

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

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Introduction

Glucocorticoids are a class of steroid hormones, produced by the adrenal cortex, that affect metabolism and have immunosuppressive and anti-inflammatory properties. The common chemical structure of these compounds is shown in Figure 1. The chemical structures of eight glucocorticoid drugs are listed in Table 1.

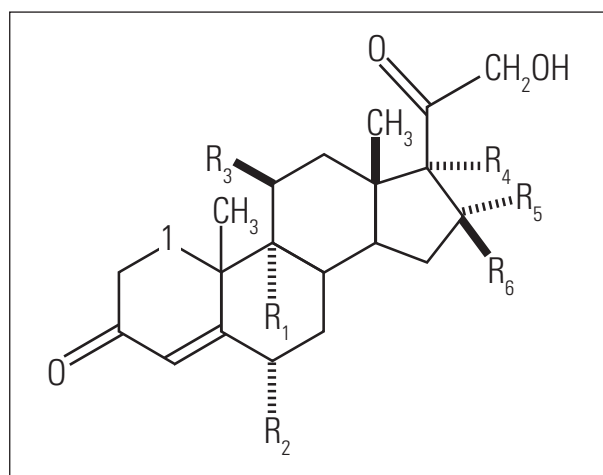


Figure 1. Common chemical structure of glucocorticoids

Table 1. Glucocorticoid drugs and their functional groups

	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
Dexamethasone	-F	-H	-OH	-OH	-CH ₃	-H
Betamethasone	-F	-H	-OH	-OH	-H	-CH ₃
Prednisone	-H	-H	=O	-OH	-H	-H
Prednisolone	-H	-H	-OH	-OH	-H	-H
Methylprednisolone	-H	-CH ₃	-OH	-OH	-H	-H
Beclomethasone	-Cl	-H	-OH	-OH	-H	-CH ₃
Hydrocortisone	-H	-H	-OH	-OH	-H	-H
Fludrocortisone	-F	-H	-OH	-OH	-H	-H

Synthetic glucocorticoids are often administered to livestock to treat inflammatory diseases, disorders of the musculoskeletal system, and other diseases common to farm animals. They can also be used to improve feed intake and stimulate growth in livestock. However, glucocorticoid residues in animal-derived food may be

harmful to humans. Therefore, the use of certain substances with a hormonal or thyrostatic action as growth-promoting additives has been banned by the European Commission.¹ Dexamethasone, betamethasone, prednisolone, and methylprednisolone are the only glucocorticoids permitted for therapeutic purposes in livestock. Maximum residue levels (MRLs) have been established for these compounds in tissues and in milk intended for human consumption by the EC²⁻⁵ and other countries. Table 2 lists the MRLs for glucocorticoids that were set by the European Union. In China, the Institute for the Control of Agrochemicals, Ministry of Agriculture, has established MRLs of 0.75 µg/kg for dexamethasone in the muscles, livers, and kidneys of cattle, pigs, and horses and 10 µg/kg for hydrocortisone in milk.

Table 2. European Union MRLs for glucocorticoids

Substance	Animal Species	MRL	Target Tissues
Dexamethasone	Cattle	0.3 µg/kg	Milk
	Cattle, Pig, Horse	0.75 µg/kg	Muscle
		2.0 µg/kg	Liver
		0.75 µg/kg	Kidney
Betamethasone	Cattle	0.75 µg/kg	Muscle
		2.0 µg/kg	Liver
		0.75 µg/kg	Kidney
		0.3 µg/kg	Milk
	Pig	0.75 µg/kg	Muscle
		2.0 µg/kg	Liver
Prednisolone	Cattle	4 µg/kg	Muscle
		4 µg/kg	Fat
		10 µg/kg	Liver
		10 µg/kg	Kidney
		6 µg/kg	Milk
Methylprednisolone	Cattle	10 µg/kg	Muscle
		10 µg/kg	Fat
		10 µg/kg	Liver
		10 µg/kg	Kidney

Goal

To develop an effective LC-MS/MS method to detect eight glucocorticoid residues in the liver and muscles of pig, cattle, and lamb, as well as in chicken, eggs and milk.

