = 20.439 × –1.106	0.9997	0.2
7	Dexamethasone	Y = 20.176 × –2.176	0.9999	0.2
8	Triamcinolone acetate	Y = 16.374 × –1.558	0.9997	0.4
9	Gestrinone	Y = 6.370 × –0.668	0.9998	1.0
10	Prednisolone acetate	Y = 15.589 × –1.627	0.9999	0.4
11	Hydrocortisone acetate	Y = 15.051 × –1.584	0.9999	0.4
12	Prednisone acetate	Y = 24.106 × –2.401	0.9997	0.2
13	Estradiol	Y = 8.709 × –0.635	0.9999	0.8
14	Cortisone acetate	Y = 19.826 × –2.336	0.9996	0.4
15	Methyltestosterone	Y = 19.980 × –2.209	0.9996	0.3
16	Estrone	Y = 10.701 × –0.847	0.9999	0.4

Recovery and Repeatability

The precision of the method was determined in terms of the recovery of spiked hormone standards in crucian meat at 2, 5, and 10 mg/kg levels. The analysis was repeated six times at each level. The chromatograms of the blank, the standards, and the spiked standard (2 mg/kg) sample are shown in Figures 2 through 4. The recovery and reproducibility data are shown in Table 3.

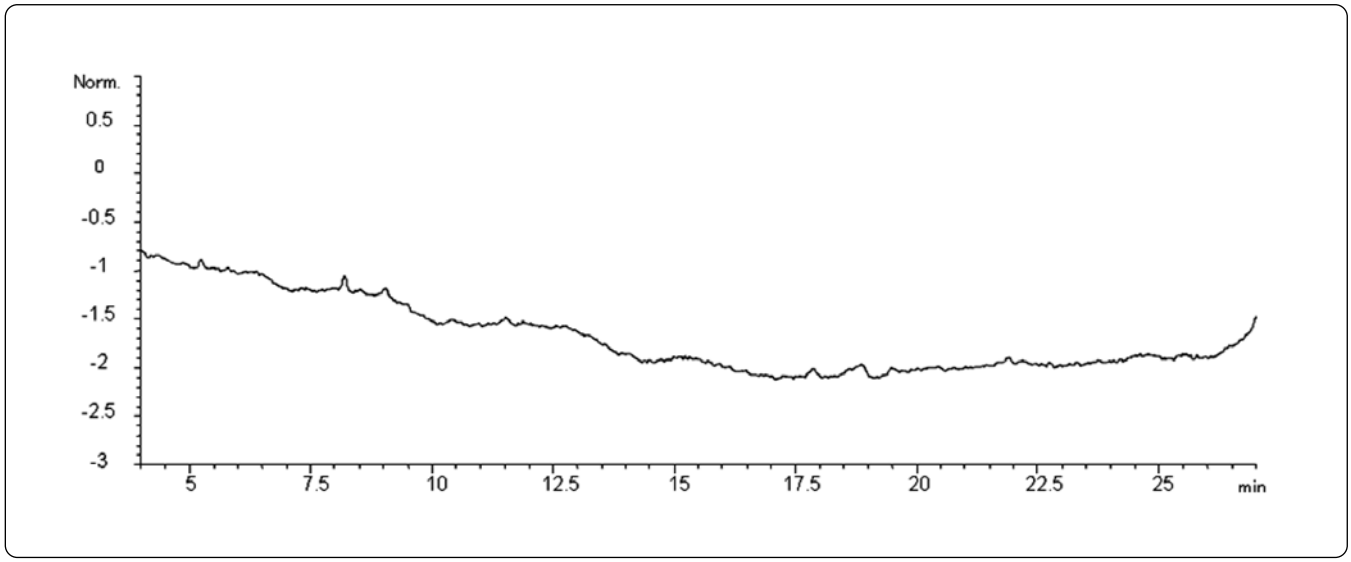
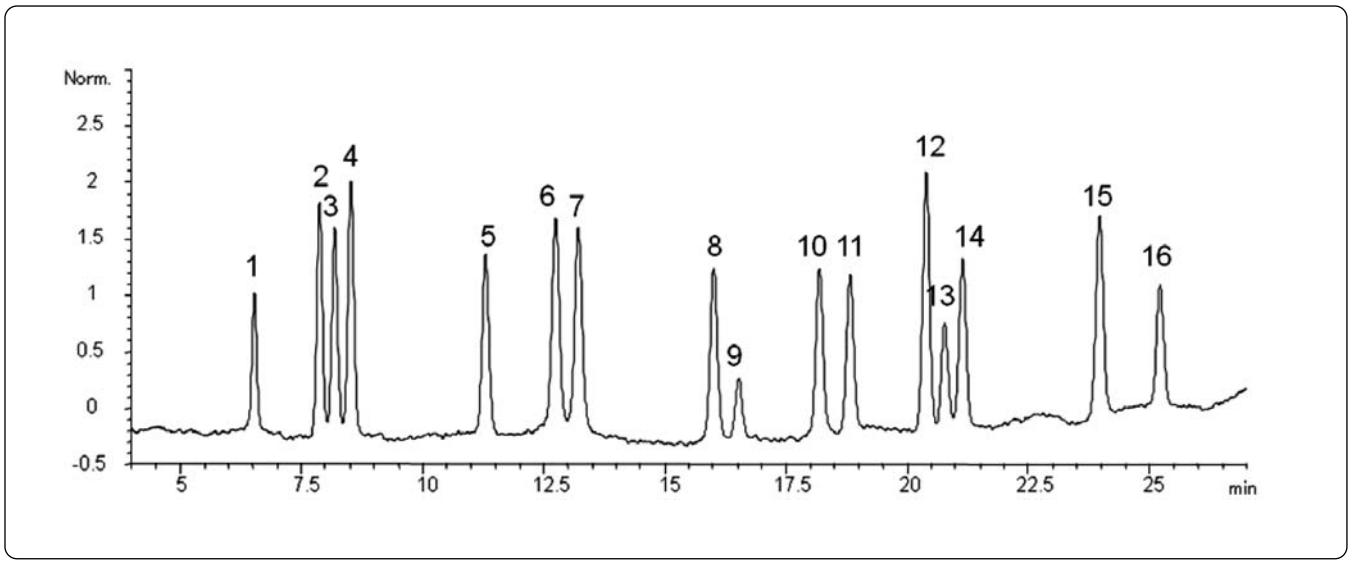
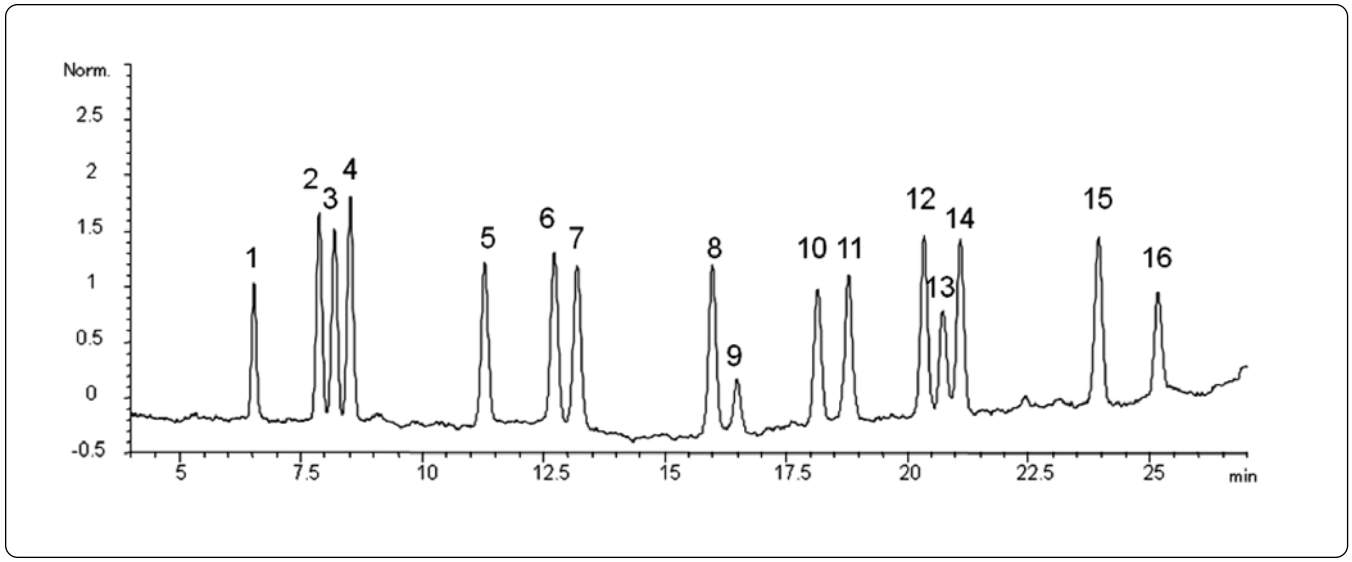


Figure 2. Chromatogram of crucian meat blank.