Key Words

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

Experimental Conditions

Sample Preparation

A 2 g tissue sample was weighed into a 50 mL centrifuge tube and 15 mL of ethyl acetate was added. The sample was vortexed for 3 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred into another 50 mL centrifuge tube. The sample was extracted again with 10 mL sodium hydroxide (0.1 M) and 15 mL ethyl acetate. The two supernatants were combined and evaporated to dryness at 40 °C. The residue was dissolved in 1.0 mL of ethyl acetate and 5.0 mL of n-hexane before being loaded to the silica extraction cartridges (SPE column). The SPE column was previously conditioned with 5 mL of n-hexane. The column was washed with 5 mL of n-hexane. After drying, the analytes were eluted with 5 mL n-hexane:acetone (40:60, v/v) and evaporated to dryness under nitrogen at 40 °C. The residues were reconstituted in 1.0 mL of 20% acetonitrile aqueous solution. The resulting solutions were vortexed for 1 minute and then centrifuged at 10,000 rpm for 10 minutes. The upper clear solutions were transferred to another sample vial for LC-MS/MS analysis.

The glucocorticoid standards were obtained from Sigma (Sigma Chemical Company, St. Louis, MO). All other chemicals were HPLC grade.

LC

HPLC analysis was performed using the Thermo Scientific Surveyor HPLC system. Each 10 µL of sample was injected into a Thermo Scientific Hypersil GOLD column (150 x 2.1 mm, 5 µm). A gradient LC method was used and mobile phases A (water with 0.1% formic acid) and B (acetonitrile) were at a flow rate of 250 µL/min together. Table 3 illustrates the gradient LC method.

Table 3. Gradient details

Retention Time (min)	A (%)	B (%)
0	70	30
18	40	60
23	40	60
23.1	70	30
28	70	30

MS

MS analysis was performed 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):	5 units
Capillary temperature:	350 °C
Collision gas pressure (Ar):	1.5 mTorr
Q1/Q3 Peak Resolution:	0.7 FWHM

The SRM transitions that were monitored are summarized in Table 4.

Table 4. SRM transitions

Drug	SRM Transitions (m/z)	
	Quantitative	Qualitative
Prednisone	403.0 → 326.9	403.0 → 357.2
Prednisolone	405.0 → 329.0	405.0 → 358.8
Hydrocortisone	407.1 → 331.1	407.1 → 361.1
Methylprednisolone	419.0 → 342.9	419.0 → 373.1
Dexamethasone	437.0 → 361.0	437.0 → 391.5
Betamethasone	437.0 → 361.0	437.0 → 391.5
Beclomethasone	453.0 → 406.9	453.0 → 376.8
Fludrocortisone acetate	467.1 → 420.8	467.1 → 349.2

Results and Discussion

Figure 2 displays the SRM chromatograms for the eight glucocorticoids. The limit of quantitation (LOQ) for prednisone, prednisolone, dexamethasone, betamethasone, and methylprednisolone in milk is 0.1 µg/L. The LOQ of these residues in muscle, eggs and livers is 0.2 µg/kg. For fludrocortisone acetate and beclomethasone, the LOQ is 0.2 µg/L in milk, and 0.2 µg/kg in eggs, and livers. For hydrocortisone, the LOQ in milk is 0.2 µg/L and in eggs and livers is 0.5 µg/kg. These LOQs easily meet the specified MRLs of the European Union and China.

The extraction recovery of glucocorticoids from the muscle and livers of cattle, pigs, and lamb; chicken; eggs; and milk is between 60% and 110%. This achieves the minimum detection requirements. Thus, the qualification method is accurate and reproducible.

The method validation data is summarized in Table 5. Linearity of the method was assumed because the R² values were greater than 0.99 for the linear regression equations (1/x weighted).

Table 5. Linearity and dynamic range

Drug	Dynamic Range (µg/L, µg/kg)	Equation	R ²
Prednisone	0.2 ~ 50	$y = 106952x + 5288$	0.9992
Prednisolone	0.2 ~ 50	$y = 100276x - 2420.7$	0.9954
Hydrocortisone	0.8 ~ 200	$y = 85500x + 6127.5$	0.9947
Methylprednisolone	0.2 ~ 50	$y = 98154x - 5291.5$	0.9995
Dexamethasone	0.2 ~ 50	$y = 106501x - 8596.1$	0.9991
Betamethasone	0.2 ~ 50	$y = 116052x - 17056$	0.9986
Beclomethasone	0.4 ~ 100	$y = 52195x - 6621.2$	0.9911
Fludrocortisone acetate	0.4 ~ 100	$y = 33779x - 2193.9$	0.9992