1 Estriol	5 Methylprednisolone	9 Gestrinone	13 Estradiol
2 Prednisolone	6 Betamethasone	10 Prednisolone acetate	14 Cortisone acetate
3 Hydrocortisone	7 Dexamethasone	11 Hydrocortisone acetate	15 Methyltestosterone
4 Prednisone	8 Triamicinolone acetate	12 Prednisone acetate	16 Estrone

Figure 3. Chromatogram of hormone standards at 2 mg/kg.



1 Estriol	5 Methylprednisolone	9 Gestrinone	13 Estradiol
2 Prednisolone	6 Betamethasone	10 Prednisolone acetate	14 Cortisone acetate
3 Hydrocortisone	7 Dexamethasone	11 Hydrocortisone acetate	15 Methyltestosterone
4 Prednisone	8 Triamicinolone acetate	12 Prednisone acetate	16 Estrone

Figure 4. Chromatogram of crucian meat sample spiked hormone standards at 2 mg/kg.

Table 3. Recoveries and RSDs of Hormones in Crucian Meat by SPE

Compund	Spiked level (mg/kg)	Recovery (%)	RSD (n = 6, %)
Estriol	2	100.4	2.2
	5	106.1	1.9
	10	102.4	4.4
Prednisolone	2	89.4	3.8
	5	90.9	7.6
	10	100.7	2.9
Hydrocortisone	2	85.3	6.7
	5	91.4	7.6
	10	101.4	3.4
Prednisone	2	82.5	7.2
	5	92.1	5.2
	10	100.7	2.9
Methylprednisolone	2	83.2	8.3
	5	93.6	3.2
	10	97.4	1.7
Betamethasone	2	88.3	8.9
	5	99.6	4.9
	10	100.8	3.8
Dexamethasone	2	79.1	4.3
	5	98.4	5.3
	10	98.4	3.9
Triamcinolone acetate	2	86.7	8.4
	5	97.6	5.9
	10	97.9	4.1
Gestrinone	2	78.0	6.6
	5	78.8	8.1
	10	85.3	8.0
Prednisolone acetate	2	86.9	7.3
	5	101.2	4.3
	10	101.9	5.7
Hydrocortisone acetate	2	87.3	6.8
	5	102.7	5.1
	10	101.5	7.9
Prednisone acetate	2	76.7	7.7
	5	94.1	3.5
	10	97.7	4.3
Estradiol	2	78.7	4.2
	5	94.7	3.5
	10	97.4	4.8
Cortisone acetate	2	82.8	6.9
	5	87.8	6.5
	10	94.4	4.1
Methyltestosterone	2	82.9	3.4
	5	91.9	4.9
	10	93.6	4.6
Estrone	2	76.2	6.4
	5	90.0	8.7
	10	93.9	5.9

Conclusions

Agilent's SampliQ OPT, a polymeric sorbent with combined hydrophilic and lipophilic characteristics that allows retention of both polar and non-polar compounds, provides a simplified and effective single cartridge method for the purification and enrichment of multiple hormone compounds in crucian carp. Recovery and reproducibility (routinely below 10%) based on solution standards are acceptable for hormone residue determination in crucian meat. Impurities from crucian were minimal and did not interfere with any of the hormones analyzed.

Product Information

Part number	Description
5982-3013	OPT Polymer - Box, 100x 1 mL tubes, 30 mg
5982-3036	OPT Polymer - Box, 50x 3 mL tubes, 60 mg
5982-3067	OPT Polymer - Box, 30x 6 mL tubes, 150 mg
5982-3096	OPT Polymer - 96 Well Plate, 10 mg
95990-902	Agilent ZORBAX Eclipse Plus C18 250 mm × 4.6 mm, 5 µm
5185-5836	Agilent PTFE 0.45 µm Premium Syringe Filter

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