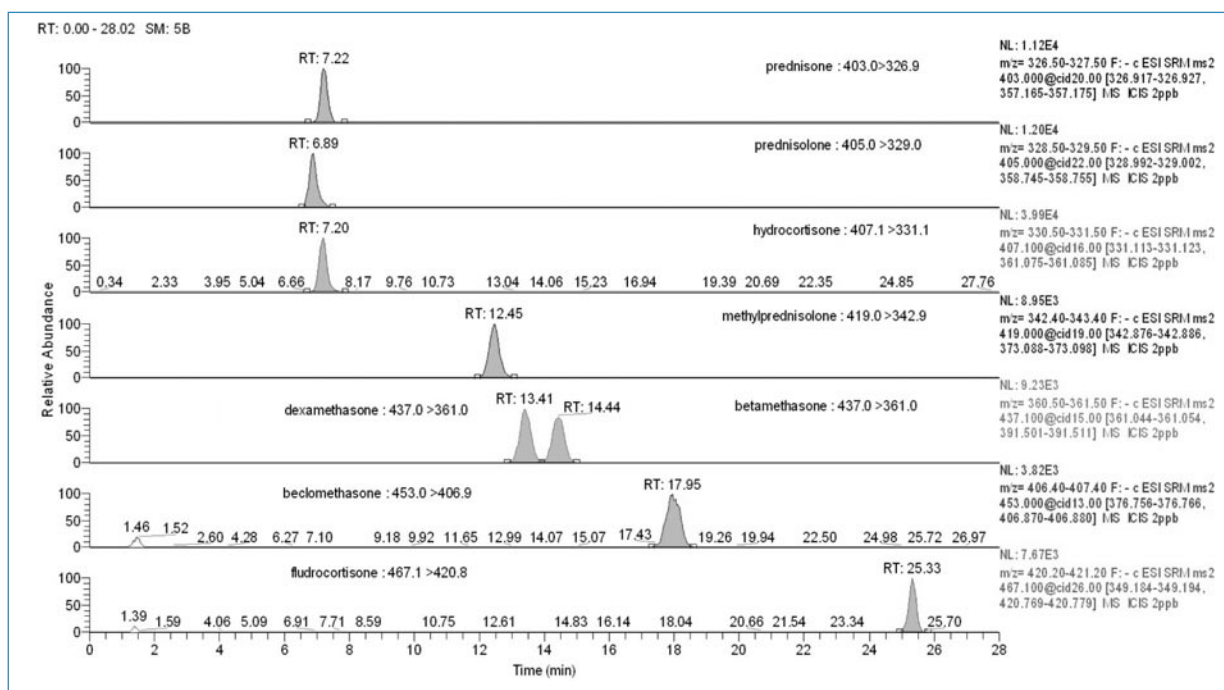


Figure 2. SRM chromatograms for the eight glucocorticoids.

Conclusion

The LC-MS/MS method described above is able to detect glucocorticoid residues with high recoveries and enable their accurate quantification. The sensitivity, extraction recovery, and reproducibility of this method meet international regulation and detection requirements.

References

1. European Community, Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of β -agonists, (Official Journal of the European Communities Official Journal L 125, 23 May 1996, pp. 3-9).
2. European Community, Commission Regulation (EC) No 508/1999 of 4 March 1999 amending Annexes I to IV of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, (Official Journal of the European Communities Official Journal L 060, of 9 March 1999, pp. 16-52).
3. European Community, Commission Regulation (EC) No 2593/1999 of 8 December 1999 amending Annexes I, II and III of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, (Official Journal of the European Communities Official Journal L 315, of 8 December 1999, pp. 26-31).
4. European Community, Commission Regulation (EC) No 2535/2000 of 17 November 2000 amending Annex I of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, (Official Journal of the European Communities Official Journal L 291, of 17 November 2000, pp. 9-10).
5. European Community, Commission Regulation (EC) No 77/2002 of 17 January 2002 amending Annexes I and III to Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, (Official Journal of the European Communities Official Journal L 16, of 17 January 2002, pp. 9-11).

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